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Article in *Laboratory Hematology* · March 2004

DOI: 10.1532/LH96.04010 · Source: PubMed

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Complete Blood Count Reference Interval Diagrams Derived from NHANES III: Stratification by Age, Sex, and Race

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Received January 15, 2004; accepted January 20, 2004

ABSTRACT

Background: Comprehensive, up-to-date “health-associated” reference interval studies of North American populations are uncommon. The third US National Health and Nutrition Examination Survey (NHANES III) was concluded in 1994 and yielded important reference interval data.

Objective: To obtain health-associated Coulter counter reference interval data from NHANES III according to age, sex, and race.

Methods: Of the 29,314 civilian noninstitutionalized US citizens who participated in NHANES III, approximately 25,000 had a complete blood count, red cell distribution width (RDW), platelet count, and automated white blood cell (WBC) differential determined on a Coulter S-Plus Jr. To determine health-associated reference intervals, we used the following exclusion criteria: pregnancy, breast feeding, obesity (body mass index [BMI] >40 and >35 for females and males, respectively), diastolic blood pressure >100 mm Hg, any smoking, any drinking of alcohol, recent treatment for anemia, creatinine level >2.5 mg/dL, glucose level >126 mg/dL, excessive thinness (BMI <8), recent surgery or hospitalization, or having antibodies to hepatitis viruses A, B, or C. The Coulter counter data (hemoglobin, hematocrit, red blood cell count, mean

corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), MCH, WBC count, platelet count, granulocyte count, monocyte count, lymphocyte count, RDW, platelet distribution width, and mean platelet volume) were separated into 6 sex/racial categories (female non-Hispanic white, female non-Hispanic black, female Mexican American, male non-Hispanic white, male non-Hispanic black, and male Mexican American) and 9 age groupings (10-14, 14-18, 18-25, 25-35, 35-45, 45-55, 55-65, 65-75, and >75 years).

Results: There was a high exclusion rate; for example, of the 20,685 individuals with measured hemoglobin levels, 12,688 (61.3%) were excluded. Percentile estimates could be derived accurately for almost all of the female age/sex categories. A few of the male Mexican American and non-Hispanic black categories contained observations for ages 45 to 75 years.

Conclusions: There are age-dependent trends for many of the tests, notably in RDW, MCV, platelet count, and granulocyte and lymphocyte percentages. Sex-dependent changes involved hemoglobin values, and race-related trends centered around mononuclear and lymphocyte percentages, hematocrit, MCHC, MCH, and hemoglobin. This study reveals the potential for using data mining of large samples to yield potentially useful reference ranges. *Lab Hematol.* 2004;10:42-53.

KEY WORDS: NHANES III · Reference interval data · Data mining · Complete blood count · Coulter S-Plus Jr

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BACKGROUND

Different approaches have been used to derive “normal ranges,” the reference intervals of clinical laboratory tests for

TABLE 1. Commonly Cited Reference Intervals for Select Hematology Tests*

Analyte	Sex	Reference Interval					
		Williams, 2001 [18]	Wintrobe, 1993 [19]	Laurells, 2001 [20]	Bick, 1993 [21]	Lewis, 2001 [22]	NORIP [10]
Hemoglobin, g/L	F	123-153	120-160	115-147 (<60 y), 113-153 (>60 y)	120-160	120-150	117-153
	M	140-175	140-180	131-163 (<60 y), 122-166 (>60 y)	140-180	130-170	134-170
Hematocrit, vol/vol	F	0.36-0.45	0.37-0.47	0.37-0.44	0.37-0.47	0.36-0.46	0.35-0.46
	M	0.42-0.50	0.40-0.54	0.39-0.49	0.40-0.54	0.40-0.50	0.40-0.50
RBC, $\times 10^{12}/L$	F	4.1-5.1		3.7-4.9	4.2-5.2	3.8-4.8	3.9-5.2
	M	4.5-5.9	4.4-6.0	4.1-5.4	4.6-6.2	4.5-5.5	4.2-5.7
MCV, fL	F/M	80-96	82-101	82-102	80-96	83-101	82-98
MCH, pg	F/M	27.5-33.2	27-34	28-35	26-34	27-32	27-33
MCHC, g/L	F/M	334-355	315-360	320-360	320-360	315-345	317-357
WBC, $\times 10^9/L$	F/M	4.4-11.3		4.0-10.0	4.5-11.0	4.0-10.0	3.5-8.8
Platelet count, $\times 10^9/L$	F/M	172-450		125-340	150-350	150-400	145-390

