NEUROPATHOLOGICAL AND SUPPLEMENTAL BIOMARKERS FROM THE 2016 HEALTH AND RETIREMENT STUDY VENOUS BLOOD STUDY (VBS)

HRS Documentation Report

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Overview

Rationale

In 2020, the HRS team was awarded an administrative supplement to the HCAP project to perform assays of neurodegeneration/ATN biomarkers on the already-collected blood samples of approximately 4,500 HRS participants from 2016 Venous Blood Study (VBS), including all Harmonized Cognitive Assessment Protocol (HCAP) participants with venous blood in repository.

Adding blood-based biomarkers of neurodegeneration and AD/ADRD in already-collected blood samples from HCAP respondents significantly enhances the available resources and opportunities for researchers interested in AD/ADRD risk and related outcomes.

This data release also includes two aging-related biomarkers, human growth differentiation factor 15 (GDF-15) and soluble urokinase plasminogen activator receptor (S)uPAR. Measures of thyroid function were measured on a smaller subsample (n=1022). These data complement the comprehensive set of cardiometabolic and immune system function measures already released on the VBS 2016 sample.

Sample

The HCAP pilot sample was selected from 2016 HRS respondents born 1959 and earlier who also participated in the 2016 VBS (VBS16, n=9,934 valid cases). All panel respondents who completed an HRS interview during the 2016 wave were asked to consent to a venous blood draw with the exception of proxy respondents and nursing home residents. The request was made by their HRS interviewer at the end of the interview (telephone and in person modes), with the offer of a fifty-dollar incentive payment to be sent by check. That incentive amount and procedure is consistent with other voluntary ancillary activities in HRS where we routinely pay in advance of completion. No higher amounts were offered.

From this group, a probability sample was selected from among respondents who were eligible for the 2016 HCAP or who are eligible for a future HCAP studies (a random half of HRS sample members assigned by household). This is the 2016 VBS Innovation (VBSI) subsample (n=4,611). The VBSI subsample includes all 2016 HCAP respondents who provided valid blood samples.

From the 2016 HCAP respondents who provided valid blood samples, a further subsample was selected for the TSH assays. The TSH subsample (n=1,058) was selected on the basis of race/ethnicity and the preliminary HCAP cognitive status indicator (diagnosis) as of April 2022. For non-Hispanic whites, 25% of respondents with normal cognition, 50% of those with mild cognitive impairment (MCI), and 100% of dementia cases were selected. For non-Hispanic blacks and Hispanics of all races, 50% with normal cognition, 100% with MCI, and 100% of dementia cases were selected.

Collection

The blood collection was managed by Hooper Holmes Health & Wellness. Hooper Holmes, now ExamOne, was provided with the names, addresses, and phone numbers of consenting respondents and contacted respondents to set appointments. Collection materials were mailed to the phlebotomists' homes in advance of the scheduled visit. The phlebotomy service reported to HRS on respondents who declined to schedule appointments or missed scheduled appointments, and HRS staff followed-up up to detect any problems and to attempt to reschedule appointments. Every attempt was made to schedule the blood draw within 4 weeks of the HRS core interview. Fasting was recommended and preferred but

not required. Phlebotomists noted the fasting status of the samples. We collected 50.5 mL of blood in 6 tubes – (1) 8 mL CPT tube, (3) 10 mL double gel serum separator tubes (SST), (1) 10 mL EDTA whole blood tube, and a 2.5 mL PAXgene RNA tube. The SST tubes are centrifuged in the field before being shipped overnight to the CLIA-certified Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing is done within 24 hours of arrival at the lab (within 48 hours of collection). More information on the blood collection procedures can be found here: https://hrsdata.isr.umich.edu/data-products/2016-venous-blood-study-vbs

Assays

HRS assayed the following neuropathological biomarkers: Aβ40, Aβ42, Phosphorylated Tau Protein 181 (pTau-181), Neurofilament Light Chain (NfL), and Glial Fibrillary Acidic Protein (GFAP). The strategy for selecting these assays of interest prioritized biomarkers that are: (1) highly reliable and replicable in blood (plasma/serum); (2) have validated correlations with AD/ADRD neuropathology from cerebrospinal fluid (CSF) or autopsy measures; (3) are found in higher concentrations in people with cognitive impairment and AD/ADRD; and (4) based on consultations with dementia experts at the NIA Intramural Research Program. What was missing from the existing cognitive biomarker literature was replication in representative population-based samples of older adults, including individuals from racial/ethnic minorities. Our project sought to address this important gap in the literature.

In addition, the soluble urokinase plasminogen activator receptor (suPAR or uPAR) and Human Growth Differentiation Factor 15 (GDF-15) were also measured from serum on this sample. Thyroid function markers, including Thyroid Stimulating Hormone (TSH), Total T3, and Thryoxine (Free T4) were measured on a smaller subsample (n=1022).

