Blood biomarkers

BRHS 40 year follow-up (Q40)

2018 - 2019

Fasting blood samples

At the 40-year follow-up physical examination in 2018-19(Q40), the BRHS study participants were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water and to attend for measurement at a prespecified time between 0800 and 1800 h.

All men were asked to provide a blood sample, collected using the BD Vacutainer system. The filled tubes were gently agitated and placed in a rack. Serum gel tubes stood for a minimum of 30 minutes before being centrifuged on site to preserve the sample during transit. The blood collection tubes were delivered overnight from the examination centres to the laboratories in London or Glasgow. The Clinical Biochemistry Laboratory (run by Health Services Laboratories) at the Royal Free Hospital in London carried out full Blood Count, HbA1c, basic Biochemical profile and Glucose. The BHF Glasgow Cardiovascular Research centre, University of Glasgow, received plasma and serum gel samples. Plasma samples were centrifuged and both plasma and serum sample were aliquoted and frozen for longer term storage at −70oC.

The blood sampling procedure is described in section 7.3.3 and the blood separation plan is included in appendix 3 of the Physical examination protocol (**BRHS 2018-19 (Q40) 40yr follow-up Physical examination protocol.pdf**)

Blood marker	units	Methods section	BRHS Variable name	Mean	SD	Min	Max	N	N Miss	Data access
Study ID			serial			2872	3408758	661	0	
Blood batch number			q40Blood_batch	36.84	24.30	1	105	661	0	
Albumin	g/L	B.1	q40Alb	45.94	2.73	36	54	659	2	yes
Alanine Transaminase (ALT)	U/L	B.2	q40ALT	22.71	9.61	8	136	659	2	yes
Alkaline Phosphatase (ALP)	U/L	B.3	q40APhos	82.12	29.96	31	462	659	2	yes
Aspartame Transaminase (AST)	U/L	B.4	q40AST	22.07	7.04	10	93	647	14	yes
Calcium	mmol/L	B.5	q40Calc	2.38	0.09	2.07	2.96	659	2	yes
Corrected Calcium	mmol/L		q40CCalc	2.39	0.09	2.15	2.97	659	2	yes
CHDR (Cholesterol/HDL ratio)			q40CHDR	3.10	1.03	1.5	8.3	659	2	yes
Cholesterol	mmol/L	B.6	q40Chol	4.37	1.05	1.9	8.6	659	2	yes
Creatinine	umol/L	B.7/8	q40Creat	102.44	30.20	56	323	659	2	yes
C-Reactive protein (CRP)	mg/L	B.24	q40CRP	2.62	8.05	0.08	124.6	657	4	yes
D-dimer	ng/ml	B.25	q40DDIMER	3284.67	14563.25	40	270970	653	8	yes
EGFR (mL/min) from LAB – method not available	mL/min	n/a	q40EGFR	67.12	16.82	16	91	656	5	yes
Eosinophils Abs	10^9/L	B.23	q40Eosin_ABS	0.19	0.14	0	1.5	660	1	yes
Eosinophils %	%	B.23	q40Eosin_PC	0.03	0.02	0	0.148	660	1	yes
Growth Differentiation Factor (GDF-15)	pg/ml	B.28	q40GDF15	2134.90	1550.85	675	27594	658	3	yes
Gamma-Glutamyl transferase (gamma-GT, GGT)	U/L	B.9	q40GGT	35.03	43.29	7	585	658	3	yes
Glucose	mmol/L	B.10	q40GLUC	5.73	1.77	2.9	18.9	618	43	yes
Haemoglobin (Hb)	g/l	B.23	q40Hb	139.66	14.50	93	184	660	1	yes
Glycated Haemoglobin (HbA1c)	mmol/mol	B.11	q40HBA1C	42.34	8.39	12.6	91.3	657	4	yes
Haematocrit - HCT	L/L	B.23	q40HCT	0.44	0.05	0.292	0.591	660	1	yes
HDLC	mmol/L	B.12	q40HDLC	1.50	0.44	0.6	3.6	659	2	yes
Insulin-like growth factor 1 (IGF-1)	ng/ml	B.27	q40IGF1	72.22	31.45	12.3	297	658	3	yes
Interleukin-6 (IL-6)	pg/ml	B.26	q40IL6	4.70	11.94	0.05	175.85	643	18	yes
Insulin	uU/ml	B.30	q40insulin	11.97	14.21	0.1	226.4	658	3	yes
LDLC	mmol/L	B.13	q40LDLC	2.29	0.93	0.4	6.8	655	6	yes
Magnesium	mmol/L	B.14	q40Magn	0.85	0.08	0.5	1.07	651	10	yes