*NORIP indicates Nordic Reference Interval Project; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; WBC, white blood cell.

ambulatory individuals in a state of good health. Compared with hematology, derivation of reference intervals in clinical chemistry is straightforward. A simple set of interview questions complemented with glucose and creatinine testing can usually exclude most patients with chronic or acute disease. With the exception of thyroidal illness, most conditions associated with abnormal clinical chemistry test results occur infrequently. Thus, in the absence of test values dependent on age and sex, the sampling of approximately 120 subjects coupled with percentile analysis can usually establish reliable reference intervals for many clinical chemistry tests [1,2]. In contrast, the variables of common hematology tests measured by multi-channel hematology analyzers are influenced by various factors, including race [3,4], smoking [5], altitude [5], iron stores, alcohol ingestion [6], and even adrenergic stimulation. The combined prevalence of these factors may be so high that exclusion or stratification of these subjects based on these factors can reduce the number of reference subjects to fewer than can be reliably summarized with percentile analysis.

Because of these complexities, there is considerable variation in the methods used to establish hematology reference intervals as well as in the resulting reference intervals themselves. Table 1 summarizes the various sets of commonly recommended reference intervals for hemoglobin, hematocrit, red blood cell count (RBC), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), MCH, white blood cell (WBC) count, and platelet count. Because most common hematology procedures are well standardized and calibrated, the variation in reference interval recommendations is most likely associated with variations in the reference interval study populations and the investigators'

overt (or tacit) requirement for either diagnostic sensitivity or diagnostic specificity (the greater the deviation of a reference limit from its actual limit, the higher will be the test's specificity but the lower will be its sensitivity).

As in clinical chemistry, the derivation of reference intervals in hematology usually involves recruiting a large sample of healthy individuals and then excluding any individuals with interviews or screening laboratory test results that indicate the presence of acute or chronic disease. Usually, a single institution recruits study subjects who live in a single region and then collects and analyzes the patient specimens. Most of the reference intervals in Table 1 were derived in this manner. Because of the multiplicity of analyzers that can be in operation in a single health region and the movements of patients within that health region, it may be more relevant to compare the reference intervals obtained by the different analyzers in use in that health region. Van den Bossche et al recently evaluated and compared the reference intervals of a group of 142 males and 175 females (age range, 19-60 years; average age, 39 years) measured by 5 different automated hematology analyzers [7].

Another approach, again using distributed laboratories, requires multiple laboratories selecting and analyzing samples from just a few healthy individuals and sending the results to a central facility that calculates reference intervals for all of the participating laboratories. This approach was first described by Koepke in 1978 [8]. More than 2000 laboratories subscribing to the College of American Pathologists proficiency survey sampled and analyzed the blood samples from an apparently healthy male and a healthy female within a specified age range. With regard to the hemoglobin level, this

novel approach provided hemoglobin reference limits that were at least 5 g/L higher than those obtained in other population studies. We attribute these higher limits to the sampling of very healthy laboratory workers. The Scandinavian Society for Clinical Chemistry has developed this style of reference interval determination, both for clinical chemistry as well as for hematology. Essentially, participating Scandinavian laboratories standardize their assays to calibrators traceable to international standards. Each laboratory then selects, samples, and analyzes a small number of subjects in a state of health [9,10]. Because relatively few subjects are sampled at each institution, the total cost and effort expended by each laboratory is minimal. The reference intervals in Table 1 from the Nordic Reference Interval Project (NORIP) were obtained in this manner. High attention to detail is associated with the NORIP work. For example, because of the slight volume dilution that occurs when specimens are drawn into liquid K₃EDTA tubes, compared with dry K₂EDTA tubes, all results were corrected with simple nonparametric statistics to expected values in the undiluted samples before the reference interval determination.