Laboratory

All assays were performed at the University of Minnesota Advanced Research and Diagnostic Laboratory (ARDL). The ADRL is CLIA certified laboratory established for improved coordination and centralization of laboratory activities of multi-center research studies contracted by the Department of Laboratory Medicine and Pathology at the University of Minnesota. ARDL served as the analytic laboratory for all of the HRS 2016 VBS.

Laboratory Methods

Amyloid beta 40 (Ab40)

Amyloid beta 40 (Ab40) was measured in EDTA plasma by an HD-X analyzer using a Multiplex Simoa Assay, Neurology 4 plex assay E (N4PE) (Quanterix, MA). The interassay laboratory CVs for this method were 7.28%, 3.97% and 8.55% at mean concentrations of 17.60, 136.72 and 42.32 pg/mL respectively for the two kit controls and the ARDL pooled serum control that were run on every plate alongside the samples.

Amyloid beta 42 (Ab42)

Amyloid beta 42 (Ab42) was measured in EDTA plasma by an HD-X analyzer using a Multiplex Simoa Assay, Neurology 4 plex assay E (N4PE) (Quanterix, MA). The interassay laboratory CVs for this method were 5.95%, 4.14% and 13.04% at mean concentrations of 5.84, 31.35 and 2.77 pg/mL respectively for the two kit controls and the ARDL pooled serum control that were run on every plate alongside the samples.

Glial Fibrillary Acidic Protein (GFAP)

Glial Fibrillary Acidic Protein (GFAP) was measured in EDTA plasma by an HD-X analyzer using a Multiplex Simoa Assay, Neurology 4 plex assay E (N4PE) (Quanterix, MA). The interassay laboratory CVs for this method were 8.64%, 7.31%, 10.03% and 11,92% at mean concentrations of 227.4, 4281.8, 40.5 and 62.7 pg/mL respectively for the two kit controls, ARDL pooled EDTA plasma control and ARDL pooled serum control that were run on every plate alongside the samples.

Neurofilament light (NfL)

Neurofilament light (NfL) was measured in EDTA plasma by an HD-X analyzer using a Multiplex Simoa Assay, Neurology 4 plex assay E (N4PE) (Quanterix, MA). The interassay laboratory CVs for this method were 9.07%, 5.67%, 12.2% and 13.24% at mean concentrations of 22.33, 410.02, 5.0 and 9.79 pg/mL respectively for the two kit controls, ARDL pooled EDTA plasma control and ARDL pooled serum control that were run on every plate alongside the samples.

PTau-181

pTau-181 was measured in serum by an HD-X analyzer using a Simoa Assay, pTau-181 V2 (Quanterix, MA). The interassay laboratory CVs for this method were 7.6%, 8.2% and 13.2% at mean concentrations of 3.0, 94.0 and 1.2 pg/mL respectively for the two kit controls and the ARDL pooled serum control that were run on every plate alongside the samples.

Urokinase-type Plasminogen Activator Receptor (uPAR)

Urokinase-type Plasminogen Activator Receptor (uPAR) was measured in serum. A Beckman Coulter Biomek NXp (Beckman Coulter, Fullerton, CA) was used for sample processing. uPAR was measured in serum by a Protein Simple ELLA (Protein Simple, San Jose, CA) using a Simple Plex microfluidic immunoassay, Human Urokinase type Plasminogen Activator Receptor (uPAR) (Protein Simple, San Jose, CA). The interassay laboratory CVs for this method were 5.4%, 8.9% and 6.4% at mean concentrations of 121.9, 6365.1 and 1778.2 pg/mL respectively for the two kit controls and the ARDL pooled serum control that were run on every plate alongside the samples. The lower limit of detection was 0.2 pg/mL per the kit insert, 1.20 pg/mL ARDL determined.

Growth Differentiation Factor 15 (GDF-15)

Growth Differentiation Factor 15 (GDF-15) was measured in serum. A Beckman Coulter Biomek NXp (Beckman Coulter, Fullerton, CA) was used for sample processing. GDF-15 was measured in serum by a Protein Simple ELLA (Protein Simple, San Jose, CA) using a Simple Plex microfluidic immunoassay, Human Growth Differentiation Factor 15 (GDF-15) (Protein Simple, San Jose, CA). The interassay laboratory CVs for this method were 4.4%, 7.6% and 7.1% at mean concentrations of 14.1, 670.3 and 771.5 pg/mL respectively for the two kit controls and the ARDL pooled serum control that were run on every plate alongside the samples. The lower limit of detection was 0.21 pg/mL per the kit insert, 1.86 pg/mL ARDL determined.

