SHARE WORKING PAPER SERIES

Collection of Dried Blood Spots in the Survey of Health, Ageing and Retirement in Europe (SHARE):

From implementation to blood-marker analyses

Martina Börsch-Supan, Luzia Weiss,

Karen Andersen-Ranberg, Axel Börsch-Supan

Collection of Dried Blood Spots in the Survey of Health, Ageing and Retirement in Europe (SHARE): From implementation to blood-marker analyses

Martina Börsch-Supan, Luzia Weiss, Karen Andersen-Ranberg
Axel Börsch-Supan

Martina Börsch-Supan, PhD, SHARE-ERIC-Biomarker Project,
80805 Munich, Germany; +49-89-32384935; *m.boersch-supan@mea.mpisoc.mpg.de*

Luzia Weiss, Munich Center for the Economics of Aging (MEA), Max Planck Institute for Social Law and Social Politics,
80799 Munich, Germany; +49-89-38602-329; *l.weiss@mea.mpisoc.mpg.de*

Karen Andersen-Ranberg, MD, PhD, University of Southern Denmark (SDU) and Odense University Hospital (OUH),
5000 Odense, Denmark; +45-65-503038; *KARanberg@health.sdu.dk*

Axel Börsch-Supan, PhD, Munich Center for the Economics of Aging (MEA), Max Planck Institute for Social Law and Social Politics; Technical University of Munich; National Bureau of Economic Research (NBER), Cambridge, MA, USA;
80799 Munich, Germany; +49-89-38602-355; *axel@boersch-supan.de*

Abstract

SHARE, the "*Survey of Health, Ageing and Retirement in Europe*", is a population-based socio-economic survey among people aged 50 and over with data from 28 European countries and Israel. It investigates individual, economic, health-related, and social impacts of the aging process in order to give answers to the challenges of population aging for individuals and society as a whole. Understanding aging per se and how we age differently given our individual background, current health and socio-economic factors is the aim of SHARE.

In order to maintain intertemporal, international and intercultural comparability, SHARE has adopted objective data collection in the health domain. SHARE measures physical performance, such as grip strength, peak expiratory flow, walking speed, chair stand and word recall and Euro-D depression among others in the cognitive and mental health modules. In 2015, SHARE has collected dried blood spot (DBS) samples as an additional objective measure of health. Eleven European countries and Israel participated in the DBS collection. The collection was harmonized in terms of designing documents, gaining consent, procuring blood-collection material, and training interviewers how to collect DBS samples while ethic and administrative regulations in all countries had to be observed in addition. Overall, about 27,200 respondents consented; the overall participation reached 72% with considerable differences between countries and interviewers.

This report describes the carefully monitored processes of consent-gaining for, and the implementation, collection and evaluation of the largest DBS sampling from a representative adult population in Europe, now awaiting the completion of blood-marker analyses. We also motivate the choice of blood markers and first assays.

Keywords:

Population-based survey, consent rates, self-reported health, objective health measurements, dried blood spots (DBS), blood markers, blood-marker analyses in DBS samples

1 Introduction

1.1 Population aging, health and SHARE, the Survey of Health, Ageing and Retirement in Europe

Population aging is one of the most pressing challenges of the 21st century. The large baby-boomer cohorts are entering old age, while the later cohorts are less fertile. A worldwide increase of life expectancy – influenced by declining old-age mortality – has not been paralleled by an equivalent increase in healthy aging. Developed as well as developing countries are facing social and economic challenges caused by the disproportional increase of older adults and the accompanying burden of chronic diseases (Chang, Skirbekk et al., 2019; Mathers, Stevens et al., 2015; World Health Organization, 2018). About one fourth of the total burden of disease is attributable to disorders in people aged 60 and over and population aging is driving the worldwide epidemic of chronic diseases (Prince, Wu et al., 2015). Socio-economic studies, which also address health in their surveys, are suited to entangle the complex interrelation of health and social status, to point out lifestyle-related risk factors in particular those, which are modifiable.