Some investigators have suggested using the data of hospitalized patients to determine reference intervals. Such logic is flawed because hospitalized patients are sick and have many different chemical and hematologic abnormalities [11].

The US government has been conducting large studies of American health status since 1971. These National Health and Nutrition Examination Survey (NHANES) studies are designed to provide nationally representative data on the health and nutritional status of the US population and are an excellent source of laboratory data for randomly selected Americans.

In NHANES I, conducted between 1971 and 1975, 14,407 US civilian, noninstitutionalized persons aged between 25 years and 74 years had standardized medical examinations and questionnaires administered. Although 24 different hematologic, blood chemistry, serologic, and urine laboratory tests were done for many of the subjects, only a few articles have addressed the laboratory findings [12].

In NHANES II, conducted between 1976 and 1980, 20,322 noninstitutionalized persons between 6 months and 74 years of age were examined from 64 sampling areas. Of these subjects, 18,981 had hemoglobin determinations done with the cyanmethemoglobin method, the hematocrit was measured after centrifugation, and the RBC count was measured with Coulter FN analyzers [13]. In addition, the Centers for Disease Control and Prevention measured iron levels, total iron binding capacity, and erythrocyte protoporphyrin levels. Besides providing a monograph summarizing the laboratory results [14], NHANES II also provided prevalences of anemia [15] and iron deficiency [16].

The third study (NHANES III) was conducted between 1988 and 1994. Data were collected on 29,314 civilian, noninstitutionalized individuals aged 2 months and older. There was deliberate oversampling of children 5 years and

younger, older adults (60 years and older), and black and Mexican American persons. Complete blood counts, red cell distribution widths (RDW), platelet counts, and automated differentials for approximately 25,000 individuals were carried out on a Coulter S-Plus Jr instrument (Beckman Coulter, Brea, CA, USA). In addition to the NHANES III laboratory data, the database contains other survey and physical examination data. We analyzed these data and derived health-associated age-, sex-, and race-specific reference interval data for the Coulter S-Plus Jr. Because newer Beckman Coulter models have generally incorporated the measurement principles and standardization techniques of previous instrument models, we believe that these reference interval data can be used as health-associated reference intervals (normal ranges) for today's hematology analyzers.

MATERIALS AND METHODS

NHANES III Data Set

Data from the NHANES III database were extracted from a National Center for Health Statistics CD-ROM (GPO 017-022-01388-6) with Microsoft Access (Redmond, WA, USA) and analyzed with Microsoft Excel.

Study Population

The sample population consisted of civilian, noninstitutionalized Americans aged 2 months and older represented within the NHANES III database. The 3 major racial categories provided were Mexican American, non-Hispanic black, and non-Hispanic white.

Hematologic Measurements

Examinees older than 11 years fasted for 10 to 16 hours before the morning phlebotomy or 6 hours before the afternoon or evening phlebotomy. Lavender-top K₃EDTA anticoagulant vacuum tubes (2- or 3-mL tubes; BD Medical Systems, San Jose, CA, USA) were used to draw 1.0 to 1.5 mL of blood. Before analysis, the blood tubes were placed on a Clay Adams Nutator mixer (BD Medical Systems) and mixed well. Samples were analyzed in duplicate on a Coulter S-Plus Jr within 20 minutes of collection. In addition to answering a detailed health history, subjects underwent an extensive physical examination.