Blood marker/cont.	units	Methods section	BRHS Variable name	Mean	SD	Min	Max	N	N Miss	Data access
Mean Cell Haemoglobin (MCH)	pg	B.23	q40MCH	30.80	1.86	22.2	41.3	660	1	yes
Mean Cell Haemoglobin Concentration (MCHC)	g/l	B.23	q40MCHU	318.71	14.90	266	375	660	1	yes
Mean Cell volume MCV	fL	B.23	q40MCV	96.76	6.15	81.1	127	660	1	yes
Monocytes Abs	10^9/L	B.23	q40Monocy_ABS	0.63	0.21	0.11	1.84	660	1	yes
Monocytes %	%	B.23	q40Monocy_PC	0.09	0.02	0.022	0.213	660	1	yes
Mean Platelet volume (MPV)	fL	B.23	q40MPV	11.18	0.99	8.8	15.1	650	11	yes
Neutrophils %	%	B.23	q40Neutro_Abs	4.61	1.50	0.84	13.49	660	1	yes
Neutrophils Abs	10^9/L	B.23	q40Neutro_pc	0.64	0.09	0.059	0.902	660	1	yes
NT-proBNP	pg/ml	B.32	q40NTproBNP	645.82	1035.56	13.6	9451	658	3	yes
Phosphate	mmol/L	B.15	q40Phosp	1.15	0.19	0.73	2.18	653	8	yes
Platelets	10^9/L	B.23	q40Plat	228.73	62.84	86	691	659	2	yes
Potassium	mmol/L	B.16	q40Potass	4.81	0.58	3.1	8.1	637	24	yes
Red blood cell count (RBC)	10^12/L	B.23	q40RBC	4.55	0.50	2.3	6.19	660	1	yes
Red Blood Cell Distribution Width (RDW)	%	B.23	q40RDW	13.91	1.32	11.8	23.6	660	1	yes
Sodium	mmol/L	B.17	q40Sodium	140.20	3.06	128	148	659	2	yes
Total Bilirubin	umol/L	B.18	q40TBili	10.83	5.59	2	48	659	2	yes
Total Protein	g/L	B.19	q40TProt	71.84	4.64	53	90	659	2	yes
Triglycerides	mmol/L	B.20	q40Trig	1.30	0.68	0.4	6.1	659	2	yes
High-Sensitivity Troponin T (hsTnT)	pg/ml	B.33	q40TroponinT	21.88	20.95	4.79	452.8	658	3	yes
Urate	umol/L	B.21	q40Urat	344.20	84.43	104	783	657	4	yes
Urea	mmol/L	B.22	q40Urea	7.23	2.55	2.7	20.7	659	2	yes
Vitamin D	ng/ml	B.31	q40VitaminD	28.28	12.30	6.3	82.1	658	3	yes
Von Willebrand factor (VWF)	IU/dl	B.29	q40VWF	175.71	64.55	29	461	658	3	yes
White blood cell count (WBC)	10^9/L	B.23	q40WBC	7.29	3.33	2.61	77.2	660	1	yes

Methodological Summaries

Routine Biochemistry and Haematology analyses were conducted by the Royal Free Hospital Clinical Biochemistry Laboratory, HEALTH SERVICES LABORATORIES (A Sonic Healthcare UK laboratory), Royal Free Hospital, Pond Street, London, NW3 2QG | UNITED KINGDOM