Departing from an individual's genetic make-up, parental conditions and SES, childhood environment and early education, the trajectories of health, economic status and social embeddedness are not determined in isolation but in mutual interaction over the entire life course. These interactions manifest their effects starting very early in life and then accumulate in positive and negative feedback cycles over the entire life course (Blane, Netuveli, & Bartley, 2007; Conti & Heckman, 2013; Health, 2006; Power & Kuh, 2006) before they determine later-life health, economic and social outcomes at older ages. Furthermore, opportunities provided by parents or choices they made cannot be changed; in adult life the individual can make unhealthy or healthy lifestyle choices that affect health negatively or positively, immediately or later in life.

Health, for instance, influences economic status because healthier bodies are likely to support higher learning capacities at younger ages and higher working loads at older ages (e.g., Deaton, 2002; Kivimäki, Batty et al., 2020; Marmot, 2002). In turn, income inequalities are likely to also cause inequalities in health because richer individuals can afford higher out-of-pocket health care costs and may have easier access to health care especially in certain health care systems (e.g., Dugravot, Fayosse et al., 2020; Lewer, Jayatunga et al., 2020; Mackenbach, 2012; Smith, 2003). Health behaviors, like physical activity, (Daskalopoulou, Stubbs et al., 2017; Marques, Peralta et al., 2018), lifestyle, environmental and occupational conditions add to these mutual interactions between health and economic status and at the same time introduce interactions with the social environment in which individuals live (Dwyer-Lindgren, Bertozzi-Villa et al., 2017). For example, there is evidence that embeddedness in a

good family background is beneficial for the health of the family members ([e.g., Fagundes, Bennett et al., 2011](#)).

SHARE is an infrastructure of micro data to address the health, social and economic impacts on growth and prosperity of the aging populations in Europe ([A. Börsch-Supan, 2013](#)). On the population level, SHARE data help to shed light on the implications of demographic aging on economic growth, living standards, social and particularly intergenerational cohesion, and how pensions and health and long-term care systems will be affected ([European Commission, 2015](#)). On the individual level, SHARE measures socio-economic, social support and health variables and studies the psychological, physiological and patho-physiological pathways, leading to physical, mental and cognitive functional deteriorations. In this respect, SHARE is especially designed to investigate the complex interactions among health, social and economic determinants over the entire life course that shape an individual's health, economic and social status in later life. With the release of Wave 7 in 2019 the SHARE database contains 380,000 interviews from 140,000 respondents (www.share-project.org; [Bergmann, Scherpenzeel, & Börsch-Supan, 2019](#); [A. Börsch-Supan, Brandt et al., 2011](#); [A. Börsch-Supan, Brandt et al., 2013](#); [A. Börsch-Supan, Brandt, & Schröder, 2013](#); [A. Börsch-Supan, Bristle et al., 2019](#); [A. Börsch-Supan, Brugiavini et al., 2008](#); [A. Börsch-Supan, Brugiavini et al., 2005](#); [A. Börsch-Supan & Jürges, 2005](#); [A. Börsch-Supan, Kneip et al., 2015](#); [Malter & Börsch-Supan, 2013](#); [Malter & Börsch-Supan, 2015](#); [Malter & Börsch-Supan, 2017](#)).

1.2 Self-reported and objective health measures

Since its first wave in 2004, SHARE respondents are asked to self-report current general health, diseases and conditions, use of medicine, as well as functional limitations and disability and are asked for height and weight. They also self-report health behaviors such as smoking, drinking, exercising, and physical fitness and self-rate their health. Although individuals are likely to evaluate their own health status differently even when they have the same objective condition ([Jürges, 2007](#)), self-reported information is of great value in itself ([Doiron, Fiebig et al., 2015](#); [Jylhä, 2009](#)).