Exclusion Criteria

Health-associated reference intervals were obtained by excluding individuals with any of the following characteristics: (1) Any alcohol consumption, any smoking, breast feeding, or birth-control pill use; (2) recent treatment for anemia, pregnancy, a body mass index exceeding 35 for females and 40 for males, blood pressure exceeding 100 mm Hg, recent hospitalization for heart problems, or chest or abdominal surgery; and (3) positivity for hepatitis B surface antibody or core antibody, positivity for hepatitis C antibody, a serum cre-

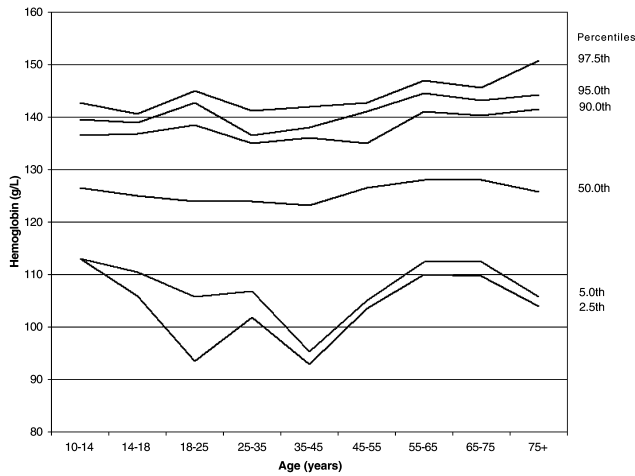


FIGURE 1. Hemoglobin values and percentiles for non-Hispanic black females.

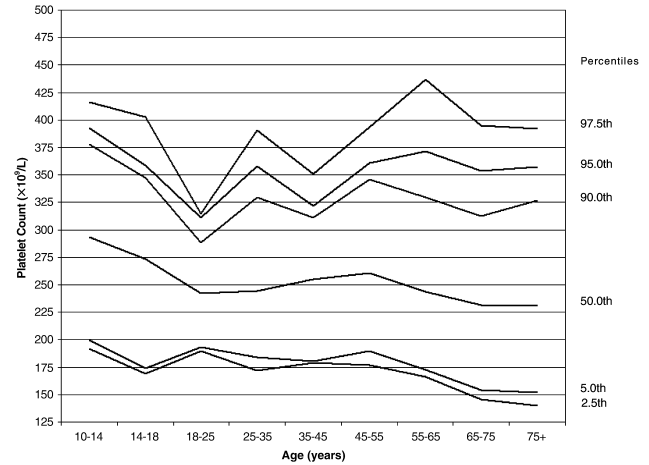


FIGURE 3. Platelet counts and percentiles for non-Hispanic white males.

atinine level exceeding 221 $\mu\text{mol/L}$ (2.5 mg/dL), or a serum glucose level exceeding 6.99 mmol/L (126 mg/dL).

Statistical Methods

For the 3 major racial categories and the 2 sexes, percentile plots were constructed for hemoglobin, hematocrit, RBC count, MCV, MCH, WBC count, platelet count, granulocyte count, monocyte count, lymphocyte count, RDW, platelet distribution width, and mean platelet volume.

RESULTS

Representative plots of hemoglobin measurements for non-Hispanic black females and Mexican American males

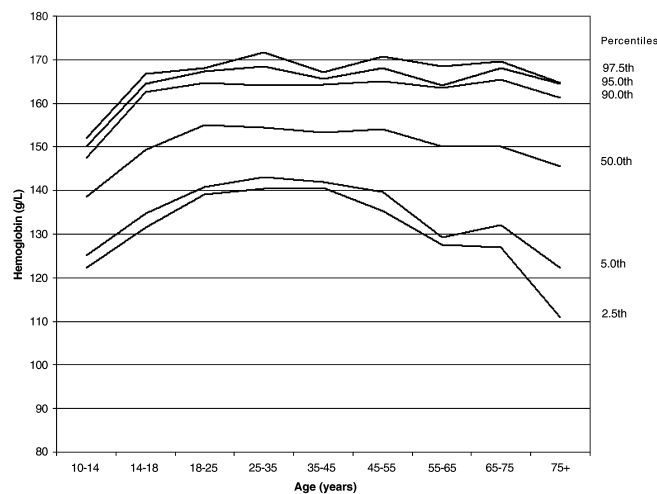


FIGURE 2. Hemoglobin values and percentiles for Mexican American males.

are shown in Figures 1 and 2, respectively, and platelet counts for non-Hispanic white males and non-Hispanic black females are presented in Figures 3 and 4, respectively. The Web site for these data (<http://www.mylaboratoryquality.com>) contains 168 reference interval diagrams (14 tests \times 6 races \times 2 unit of measures [SI and USA units]).