Routine Biochemistry

Laboratory: Royal Free Hospital Clinical Biochemistry Laboratory
HEALTH SERVICES LABORATORIES (A Sonic Healthcare UK laboratory)
Royal Free Hospital, Pond Street, London, NW3 2QG | UNITED KINGDOM

B1. Albumin g/l

Principal: Endpoint colorimetric assay. In acidic conditions, albumin can bind bromocrescol green to produce a coloured complex, the intensity of which is directly proportionate to the concentration of albumin.

Between batch Imprecision: <2%

Reference: Hill PG, 1985. "The measurement of albumin in serum and plasma." Ann Clin Biochem 22:565–78

B2. Alanine Aminotransferase (ALT) u/l

Principal: Kinetic enzymatic assay. ALT is able to form pyruvate and L-Glutamate from alpha-ketoglutarate and L-aspartate. Pyruvate can then be utilised with NADH and H+ by lactate dehydrogenase to produce L-Malate and NAD+. The rate of consumption of NADH can be measured spectrophotometrically and is proportionate to the concentration of ALT.

Between batch Imprecision: 3.2%

Reference: Bergmeyer HU, Horder M, Rej R. 1986 "Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes" J. Clin. Chem. Clin. Biochem. 24: 481-495.

B3. Alkaline Phosphatase u/l

Principal: Enzymatic colorimetric assay. In the presence of zinc and magnesium, ALP is able to convert p-nitrophenyl phosphate into p-nitrolphenol, the concentration of which is measured spectrophotometrically and is proportionate to the concentration of ALP.

Between batch Imprecision: <2%

Reference: Bowers GN Jr, McComb RB. 1975 "Measurement of total alkaline phosphatase activity in human serum." Clin Chem 21: 1988-1995.

B4. Aspartate Transaminase (AST) u/l

Principal: Kinetic enzymatic assay. AST is able to produce oxaloacetate when L-aspartate and alpha-ketoglutarate are present. Oxaloacetate can then be utilised by malate dehydrogenase along with NADH and H+ to produce L-Malate and NAD+. The rate of consumption of NADH is measured spectrophotometrically and the decrease in absorbance is directly proportionate to the rate of production of oxaloacetate and therefore the AST concentration.

Between batch Imprecision: 3.2%

Reference: Bergmeyer HU, Horder M, Rej R. 1986 "Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes" J. Clin. Chem. Clin. Biochem. 24: 497-510.

B5. Calcium mmol/L

Principal: The method is based on a blanked colorimetric endpoint assay using o-cresolphthalein complexone which produces a purple coloured complex whose intensity is directly proportional to the calcium concentration.

Between batch Imprecision: <2%

Reference: Gindler EM & King JD. 1972 "Rapid colorimetric determination of calcium in biologic fluids with methythymol blue." Am JClin Pathol 58: 376 – 382

B6. Cholesterol mmol/l

Principal: Enzymatic colorimetric assay. Cholesterol and cholesterol esters are converted via a series of enzymatic reactions to hydrogen peroxide which is then utilised to produce a red dye via a peroxidase enzyme. The colour intensity of the dye is directly proportionate to the concentration of cholesterol.

Between batch Imprecision: <2%

Reference: Deeg R, Ziegenhorn J. 1983 "Kinetic enzymic method for automated determination of total cholesterol in serum." Clin Chem 29: 1798-1802.

B7. Creatinine - Jaffe µmol/l

Principal: Rate blanked kinetic assay. Creatinine reacts with picric acid in alkaline conditions to produce an orange-red product which can be measured photometrically. The rate blank step is introduced to minimise interferences from bilirubin. A negative factor of -26 µmol/L is applied to reduce non specific interferences.

Between batch Imprecision: 2.3%

Reference: Peake M, Whiting M. 2006. "Measurement of Serum Creatinine – Current Status and Future Goals" Clin Biochem Rev. 27: 173-184.

