Hemoglobin

Blood Spot Measurement of Hemoglobin in Wave I of the National Social Life Health & Aging Project

Authors:

Alina Fomovska, University of Chicago, Department of Ob/Gyn

Thomas McDade, Northwestern University, Departments of Anthropology and Cells to Society (C2S)

Sharon Williams, Purdue University, Department of Anthropology

Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics, Chicago Core on Biomeasures in Population-Based Health and Aging Research at NORC and the University of Chicago Center on Demography and Economics of Aging *

* Corresponding author.
E-mail address: slindau@uchicago.edu (S.T. Lindau).
Fax: +1 773 834 5664

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Rationale

Hemoglobin is the red blood cell protein that carries oxygen in the blood. Anemia is an unhealthy condition indicated by low hemoglobin levels, and can ultimately be the result of iron, folate or B_{12} deficiency, renal insufficiency, chronic inflammation, acute or chronic bleeding, or other causes. About a third of cases of anemia among older individuals is caused by iron, folate, or B_{12} deficiency. Other causes include chronic inflammation (19.7%), chronic renal failure (8.2%) or both (4.3%). Another third of cases are unexplained(Guralnik, Eisenstaedt et al. 2004).

Anemia is associated with an increased risk of cardiovascular disease, cognitive dysfunction, reduced bone density, longer hospitalization for elective procedures(Eisenstaedt, Pennix et al. 2006), as well poor outcomes in many chronic diseases(Goodnough and Nissenson 2004). Among the older population, anemia can be an independent risk factor for death(Lipschitz 2003). Anemia is also associated with fatigue and decline in physical performance, which may lead to a loss of independence and other social and economic effects(Penninx, Guralnik et al. 2003). As such, anemia can have a significant effect on the quality of life of older individuals(Eisenstaedt, Pennix et al. 2006). The prevalence of anemia increases with age(Guralnik, Eisenstaedt et al. 2004). Estimated prevalence rates for older populations in the United States vary widely from 3.9% - 59.9% depending on the population studied and criteria used for defining anemia(Beghe, Wilson et al. 2004). Some studies suggest that even "low normal" hemoglobin levels may be associated with poor performance on mobility tests and physical function(Eisenstaedt, Pennix et al. 2006).

In healthy older individuals between 60 and 98 years of age, hemoglobin levels do not change significantly(Balducci 2003). Normal range of hemoglobin levels is defined by the World Health Organization as between 12 and 16 g/dL. By WHO criteria, anemia is defined as a hemoglobin concentration lower than 13 g/dL in men and lower than 12 g/dL in women. Severe anemia is characterized as Hb<10 g/dL(Charves et al. 2004).

Measurement

In NSHAP, blood was collected by finger-stick using a disposable lancet, and then captured on filter paper for transport and storage. (Details about collection equipment are described in the "Specimen Collection" section, below.)

NSHAP's in-home biological data collection design was based on the principles of minimal invasiveness(Lindau and McDade 2007). For this reason, blood was collected by a finger-stick using a disposable lancet (as opposed to venipuncture), and then captured on filter paper for transport and storage.

Population Norms

Table 1. Prevalence of anemia, 65 and older in the U.S., by gender

Race/ethnic group	Men	Women
Non-Hispanic/white	9.2 %	8.7 %
Non-Hispanic/black	27.5 %	28.0 %
Mexican American	11.5 %	9.3 %
Other	20.4	7.5
Total	11.0	10.2

(Guralnik, Eisenstaedt et al. 2004)

Specimen Collection

The blood spot module was randomized to 5/6 of the total NSHAP sample (N=2494), of these, 84.5% (2,105) participated. There were no significant differences between those who agreed to provide blood spots and those who did not with respect to gender, race, ethnicity, age, education, income level, marital status, self-reported mental or physical health, or the reported number of doctor's visits. Due to collection difficulties, blood spots were not collected from an additional 57 individuals who originally agreed to participate. In total, dried blood spots were collected from 2,048 individuals. From these, 1940 CRP values were obtained.

Blood was obtained from free-flowing capillary blood via a single fingerstick using a retractable-tip, single-use disposable lancet. NSHAP acquired two lancets (model details in Product Availability section below). The Surgilance lancet was used routinely, contained a 2.3 mm blade and was slightly less penetrative. The BD lancet, which contains a slightly larger blade and is commonly used for newborn heel-sticks, was used for cases of thick calluses or where the fingerstick specimen was difficult to obtain with the Surgilance. The blood was allowed to pool on the respondents' finger and four drops of blood were dropped onto filter paper, the first drop being reserved for future genetic analysis. Overall, the procedure required approximately 8 minutes.

Interviewer instructions

- o Angle Respondent's hand below their lap.
- o Warm finger and stimulate circulation by gently kneading and squeezing the appropriate finger.
- o Ask Respondent to gently shake their hand a few times.
- o Wipe the index finger of the right hand with alcohol swab and wait a few seconds for the alcohol to dry (DO NOT blow on finger, wave hand, etc. to speed up drying).