Table 2 shows the number of individuals with Coulter instrument results for each of the sex/race and age groupings as well as the number of individuals remaining in each of the groupings after the exclusion criteria were applied. The Appendix shows composites of reference interval plots for females and males for hemoglobin, MCV, platelet count, WBC count, and granulocyte number. The 2.5th, 50th, and 97.5th percentiles are presented.

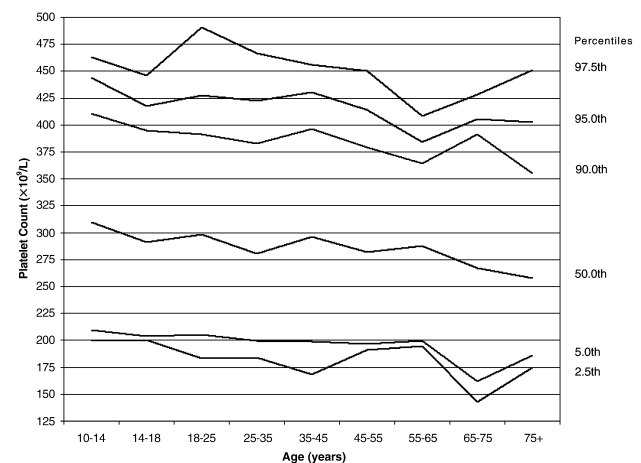


FIGURE 4. Platelet counts and percentiles for non-Hispanic black females.

TABLE 2. Remaining/Total Numbers of Subjects per Sex/Race and Age Interval*

	Age Interval, y									
Sex/race	10-14	14-18	18-25	25-35	35-45	45-55	55-65	65-75	≥75	Total
FMA, n	122/356	177/274	238/480	301/583	237/474	126/456	112/250	119/242	52/104	1484/3219
FNHB, n	129/358	196/294	245/462	236/632	226/606	101/301	86/297	88/247	66/178	1373/3375
FNHW, n	82/247	131/249	106/350	192/571	274/563	235/452	272/503	346/610	512/898	2150/4443
MMA, n	102/351	133/266	128/479	128/585	75/426	52/251	67/280	64/245	36/112	785/2995
MNHB, n	94/338	187/279	144/379	130/498	96/476	45/228	44/269	70/268	35/127	845/2862
MNHW, n	72/240	100/185	47/265	99/445	124/448	136/407	178/455	235/586	369/760	1360/3791
Total, n	601/1890	924/1547	908/2415	1086/3314	1032/2993	695/2095	759/2054	922/2198	1070/2179	7997/20,685

*FMA indicates female Mexican American; FNHB, female non-Hispanic black; FNHW, female non-Hispanic white; MMA, male Mexican American; MNHB, male non-Hispanic black; MNHW, male non-Hispanic white.

DISCUSSION

Large numbers of individuals were excluded to obtain a health-associated sample population, as is shown in Table 2. This large exclusion number may be due to our stringent criteria rather than disease pervasiveness in the population considered.

The Coulter instrument data, including hemoglobin levels, hematocrits, RBC counts, MCV, MCHC, MCH, WBC counts, platelet counts, granulocyte counts, monocyte counts, lymphocyte counts, RDW, platelet distribution widths, and mean platelet volumes, have been tabulated, and several trends are present.