B8. Creatinine - Enymatic µmol/l

Principal: Enzymatic colorimetric assay. Creatinine is converted via a series of enzymes to hydrogen peroxide, which can be measured spectrophotometrically at 546nm following a modified Trinder reaction with absorbance blanking at 700nm.

Between batch Imprecision: 2.1%

Reference: Peake M, Whiting M. 2006. "Measurement of Serum Creatinine – Current Status and Future Goals" Clin Biochem Rev. 27: 173-184.

B9. Gamma-Glutamyltransferase (GGT) u/l

Principal: Enzymatic colorimetric assay. Following addition of substrate, GGT catalyses the conversion of reagent into 5-amino-2-nitrobenzoate which can be measured via spectrophotometry at 410nm.

Between batch Imprecision: <2%

Reference: Tietz, N.W., 1986. "Fundamentals of Clinical Chemistry", 3rd Edition, W.B. Saunders,

---

B10. Glucose mmol/l

Principal: Enzymatic colorimetric method. Glucose is enzymatically converted into hydrogen peroxidase which can be utilised by a peroxidase enzyme to produce a red dye which is read via spectrophotometry at 505nm. The concentration of the dye is directly proportionate to the concentration of glucose in the sample.

Between batch Imprecision: 3.8%

Reference: Trinder P. 1969 “Determination of blood glucose using 4-amino phenazone as oxygen acceptor.”

B11. Glycated Haemoglobin (HbA1c) %

HbA1c is analysed using the TOSOH G11 analyser, method principle HPLC

B12. High Density Lipoprotein Cholesterol (HDL) mmol/l

Principal: Homogenous enzymatic colorimetric assay. A detergent containing a sugar compound in conjunction with magnesium is used to favour the conversion of HDL cholesterol esters to cholesterol over other sources of cholesterol such as LDL-cholesterol. This liberated HDL-cholesterol is further converted to hydrogen peroxide which can react with a dye via peroxidase to form a purple-blue pigment which can be detected spectrophotometrically. The concentration of the purple-blue pigment is directly proportionate to the concentration of HDL-Cholesterol.

Between batch Imprecision: <2%

Reference: Nauck M, März W, Jarausch J, Cobbaert C, Sägers A, Bernard D, Delanghe J, Honauer G, Lehmann P, Oestrich E, von Eckardstein A, Walch S, Wieland H, Assmann G. 1997 “Multicenter evaluation of a homogeneous assay for HDL-cholesterol without sample pretreatment.” Clin Chem. 43: 1622-1629.

B13. Low Density Lipoprotein Cholesterol (direct) (LDL) mmol/l

Principal: Homogenous enzymatic colorimetric assay. A detergent containing a sugar compound in conjunction with magnesium is used to favour the conversion of LDL cholesterol esters to cholesterol over other sources of cholesterol such as HDL-cholesterol. This liberated LDL-cholesterol is further converted to hydrogen peroxide which can react with a dye via peroxidase to form a purple-blue pigment which can be detected spectrophotometrically. The concentration of the purple-blue pigment is directly proportionate to the concentration of LDL-Cholesterol.

Between batch Imprecision: <2%

Reference: Esteban-Salán M, Guimón-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Río E. 2000 “Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients.” Clin Chem 46:1121¬1131.

B14. Magnesium mmol/L

Principal: Colorimetric endpoint assay. The sample is incubated with EGTA to minimise calcium interference and an alkaline buffer, after which xylidyl blue is added which will react with magnesium to produce a purple product. The decrease in absorbance at 600nm by xylidyl blue is measured and used to determine the magnesium concentration.

Between batch Imprecision: <2%

Reference: D. Stankov, T. Jovanović, M. Jelikić-Stankov. 1997 “Spectrophotometric Determination of Magnesium in Serum Using Xylidyl Blue Reagent in Micellar Medium” In: “Magnesium: Current Status and New Developments” p51-52. Kluwer Academic Publishers.