Nevertheless, self-reported data on past and current health conditions and disease symptoms rely on recognizing those symptoms, having access to medical care and being diagnosed, as well as being able to recall the diagnoses. Moreover, in older adults many diseases may have subtle symptoms, which may be misinterpreted as signs of aging rather than disease onset, thereby delaying diagnosis and treatment. Other diseases, like diabetes and hypertension, can go without symptoms for quite some time, causing organ damage before being diagnosed. Older age is characterized by the emergence of several complex health states that do not fall into discrete disease categories, commonly called geriatric

syndromes, which are often the consequence of multiple underlying factors. Hence, the accuracy of self-reported health data depends on individual health literacy and access to health care and treatment, which are correlated with socio-economic factors. This correlation may lead to spurious associations between health outcomes and socio-economic factors in the observed data (Bound, 1991; Sen, 2002). Therefore, SHARE has decided to spend major efforts to collect objective information on physical, cognitive and mental health in addition; such as grip strength, peak expiratory flow, walking speed, and chair stand as physical measurements and using e.g., word recall, Euro-D depression battery in the cognitive and mental health modules.

While the collection of health data in a population-based survey is already a complex enterprise in a single country, the effort in a pan-European study like SHARE is even greater and requires extensive planning, as it is crucial to ensure the comparability of collection protocols across countries (Weiss, Sakshaug, & Börsch-Supan, 2018). This applies in particular to obtaining blood-based biological markers detectable in dried blood spots (DBS) as has been done in Wave 6 in 2015 (M. Börsch-Supan & Andersen-Ranberg, Chapter 6.1, in: Malter & Börsch-Supan, A (Eds.) 2017).

1.3 Combining bio-medical with socio-economic research: Collection of Dried Blood Spot samples in a multi-national population survey

In spite of this effort, the motivation to combine bio-medical and socio-economic research is manifold: Blood biomarkers e.g., addressing conditions and diseases of older age can help to identify pre-disease physiological processes, which are below the threshold of an individuals' perception. They capture health aspects (yet) unknown to the survey participants and they can help to understand complex relations between socio-economic conditions, health, cognition, and physiological pathways. Cardiovascular, metabolic, inflammatory, and other biomarkers have been shown to be predictors of mortality and morbidity by themselves or combined with self-assessed health (Gruenewald, Seeman et al., 2006; Ridker, 2007). Furthermore, blood biomarkers shed light on the mechanisms that relate socio-economic factors influenced by lifestyle and environment to morbidity and mortality, which are not confounded by the shortcomings related to self-reported health information.

Collecting blood is the method of choice when combining bio-medical and socio-economic research (Williams & McDade, 2009). From a laboratory point of view, venous blood samples are best suited for analyzing blood biomarkers. However, collecting venous samples in very large community-based setting in so many countries as in SHARE entails major logistic challenges of collection, transportation and timely laboratory analyses as well as large expenses for venipuncture by certified health professionals. Moreover, a venipuncture is invasive and may potentially cause harm to the individual. It may also be regarded as

intrusive by the respondent and thus yield higher proportions of non-participation in the SHARE survey itself.

Instead of drawing venous samples, SHARE decided to collect blood using a method more adaptable to the circumstances of a very large international population survey. The sampling of DBS offers numerous advantages over conventional blood collection (Wenkui Li & Lee, 2014; Wenkui Li & Tse, 2010). DBS samples are drops of capillary whole blood collected on filter paper from a simple finger prick. The method is regarded less intrusive than venipuncture and easily applied. Blood collected on filter cards can be shipped by regular mail to a laboratory and stored frozen until analyses. This makes DBS samples well suited for many screening programs or population-based studies in the area of public health research.

The analytes, which can be measured in DBS samples today, have gradually increased over the years thus making the collection method increasingly interesting. They range from DNA analyses (viral / human) to a variety of proteins, lipids and vitamins (e.g., Brindle, O'Connor, & Garrett, 2014; Crimmins, Kim et al., 2014; Eyles, Anderson et al., 2009; McDade, Williams, & Snodgrass, 2007; Mei, Alexander et al., 2001; Skogstrand, 2012; Skogstrand, Thorsen et al., 2005) as well as multiple elements helping to identify environmental or workplace-related heavy-metal pollution (Pedersen, Andersen-Ranberg et al., 2017).