Age-Related Trends

The RDW, a measure of the size heterogeneity of corpuscles, tends to increase with age. Given our criteria for exclusion, we may not be excluding a population of patients who are having chronic gastritis due to nonsteroidal anti-inflammatory drug administration for painful conditions such as arthritis, occult gastrointestinal malignancies, or nutritional deficiencies. Conversely, and more likely, we see an increasing MCV with age. This finding may be due to age-related nutritional deficiency or myelodysplasia. Hemoglobin level appears to increase and peak from approximately the mid 20s to the late 40s in males and then decreases. Again, well-known etiologies of nutritional deficiency, occult malignancy, and anemia of chronic disease may contribute to this trend. This age-related hemoglobin trend does not appear to exist in the female population. Platelet count appears to decrease with increasing age in both males and females, perhaps due to marrow hypoproduction, nutritional deficiencies, or a chronic consumption of platelets due to a chronic inflammatory process. The granulocyte percentage appears to increase with age, and the lymphocyte percentage appears to decrease with age. This trend may be a physiologic phenomenon of aging; however, it may also be due to our exclusion criteria not excluding

patients who are on medication and/or have chronic inflammatory diseases.

Sex-Related Trends

There were few sex-related trends apparent in our data. Hemoglobin values in females appear to exhibit a slight rise over time, whereas males have hemoglobin values that peak during middle age and then decrease. This trend appears almost paradoxical, because one would expect females to have decreased hemoglobin levels during menstruation age and rising hemoglobin levels as menstruation stops. Perhaps dietary supplementation and education have lessened iron deficiency anemia in the pubertal female. Conversely, males appear to have drops in hemoglobin level at the age extremes that reflect dietary supplementation during periods of growth and occult malignancy during the latter part of life. In addition, hemoglobin values are higher for males than for females for most age ranges. Again, this trend is due to chronic menstrual blood loss in females.

Race-Related Trends

There are race-related differences, predominantly in the black population. The WBC count is lower in both sexes, and this trend is a commonly accepted physiologic norm. Mononuclear and lymphocyte percentages in this population are also increased, and there are decreases in granulocyte percentage for all ages and sexes. Finally, hematocrit, MCHC, MCH, and hemoglobin are also decreased across all age and sex categories. These race-related findings are commonly accepted as physiologically normal for the black population and are reflected in our data [17]. There did not appear to be any trend differences between the non-Hispanic white and Mexican American populations in the study.

This study represents an application of data mining that can be used to obtain hematology reference ranges for various sexes, races, and ages. The NHANES III data represent a treasure trove of information that can be used by laboratories to help improve test use. Whenever a laboratory

assesses the validity of its reference intervals (acquisition of new analyzer, a new medical director, a new laboratory information system, complaints of the medical staff, laboratory merger, and so forth), reference interval data such as these NHANES summaries should be consulted and even incorporated into a laboratory's reference intervals.