- Squeeze the finger just below the area to be pricked.
- Firmly prick finger in the fleshy part of the pad, just off the center.
- IMMEDIATELY dispose of the lancet into the sharps container.
- Allow blood to well on tip of finger.
- If necessary, apply gentle pressure below the site of the prick
- Place first drop in discard circle of filter paper – marked D
- Place 3 (if possible) additional drops on filter paper.
- If unable to fill 3 spots (+ discard spot): Prick another finger. Place first drop in discard circle of filter paper (marked D) and place additional drop(s) in remaining circles.
- If necessary: Ask Respondent to hold cotton ball on finger and apply pressure until bleeding stops.
- Offer Respondent a bandage.
- Label filter paper with su_id.
- Fill out blood spot collection form.
- Place filter paper in plastic bag with desiccant and seal.
- Store at 4°C until shipment.

The filter paper was allowed to dry for the remainder of the interview before being placed in a plastic bag with desiccant for transportation.

Storage and Shipping

After the interview, the filter paper was placed in a Ziploc bag. Once the field investigator reached home, the filter paper was removed from the Ziploc bag and placed in a clear plastic container with the filter paper cover flipped up. A desiccant pack was placed in the container which was then sealed overnight and left at room temperature to ensure drying of the blood spots. In the morning, the filter paper was stored in a sealed Ziploc bag along with the desiccant pack and placed in a storage container. The storage container was kept in the refrigerator at 4°C until shipping day. Blood spots were shipped at room temperature to the designated location. Upon arrival at the laboratory, the specimens were catalogued, analyzed for quality and quantity, and stored at -25°C until analysis.

Shipping Address	Thomas McDade, PhD Northwestern University Department of Anthropology Laboratory for Human Biology Research 1810 Hinman Ave. Evanston, IL 60208

Assay

The assay is a colorimetric method based on the positive and highly linear relationship between level of hemoglobin in the sample and amount of color development that occurs after reaction with a single reagent, Drabkin's solution. All hemoglobins in the sample (except sulfhemoglobin) are converted to cyanmethemoglobin which can be read photometrically at 540 nM. Potassium cyanide in Drabkin's reagent converts Hb iron from the ferrous to the ferric state to form methemoglobin. Methemoglobin combines with potassium cyanide (also in Drabkin's solution) to produce the stable and easily measurable cyanmethemoglobin. Because the color development is directly proportional to the concentration, unknown samples are easily quantified by comparison to known concentrations in a standard curve. A single disc of blood was punched from each sample using 3.2mm hole punch.

Table 2. NSHAP Summary statistics for hemoglobin levels (g/dL)

Hemoglobin (mg/dL)	Range	Mean (weighted)	Standard Deviation
Men			
Ages 57-65	8.89-18.5	13.8	1.76
Ages 66-75	7.65-18.7	13.2	1.92
Ages 76+	7.56-17.0	12.6	1.72
Women			
Ages 57-65	7.89-18.3	12.6	1.63
Ages 66-75	4.50-18.4	12.3	1.81
Ages 76+	6.58-16.4	12.0	1.66

Table 3 NSHAP Blood Spot Hemoglobin Testing Performed at Northwestern University

Units	Highest Calibrator*	Lowest Calibrator*	Lower limit of sensitivity
g/dL	8	18.9	NA

(Williams and McDade 2007)

Scoring & Usage

- Results are reported in g/dL (grams per deciliter).
- Anemia is defined as <12 g/dL in women and 13 g/dL in men based on WHO(World Health Organization 1968).

Quality Control

Blood spots were obtained on a card containing five pre-printed circles of standard size (1/2 inch/12.7mm diameter). Excellent samples filled the entire circle. Good samples filled a large (6.0mm) hole-punch. Poor samples were ones that did not fill a small (3.2mm) hole punch. Cards were adequate if they allowed for one large and three small punches and excellent if they allowed for more. Throughout the study,

the number, quality and condition of the blood spots was recorded by personnel, and if consistent problems were observed from a single field interviewer, they were contacted to discuss problems and techniques to improve the quality of blood spot collection (Williams and McDade 2007).

Three control samples were used for estimating between-assay variation:

Average	6.764286	13.74446	22.47839
Standard deviation	0.523954	0.655389	1.402853
% coefficient of variation	0.077459	0.047684	0.062409

Product Availability

Product Name	903™ Multiple-part Neonatal Card
Manufacturer	Schleicher & Schuell BioScience
Location of Manufacturer	Keene, NH 03431 Contact: Judy Peter 800- 437-7003
Catalogue No.	10 537 279
Product Name	SUD-CHEMIE Performance Packaging ; SORB-IT Harmless Absorbant
Manufacturer	Süd-Chemie Performance Packaging
Location of Manufacturer	926 S. 8th St. PO Box 610 Colton, CA 92324 Tel: +1 909 825 1793 +1 800 966 1793 Fax: +1 909 825 6271
Catalogue No.	n/a
Product Name	SurgiLance Safety Lancet, 2.3 mm (blue)
Manufacturer	SurgiLance, Inc.
Location of Manufacturer	Norcross, GA Tel: +1 (770) 448 9493 (US) Fax: + 1 (877) 804 5240 (Toll Free) (US)
Catalogue No.	SLB250
Product Name	BD Quikheel™ Preemie Lancet (pink)
Manufacturer	Becton, Dickinson and Company
Location of Manufacturer	Franklin Lakes, NJ
Catalogue No.	368100

Sources

Procedure developed December 2004 from recommendations by Stacy T. Lindau, University of Chicago, Thomas McDade, Northwestern University and Sharon Williams, Northwestern University. Assay developed by Thomas McDade at Northwestern University Laboratory for Human Biology Research (currently unpublished).

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