The blood-based biomarkers shall provide SHARE and its users with additional objective health measures to invest more closely the most common aging-related health conditions in the context of the socio-economic information gained from its survey. Those health conditions and aging-related diseases include diabetes (e.g., Kalyani, Golden, & Cefalu, 2017), cardiovascular disease (CVD) (e.g., North & Sinclair, 2012), decline of kidney function, cognitive decline, but also loss of strength and muscles (sarcopenia). Many of these conditions have several underlying factors, both biological and environmental, and with a complex interrelationship, which, in addition, is dependent on the genetic make-up of an individual. Among the biological factors, metabolic, inflammatory and neuroendocrine pathways are considered to be among the most important. Especially elevated blood-fat levels but also inflammation and the subsequent inflammatory cascade are particularly important in the atherosclerotic process and the development of CVD (Boekholdt & Stroes, 2012; Brüünsgaard & Pedersen, 2003). There are reports that elevated levels of pro-inflammatory markers are present in mild cognitive decline (MCI) (Saleem, Herrmann et al., 2015) and they may play a role in the neurodegenerative cascade in established Alzheimer's' disease (AD) (Swardfager, Lanctôt et al., 2010). The same body's own system can also react to activities like physical training (Nascimento, Pereira et al., 2015; Palmefors, DuttaRoy et al., 2014) and social contacts and support (Fagundes, Bennett et al., 2011; Salinas, 2016) with beneficial and anti-inflammatory signals. Age-related health conditions are not

independent from each other. CVD ([Dregan, Stewart, & Gulliford, 2013](); [Stampfer, 2006]()), hypertension / high blood pressure ([Skoog, Lernfelt et al., 1996]()), as well as type-2 diabetes ([Wei Li & Huang, 2016]()) are risk factors for cognitive decline and AD. Yet, diabetes, obesity and hypertension are important risk factors for CVD as is impaired kidney function ([Sarnak, Katz et al., 2008](); [Yaffe, Lindquist et al., 2008]()). These and further considerations guided the selection of markers to be analyzed from the SHARE-collected blood spots. A detailed description of the chosen markers will be given in Section **5** below.

However, surveys such as SHARE operate in the field i.e., respondents are interviewed in their homes. Trained interviewers instead of certified health professionals collect the samples according to a protocol but not under controlled and standardized laboratory conditions. The conditions during fieldwork and shipment may vary namely the environmental temperature and humidity; those conditions are hard to control. In addition, best care and attention exercised by the interviewer while taking the blood and handling the sample is very important ([Crimmins, Zhang et al., 2020]()). The interviewer may influence the drying time of the blood samples, humidity protection during shipment or other quality measures, shortcomings, which lead to avoidable damage (see **2.2.3** and **4.2**).

Furthermore, not all collected samples contain enough material – as not all respondents bleed well - or bad sample quality does not allow analyzing all biomarkers of interest. This and certain medical conditions, e.g., bleeding disorders prohibiting blood-taking, and non-consenting lead to biomarker values available only from a subsample of the DBS-eligible respondents.

In awareness of the shortcomings of blood collection during fieldwork, SHARE and the two laboratories in Seattle and Copenhagen (entrusted with the blood-marker analyses; see Section **5**) performed validation experiments in 2018 and 2019, respectively. The influence of single conditions, like temperature, humidity, drying and shipping times was tested as well as interactions of these conditions ([Börsch-Supan, Weiss et al., 2019](); [Weiss & Börsch-Supan, Chapter 37, in: Börsch-Supan, A et al. (Eds.), 2019]()). A detailed description of the validation experiments will be published elsewhere ([A. Börsch-Supan, Weiss et al., 2020 - submitted for publication]()).

In Section **2.1** below we will describe the steps preceding the blood collection: the preparation of all legal, ethical and administrative documents required, the compilation of the DBS collection kit, the implementation of a DBS module in the CAPI instrument, the training of the interviewers how to collect DBS on a filter card and send it to the biobank. Section **2.2** is dedicated to the collection of DBS during the survey and the fieldwork monitoring. In Section **3** we will discuss the participation of the respondents and the consent rates followed by the presentation of the data (number and quality of the collected samples) and the first

steps of data cleaning, i.e., samples which had to be excluded in Section **4**; here, we also discuss environmental and other influences the samples were exposed to. In Section **5.1** we present the blood biomarkers SHARE has chosen to analyze and motivate our decision. In the final sections **5.2** to **5.3** we describe the preparation of the DBS samples for laboratory work, the analyses of a subset of 8000 samples and the necessary adjustments for field conditions, which has to precede the release of the data once the analyses are completed. Section **6** summarizes and presents an outlook on the remaining work.