ACKNOWLEDGMENT

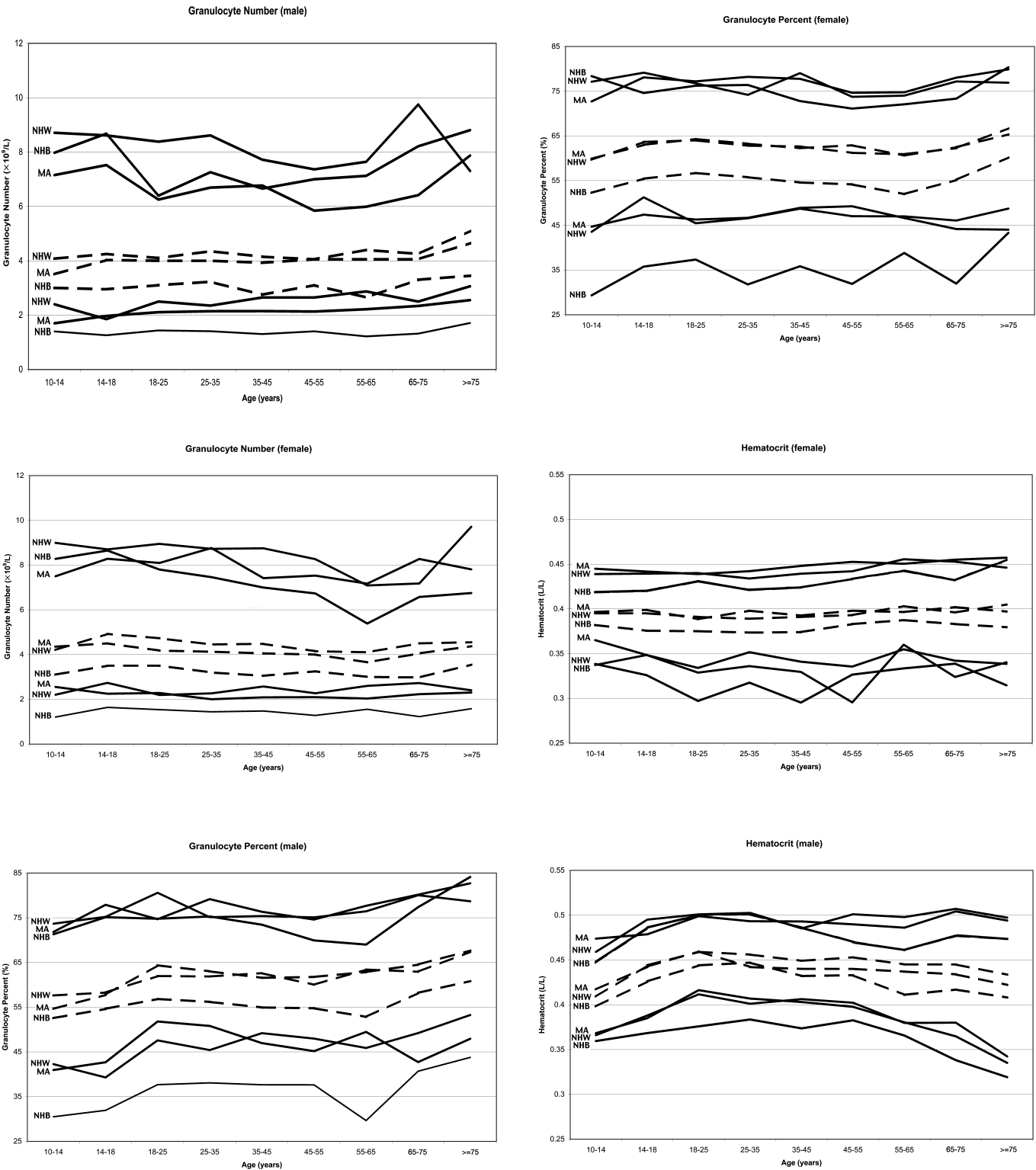
Thank you to Mark Cembrowski for helping with the creation of the figures and the data analysis.

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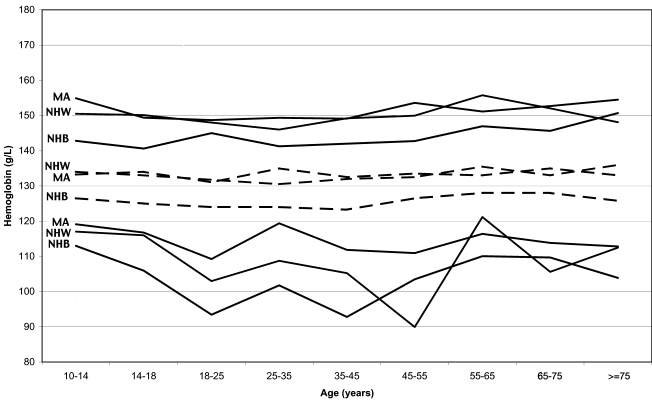
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APPENDIX

