

Validating and Developing Duffy Null Specific Absolute Neutrophil Count Reference Ranges for Adults and Pediatrics

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A. OVERVIEW

Background and Significance

People of African or Middle Eastern genetic ancestry often have lower peripheral absolute neutrophil counts (ANC) than those with European or Asian genetic ancestry without any increased risk of infection or negative clinical sequelae. We now know that this lower ANC is closely linked to the Duffy null [Fy(a-b-)] red blood cell antigen phenotype. The Fy(a-b-) phenotype occurs in as many as 80-100% of those of African ancestry, 50-60% of those from the Arabic peninsula, and <1% of those of European or Asian genetic ancestry. Approximately 2 of 3 people identifying as Black in the USA are expected to have the Duffy null phenotype. Of note, race and ethnicity are not biological facts but sociopolitical constructs. Thus, genetic traits do not obey racial boundaries or geographic constructs and there should be extreme caution for use as a proxy. Although the Duffy null phenotype is not limited to those only of African or Arabian Peninsula genetic ancestry, it is most commonly found among these populations. One study of healthy Black individuals in the USA showed that the median ANC for Duffy null individuals was 2,820/uL (IQR: 2088-3490) compared to Duffy non-null individuals with median ANC of 5005 (IQR: 3675-5828). There was a significant difference in median ANC and institutional reference range ANC among Duffy null individuals, but not among Duffy non-null individuals. A single center study developed and published a new ANC reference range for Duffy-null individuals that suggest 1210/uL-5390/uL which is significantly different than the institutional reference range of 1920/uL-7600/uL.

Reference ranges that accurately reflect the normal values of the population are essential to deliver optimal care including appropriate initiation of testing for values out of range, admittance to clinical trials, and optimized treatment with chemotherapeutics and other myelosuppressive medications. We aim to accelerate the adoption of Duffy null specific reference ranges by validating the adult reference range and developing pediatric ranges and publishing these for use in clinical practice.

B. Methods

We will perform a single-arm prospective blood sample collection study in healthy Participants in order to validate Duffy null specific ANC reference ranges that are acceptable for clinical use. This is a multi-site project, and there are multiple acceptable ways of recruiting individuals and obtaining the data.

Patient Selection and Eligibility

Inclusion criteria:

- i. self-identification as Black or African American or Middle Eastern ancestry
- ii. blood draw obtained when in the outpatient setting, pre-operative elective surgery, or community setting

Exclusion criteria:

- i. use of medications known to cause neutropenia (table attached)
- ii. history of a congenital neutropenia syndrome

- iv. acute illness
- v. temperature >38°C
- vi. being seen for an urgent visit or undergoing emergency surgery
- vii. history of rheumatologic disorders, malignancy, chronic infection, or autoimmune disease
- viii. history of bone marrow transplant or any immunodeficiency
- ix. undergoing work-up for infection, cancer, or autoimmune disease.
- x. active pregnancy

Recruitment

Participants can be recruited from any participating site where they can reasonably be expected to be healthy, including outpatient clinic, in the community, or in elective preoperative settings. Participants should be screened by the study team for eligibility before they are approached, as outlined in the exclusion and inclusion criteria.

Informed Consent

Individuals who meet the inclusion criteria may be approached by a member of the study team in-person, by telephone, or electronically. The individual will be asked if they are interested in participating in the study and if interested, the study team at the participating site will review the informed consent form in-person or virtually. The study team will address any questions or concerns the patient has prior to obtaining verbal or written informed consent. A waiver of consent will be requested from local IRB for sites who opt to use residual blood. The site is responsible for verifying that consent will be obtained from adults, or the parent/legal guardian of children, according to IRB requirements and applicable local regulations. Verbal and/or written assent will be obtained from children based on local IRB requirements.

Withdrawal of Consent

Individuals may decide to withdraw their consent at any time before blood draw is obtained. After the blood draw is obtained, the data is de-identified and specific patient data will not be able to be identified for withdrawal.

Participant Costs and Compensation

There is no cost for participating. Compensation schemes will vary by participant population and institution-specific preferences.

Patient Risk/Benefit Assessment

The risks to the subjects include the discomfort of a needle stick for blood work. Every attempt will be made for the participants' study sample to be collected at the time of clinically relevant blood work to minimize needle sticks and the subsequent risks. Another risk associated with this project is the potential for a breach of patient confidentiality. However, we established a robust plan for ensuring appropriate and commercially reasonable physical, technical, and administrative safeguards are in place to prevent, preempt, and mitigate any potential risks. Some institutions may make Duffy typing results available for participants and clinicians through the electronic medical record which could be potentially beneficial in understanding their Duffy phenotype if new Duffy null specific ANC reference ranges are published.