2 Methods

2.1 Implementation

2.1.1 Legal, ethical and administrative requirements

Collecting data from respondents in a survey, particularly collecting blood samples, needed the approval of ethical committees in all participating countries. DBS collection is considered an invasive, albeit only minimally invasive method. It therefore requires careful legal, ethical and administrative preparation.

For the DBS collection in SHARE Wave 6 the CAPI[1]-assisted questionnaire around blood taking, consent documents, and other training and field material as well as a survey protocol were prepared centrally in English language. They were translated into the national languages of the SHARE countries and – depending on the requirements of each country - compiled, adapted, extended and then presented to the responsible national ethics committees and/or other authorities to obtain ethics approval (Schmidutz, 2016; Schmidutz, Ryan et al., 2013; Schmidutz & Weiss, 2017).

Obtaining informed written consent was obligatory in all participating countries. The consent defined how the sample collection had to be conducted in the field and how the samples can be handled and analyzed in the future. However, the countries differed regarding the information that had to be provided to the respondents and regarding the content and form of the consent documents. In some countries (Switzerland, Denmark, Italy, Sweden) the respondents had to be informed about the blood taking with an advance letter; in other countries it was sufficient to inform the respondents during the interview prior to the planned DBS collection.

SHARE decided not to report lab values to the respondents in case of a disease-indicating level of a blood-marker value. This was done for several reasons: The methods of blood collection used in SHARE are not suited for individual disease diagnosis; instead, they will shed light on population health. Diagnosis of a serious health condition has to be performed

---

[1] CAPI: Computer-assisted personal interviewing

under standard clinical conditions with multiple testing. Besides, the blood values gained during the survey are only available a long time after blood was taken; at the time of back-reporting, conditions of the respondent may be different. In addition, experiences in Germany, while piloting the DBS collection in Wave 4, have shown that back-reporting is complicated as the data is anonymized; the respondents had to consent to reporting of the blood-marker values to a third institution/person, e.g., via his/her general practitioner. In many cases, the contact to the GP had failed.

All ethic committees followed our reasoning not to report blood values to the respondents, with a single exception: In Denmark, the respondent had to be notified in case unknown diabetes was detected by the SHARE DBS analyses; this had to be carried out via the Danish survey agency, as SHARE has no access to respondent addresses.

The interviewers play a crucial role in the process of gaining consent, because they are the only ones being in direct contact with the respondent and those collecting the DBS sample during the interview. Hence, the aspect of successful consent gaining was an important part of the DBS collection training (Korbmacher, 2014; Schmidutz & Weiss, 2017).

Most countries allowed the collection of blood by trained interviewers. In France, however, interviewers were not allowed to touch the respondents. French respondents therefore pricked their own finger with the lancet, guided by the interviewers or could decide to be pricked by a third person e.g., a confidant. In Austria, the Czech Republic, Poland, and Luxembourg even this was not possible. Only medically trained personnel are allowed to take any kind of blood samples. Since this was neither practical nor financially feasible, no DBS samples could be taken in these countries. And, lastly, Croatia, Hungary, The Netherlands, and Portugal could not be included in the DBS collection of the main survey due to financial reasons. Twelve SHARE countries[2] (Belgium, Denmark, Estonia, France, Germany, Greece, Israel, Italy, Slovenia, Spain, Sweden, and Switzerland) remained and a total of ca. 27,200 DBS samples were collected. In addition to the main DBS collection, we conducted a small study in Poland in 2016 with 37 participants, where nurses collected venous and capillary (DBS) blood. The study was used for validation purposes (see 5.4 and Weiss, Börsch-Supan et al., 2019).