B15. Phosphate mmol/l

Principal: A colorimetric assay with endpoint determination and sample blanking. Inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate in the presence of sulphuric acid. The complex is determined photometrically in the ultraviolet region (340 nm).

Between batch Imprecision: <2%

Reference: Weissman N, Pileggi VJ. 1974 "Inorganic anions." In: Henry RJ, Cannon DC, Winkelman JW, eds. "Clinical chemistry: principles and techniques" 2nd ed. New York: Harper and Row:723 – 727

B16. Potassium mmol/l

Principal: Potassium is measured by an ion selective electrode. A selective membrane composed of valinomycin creates an electrical potential as only potassium ions traverse the membrane. The electrical potential can be compared to a reference electrode to determine the potassium ion concentration.

Between batch Imprecision: <2%

Reference: Fiedler U, Růžička J. 1973. "Selectrode—the universal ion-selective electrode: Part VII. A valinomycin-based potassium electrode with nonporous polymer membrane and solid-state inner reference system". Analytica Chimica Acta. 67: 179-193.

B17. Sodium mmol/l

Principal: Sodium is measured by an ion selective electrode. A membrane composed of crown ether with a neutral PVC carrier forms a selective membrane for sodium ions, creating an electrical potential as sodium ions traverse the membrane. The electrical potential can be compared to a reference electrode to determine the sodium ion concentration.

Between batch Imprecision: <2%

Reference: GB Levy,. 1981. "Determination of Sodium with Ion-Selective Electrodes." Clin Chem. 27: 1435-1438.

B18. Total Bilirubin µmol/l

Principal: Colorimetric assay. Indirect/Unconjugated bilirubin is liberated following interaction with detergents. After this step, the bilirubin reacts with diazonium ions under strongly acidic conditions to produce azobilirubin, which can be measured spectrophotometrically.

Between batch Imprecision: <2%

Reference: Watson D, Rogers JA. 1961 "A study of six representative methods of plasma bilirubin analysis" J Clin Pathol. 14: 271-278.

B19. Total Protein g/l

Principal: Colorimetric assay. In alkaline conditions protein can complex with copper ions to produce a copper protein complex, the concentration of which is determined spectrophotometrically and is directly proportionate to the concentration of protein in the sample.

Between batch Imprecision: <2%

Reference: Savory J, Heintges MG, Sonowane M, Cross RE. 1976 "Measurement of total protein and albumin in serum with a centrifugal analyzer" Clin Chem. 22: 1102-1104.

B20. Triglycerides mmol/l

Principal: Enzymatic colorimetric assay. Triglycerides are converted via a series of enzymatic reactions to produce hydrogen peroxide, which is then utilised in a Trinder endpoint reaction to form a red dye. The concentration of the dye is used to determine the concentration of triglyceride.

Between batch Imprecision: <2%

Reference: Wahlefeld, A. W. 1974. Triglycerides. Determination after enzymatic hydrolysis. In: "Methods of Enzymatic Analysis." H. U. Bergmeyer, editor. Academic Press, New York, NY.

B21. Urate umol/l

Principal: Enzymatic colorimetric assay. Uric acid is converted via uricase to hydrogen peroxide which can react via a peroxidase to a quinone-diimine dye which can be measured spectrophotometrically. The concentration of the dye is proportionate to the urate concentration.

Between batch Imprecision: <2%

Reference: Zhao Y, Yang X, Lu W, Liao H, Liao F. 2009 "Uricase based methods for determination of uric acid in serum" Microchimica Acta 164: 1-6.

B22. Urea mmol/l

Principal: A kinetic UV assay. Urea is enzymatically converted via urease to ammonium ions which are utilised by the enzyme glutamate dehydrogenase. This enzyme consumes NADH, the decrease of which is measured kinetically.