Oversight

Management of the project will be conducted in accordance with applicable U.S. Food and Drug Administration Regulations and guidelines including Good Pharmacovigilance Practices and

Pharmacoepidemiologic Assessment, International Council for Harmonisation guidelines on Good Clinical Practice, and the principles of the Declaration of Helsinki, as well as all other applicable national and local laws and regulations.

The American Society of Hematology (ASH) has an Oversight Committee to oversee the operations of this project. This expert panel directs the efforts of ASH staff in developing and executing this project in a manner that preserves patient trust and complies with all international, federal, state, and local guidelines. Doris Duke Foundation is a collaborator on this project.

Design and Procedures

Once the individual has agreed to participate, s/he will be assigned a unique study code. Participants may undergo dedicated blood draw for research purposes or testing may be added on to previously planned blood draw. Residual blood is also acceptable. Blood will be processed at the local site. Blood will not be removed from the site nor stored for research purposes. One 3 mL lavender top (EDTA) tube of volume blood will be collected from adults and infants participants to perform Duffy genotyping, phenotyping, and absolute neutrophil count (ANC) analysis. Some labs require two separate 3 mL lavender top tubes: one to perform ANC analysis and one for Duffy typing. Duffy phenotype or Duffy genotype by sequencing are acceptable methods to obtain typing. Complete blood count (CBC) with differential must be obtained within 72 hours of screening to ensure that inclusion and exclusion criteria are met. Duffy typing is ideally obtained at the same time as CBC with differential to minimize patient discomfort but is not required concurrently. Duffy typing can be obtained at any time in the research timeline. Local IRB will determine if subjects receive their results over electronic medical record or if the results will be used for research purposes alone.

The following data will be recorded in RedCap Cloud, a secure database where individual sites will input de-identified data and will be the responsibility of ASH as study sponsor:

1. Study ID
2. Age at time of blood draw
3. Sex
4. Race (if available)
5. Ethnicity (if available)
6. Duffy phenotype or genotype result
7. Absolute neutrophil count
8. White blood cell count

Inclusion

The age range of all participants is 0-100 years, categorized into pediatric and adult age groups.

Pediatrics

Different reference ranges by age must be developed for pediatrics. The age categories include: 0-3 months, 3-6 months, 6 mo-5 years, 5-12 years, 12-18 years. We will enroll individuals until there are at least 120 Duffy null samples obtained in each age category. Assuming 60% of individuals are Duffy null, we will need at least 200 samples from each age category. Individual sites will not be responsible for obtaining all of the pediatric samples in any category. Rather, these data will be pooled from all sites in order to more efficiently reach the target number of samples and minimize the number of children who are tested. The 120 samples for development of a reference range are obtained from the Clinical

Laboratory Standards Institute (CLSI) guidelines. CLSI guidelines will also be used to establish a 95% reference interval by a nonparametric percentile method. A 1-sample, 2-sided Wilcoxon signed-rank test will be used to compare ANC reference median with the Duffy null cohort median, with a preset significance level of 0.05.

Adults

We will attempt to enroll 100 adult (≥ 18 years old) participants in each center. Assuming 60% of participants have the Duffy null phenotype, we will have 60 Duffy null subjects which is acceptable for validating a previously published reference range. Typically, 40-60 samples are needed to validate a previously published reference range per Clinical Laboratory Standards Institute (CLSI) guidelines. Local pathology policies will determine the exact number of samples needed to validate and implement an adult Duffy-null ANC reference range at each institution.

ASH is the study sponsor. ASH staff will be responsible for managing the operational aspects and data storage. Data included will be used for validation, development, and dissemination of adult and pediatric Duffy-null specific reference ranges. Separate data collection protocols, informed consent documents, and any other documents as appropriate must be approved by the IRB as amendments or new protocols.

All responsible parties provide a written statement agreeing to the content of the proposal and the confidential nature of the documentation and acknowledging that ASH has the right to discontinue and/or amend this protocol at any time as appropriate. Participant information collected will comply with the standards for protection of privacy of individually identifiable health information according to local privacy laws. Informed consent and assent forms, as applicable, will be prepared according to the institutional requirements for informed consent and the applicable regulations.

C. Data Collection and Management

Data Collection

Data collection will be done from individuals' existing medical records and/or manual data entry into an electronic data capture form (RedCap Cloud). Participating sites will submit HIPAA-compliant retrospective de-identified datasets of a participants' information on a one-time basis per participant and limited only to age, sex, race, ethnicity, Duffy typing results, WBC and ANC. Sites will submit data to RedCap Cloud at least once every 2 weeks, but are recommended to submit data weekly or in real time. ASH study staff and PI Lauren Merz will manage the data and be responsible for the integrity and confidentiality of the data.