We developed a detailed plan how to process and store the blood samples as well as corresponding laboratory data. To ensure data privacy of the respondents the signed consent forms, the blood material, and the CAPI interviews are each stored at separate locations: with the national survey agencies, the biobank and on the SHARE server, respectively. All three data sources (the sample itself, the consent form and the interview

---

[2] Country Code of all SHARE countries in wave 6: AT Austria, BE Belgium, CH Switzerland, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES-sp Spain, ES-cat Catalan province of Girona, FR France, GR Greece, HR Croatia, HU Hungary, IL Israel, IT Italy, LU Luxembourg, NL Netherland, PL Poland, PT Portugal, SE Sweden, SI Slovenia.

data) are labelled with a barcoded identification numbers for each participating respondent. This number is used for the future linkage of the biomarker analyses results to the survey data.

For the secure storage of DBS samples from all participating SHARE countries, the SHARE biobank had been established at the Department of Public Health at the University of Southern Denmark (SDU) in Odense, Denmark. It is affiliated to the biobank of the Danish Twin Registry, hosted by SDU. All blood samples collected from respondents during the field phase of Wave 6 have been sent there, where they are stored at -20°C, each together with desiccant for humidity protection in SHARE-ERIC owned laboratory-style freezers. For surveillance, all freezers are connected to an alarm system, which notifies a biobank staff member in the case of a temperature rise or another hazard. The biobank also provides a back-up freezer in the case of emergency.

2.1.2 Required documents

The SHARE biomarker team centrally prepared and compiled the material needed by the SHARE interviewers to collect the DBS from the survey respondents during the interview. This material included the blood-sampling equipment, the DBS collection kit (see **2.1.3**) but also documents to inform the respondent about the DBS collection, processing and subsequent analyses of the blood-samples and the consent forms, which had to be signed by consenting respondents before DBS taking.

- (1) Information leaflet
- (2) Consent form
- (3, 4, 5) Interviewer instructions: DBS manual, step-by-step instructions written and as illustrated flyer

The *information leaflet (1)* explained the purpose and conduction mode of the DBS collection and provided respondents with contact details as well as information about their rights and the privacy practices within the project. The DBS collection *consent form (2)* recalled the information in an abbreviated version and had to be signed by both, the respondent and the interviewer. The leaflet and one copy of the consent form remained with the respondent; the second copy was mailed to the national survey agency by the interviewer.

In addition, *information material (3)* with a *step-by-step guide (4)* of the blood collection was prepared for the interviewers. As a very quick reference, interviewers also received a brochure – an illustrated flyer (5) – showing the most important steps of the blood collection as illustrations.

2.1.3 The collection kit

We equipped the interviewers of each country with ready-to-use DBS collection kits (Fig. 1). The coordinating SHARE biomarker team in Munich designed it, as no suitable kit was commercially available. It contained all items needed for the blood collection and subsequent shipment of the samples to the SHARE biobank. Before a decision for some of the items was made, we investigated various shipment formats – namely envelopes, bags and different desiccant materials – with regard to their temperature- and humidity-protection capacity (see **2.1.4**). To learn more about the temperature exposure of the samples during shipment, we used temperature-tracking devices in the field during pretest of SHARE Wave 6, and Israel used long-run temperature tracking starting with the summer months during SHARE Wave 6 main survey (see **2.1.4** and **2.2.3**).