Thyroid Stimulating Hormone (TSH)

Thyroid Stimulating Hormone (TSH) was measured in serum by a Roche COBAS 8000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) using an electrochemiluminescence immunoassay / sandwich principle, Elecsys TSH (Roche Diagnostics, Indianapolis, IN). The interassay laboratory CVs for this method were 2.8% and 4.9% at mean concentrations of 2.1 and 8.3 uIU/mL respectively for the two kit controls that were run daily. The lower limit of detection was 0.005 uIU/mL.

Total T3

Total T3 was measured in serum by a Roche COBAS 8000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) using a competitive principle/electrochemiluminescence assay, Elecsys T3 (Roche Diagnostics, Indianapolis, IN). The interassay laboratory CVs for this method were 5.0% and 3.4% at mean concentrations of 101.8 and 323.9 ng/dL respectively for the two kit controls that were run daily. The lower limit of detection was 19.5 ng/dL.

Thryoxine, Free T4

Free T4 was measured in serum by a Roche COBAS 8000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) using a competitive principle/electrochemiluminescence assay, Elecsys T4 (Roche Diagnostics, Indianapolis, IN). The interassay laboratory CVs for this method were 2.1% and 3.6% at mean concentrations of 1.1 and 2.8 ng/dL respectively for the two kit controls that were run daily. The lower limit of detection was 0.04 ng/dL.

Quality Control

General Laboratory Quality Control

To ensure accurate and precise laboratory results, new testing or instruments are verified prior to implementation and modifications of existing methods and laboratory-developed methods are validated prior to implementation. Analytical verification is defined as the process by which the laboratory determines that an unmodified FDA-cleared/approved test performs according to the specifications set forth by the manufacturer when used as directed. In addition, for all FDA approved tests, the laboratory participates in CAP proficiency testing. Analytical validation is defined as the process used to confirm with objective evidence that a laboratory developed or modified FDA-cleared/approved test method delivers reliable results for the intended application.

The laboratory follows the manufacturer's instructions for the instrument and test system operation. Instructions for calibration and control frequency, maintenance, acceptable specimen type, reagent storage and expiration dates, and test procedures are followed as specified in the package insert or operator's manual. If there's a difference between the manufacturer's requirements and a regulatory agency such as CAP or New York State requirements, the laboratory follows the more stringent requirement.

Quality control data is measured for each assay and records are maintained. Each assay includes at least two levels of control with each run. Calibration occurs at least every 6 months. Quality control data are reviewed at least monthly by the laboratory manager or the technical supervisor. Written procedures include corrective action to take when calibration or controls fail to meet acceptability criteria. The analyst's competency must have been assessed within the past 12 months. When a procedure is removed from production and then returned to production, verification must occur within 30 days prior to restarting.

Duplicate Quality Control Samples

For the Neurology 4 plex assay E (N4PE) assay, 24 samples were run in duplicate. CVs for all assays fell within the acceptable range. For the pTau-181 V2 assay, sample sets of 13-25 samples were run in duplicate with CVs ranging from 4.5% to 10.8%.

Pilot Studies Assessing Validity and Reliability of Methods

To ensure the feasibility of our approach, we conducted a pilot study (n=41) demonstrating that our collection protocol, which involves a 24-48 hour delay in processing, was effective for testing relevant biomarkers by the Quanterix method. Plasma samples were used to assess the Aβ42/40 ratio, neurofilament light (NfL), and glial fibrillary acidic protein (GFAP). Serum samples were used for phosphorylated tau-181 (pTau181) due to stability concerns. This work builds on findings related to pre-analytical variables impacting the stability of blood-based biomarkers of neuropathology, as outlined by Panikkar et al. (2023).

If You Need to Know More

This document is intended to serve as a brief overview to the 2016 Neuropathological and Supplemental Biomarker data product. If you have questions or concerns that are not adequately covered here or on our Web site, or if you have any comments, please contact us. We will do our best to provide answers.

HRS Internet Site

Health and Retirement Study public release data and additional information about the study are available on the Internet. To access public data or to find out more about restricted data products and procedures, visit the HRS Web site.

Contact Information

If you need to contact us, you may do so by one of the methods listed below.

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Citing this Document

Please include the following citation in any research reports, papers, or publications based on these data along with the citation for the reference epigenetic clock:

In text: "The HRS (Health and Retirement Study) is sponsored by the National Institute on Aging (NIA U01AG009740) and is conducted by the University of Michigan. This study was also supported by The Alzheimer's Association (HRS-18-586069C)."

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References

Crimmins, E., Faul, J., Thyagarajan, B., & Weir, D. (2017). Venous blood collection and assay protocol in the 2016 Health and Retirement Study 2016 Venous Blood Study (VBS). In (pp. 1-73). Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, Michigan.

Panikkar, D., Vivek, S., Crimmins, E., Faul, J., Langa, K. M., & Thyagarajan, B. (2023). Pre-Analytical Variables Influencing Stability of Blood-Based Biomarkers of Neuropathology. J Alzheimers Dis, 95(2), 735-748. https://doi.org/10.3233/JAD-230384