Between batch Imprecision: 3.4%

Reference: Kaplan, L.A., 1984. Urea. In: Clinical Chemistry; Theory, Analysis and Correlation, Kaplan, L.A. and A.J. Pesce (Eds.). CV Mosby Co., St. Louis, pp: 1257-1261.

B.23 Routine Haematology

Laboratory: Royal Free Hospital Clinical Biochemistry Laboratory
HEALTH SERVICES LABORATORIES (A Sonic Healthcare UK laboratory)
Royal Free Hospital, Pond Street, London, NW3 2QG | UNITED KINGDOM

The haematology tests were performed on the Sysmex XN-series analysers and the methods used are a mixture of flowcytometry and colorimetric.

Haematology marker	**units**
Haemoglobin	(g/l)
White Blood Cell Count	(10^9/L)
Platelets Red Blood Cell Count	(10^9/L)
Red blood cell count (RBC)	(10^12/L)
Red Blood Cell Distribution Width	(%)
Haematocrit	(L/L)
Mean Cell Volume (MCV)	(fL)
Mean Cell Hemoglobin (MCH)	(pg)
Mean Cell Hemoglobin Concentration (MCHC)	(g/l)
Neutrophils Absolute Value	(10^9/L)
Monocytes Absolute Value	(10^9/L)
Eosinophils Absolute Value	(10^9/L)
Mean Platelet Volume (MPV)	(fL)

Additional Blood biomarkers and laboratory methods

Analyses carried out by Dr Paul Welsh, Institute of Cardiovascular & Medical Sciences), BHF Glasgow Cardiovascular Research Centre, 126 University Place, Glasgow, G12 8TA

Laboratory methods

B.24 CRP

Unit: mg/L

Measured using an automated analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturer's calibrators and quality controls. A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection. For CRP lowest limit of detection was <0.15. Therefore, if CRP (mg/L) reported as <0.15 then CRP was given the value of ½*0.15=0.075

B.25 D-Dimer

Unit: ug/mL

Measured using an automated analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturer's calibrators and quality controls. A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection. For D-Dimer lowest limit of detection was <0.80. Therefore, if D-dimer (ng/ml) was reported as <80 then D-Dimer was given the value of ½*80=40

B.26 Plasma Interleukin-6 (IL-6)

Unit: pg/mL

Measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA)
(R&D Systems, Abingdon, UK) with the manufacturers quality controls.
A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection. IL-6(pg/ml) lowest limit of detection was <0.10. Therefore, if IL-6(pg/ml) was reported as <0.10 then IL-6(pg/ml) was given the value of ½*0.10=0.05.

B.27 Insulin-like growth factor 1 (IGF-1)

Unit: ng/ml

Measured using an enzyme-linked immunosorbent assay (ELISA)
R&D Systems, Abingdon, UK) with the manufacturers quality controls

---

B.28 Growth Differentiation Factor (GDF-15)

Unit: pg/ml

Measured using an enzyme-linked immunosorbent assay (ELISA)
R&D Systems, Abingdon, UK) with the manufacturers quality controls

---

B.29 Von Willebrand factor (VWF)

Unit: %

Measured using high-sensitivity enzyme-linked immunosorbent assays (ELISA)
(Asserachrom , Stago, Theale, UK).

B.30 Insulin

Unit: uU/mL

Measured using an automated analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers calibrators and quality controls. A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection. Insulin lowest limit of detection was <0.2. Therefore, if insulin) was reported as <0.2 then Insulin was given the value of ½*0.2=0.01

B.31 Vitamin D

Unit: ng/mL

Measured using an automated analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers calibrators and quality controls.

B.32 NT-proBNP

Unit: ng/L

Measured using an automated immunoassay analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers' calibrators and quality controls. The lower limit of sensitivity was 5 pg/ml

---

B.33 High-Sensitivity Troponin T (hsTnT)

Unit: ng/L

Measured using an automated immunoassay analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers' calibrators and quality controls. The lower limit of sensitivity was 3 pg/ml

---