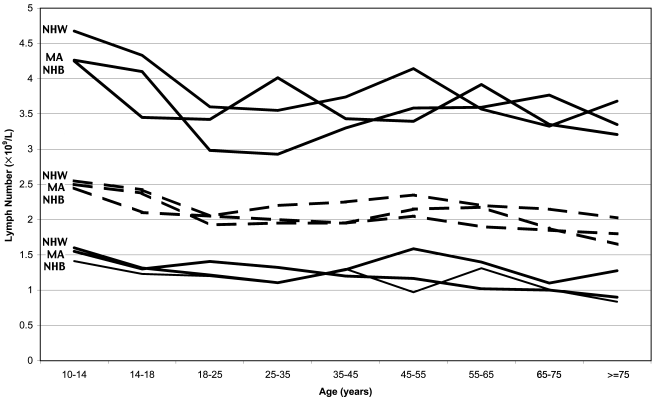
Composite reference interval diagrams according to race for non-Hispanic blacks (NHB), non-Hispanic whites (NHW), and Mexican Americans (MA). Top solid lines represent 97.5th percentile, dotted lines represent median, and lower solid lines represent 2.5th percentile.



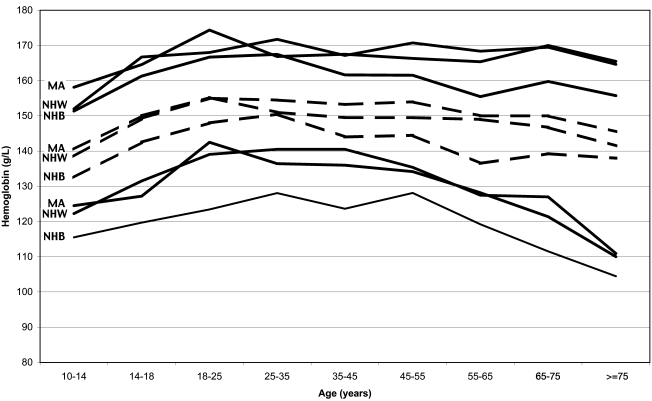
Hemoglobin (female)



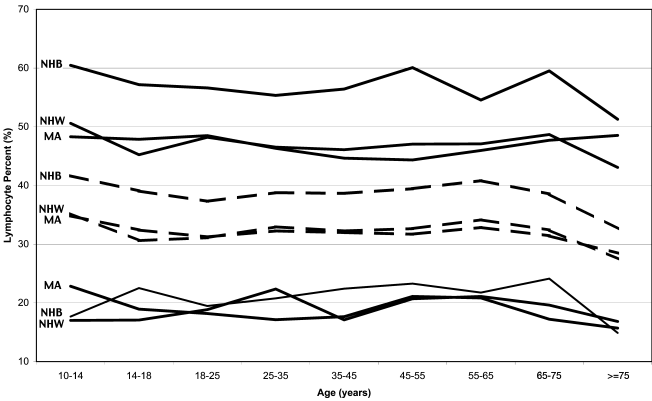
Lymph Number (male)



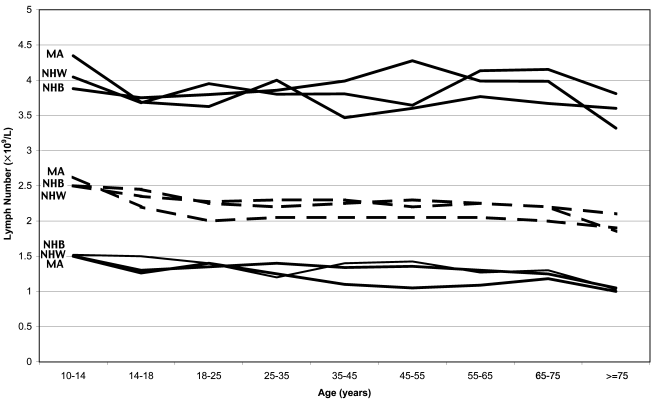
Hemoglobin (male)



Lymphocyte Percent (female)



Lymph Number (female)



Lymphocyte Percent (male)