The blood-collection items were bought centrally and assembled into kits by a local Munich company. This ensured the usage of identical material in all countries and by all interviewers. Altogether, 40,000 kits were delivered to the national survey agencies that were commissioned with the SHARE data collection in Wave 6 in the participating countries.
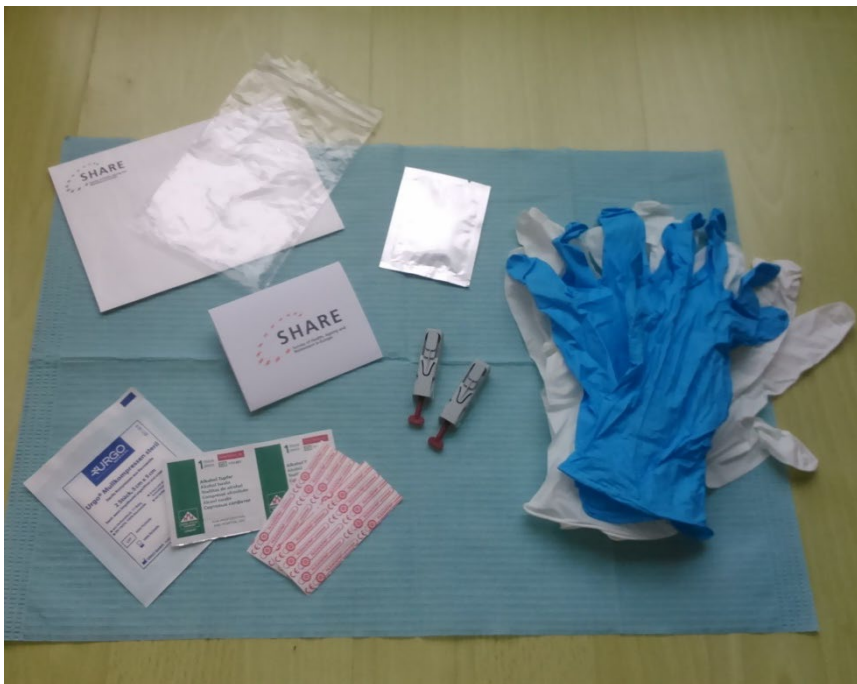
Figure 1: Items in a SHARE W6 DBS collection kit. A large Ziploc plastic bag for all necessary material, which later served as disposal bag for used items after the DBS collection; the SHARE DBS filter card with pre-printed 1-cm circles (see Fig. 6) for placing the blood drops; blue disposable protective cloth used as a clean base for material and as surface protection from any small blood stains; disposable protective gloves; disposable disinfecting wipes; two semi-automatic, one-time use lancets; sterile gauze pads; plasters. 2g of molecular-sieve served as desiccant contained in a small Tyvek® bag; to prevent untimely humidity absorption and extend the shelf life, the desiccant bags were individually sealed in a larger aluminum pouch, from which the small – 25x55mm - Tyvek® bag had to be removed before use. For later shipment of the sample to the biobank the kit also contained a small plastic bag for the DBS card and desiccant and a special tear-proof and water-resistant Tyvek® mailing envelope.

Not shown in Figure 1, but also part of the interviewers' collection material: Reusable hand warmers to be used to warm the respondents' hands before the DBS collection, since warm hands show better blood flow. The hand warmers stayed with the respondents after the interview as a small token of appreciation. In addition, the interviewers were provided with a sheet with barcode stickers (all with the same barcode number) for each respondent; pre-printed address stickers for mailing the sample to the SHARE biobank; and stamps, which were supplied by the survey agencies of each country for international postage to Denmark. The temperature strips used in Israel were bought while the survey was already in the field and added to the Israeli test kits.

2.1.4 Investigating envelops, temperature-control devices and desiccant materials

Envelopes and bags: Different shipment-material formats have been tested before preparing the collection kits in order to investigate temperature and humidity protection for the DBS samples. We were interested to learn, how well envelopes made of different material and different kind of plastic bags would protect the sample against temperature change. We acquired various kinds of envelopes (paper padded, bubble-foil padded, unpadded Tyvek®) and bags (polyethylene PE, Mylar, insulating material). We equipped various combinations of envelope/bag each with an "empty" DBS card and desiccant inside the bags – as if ready for shipment. A temperature/humidity logger was added to the envelopes, which were then exposed to various controlled temperatures between room temperature and up to 35°C for different periods in a laboratory oven[3]. Different material provided temperature protection within a very narrow range (Fig. 2). The temperature inside an envelope/bag combination exposed to 35°C reaches a threshold of 33°C in a time span between 21 and 44 minutes. In summary, compared to the expected shipping times of international mailing the environmental temperature was reached inside the envelopes within a very short time. Therefore, we decided for the sturdy envelope material Tyvek® being tear- and water-resistant, light and easy to close in combination with a simple PE bag for the DBS card.

---

[3] The oven was kindly loaned to us by the Max Planck Institute of Neurobiology in Munich.
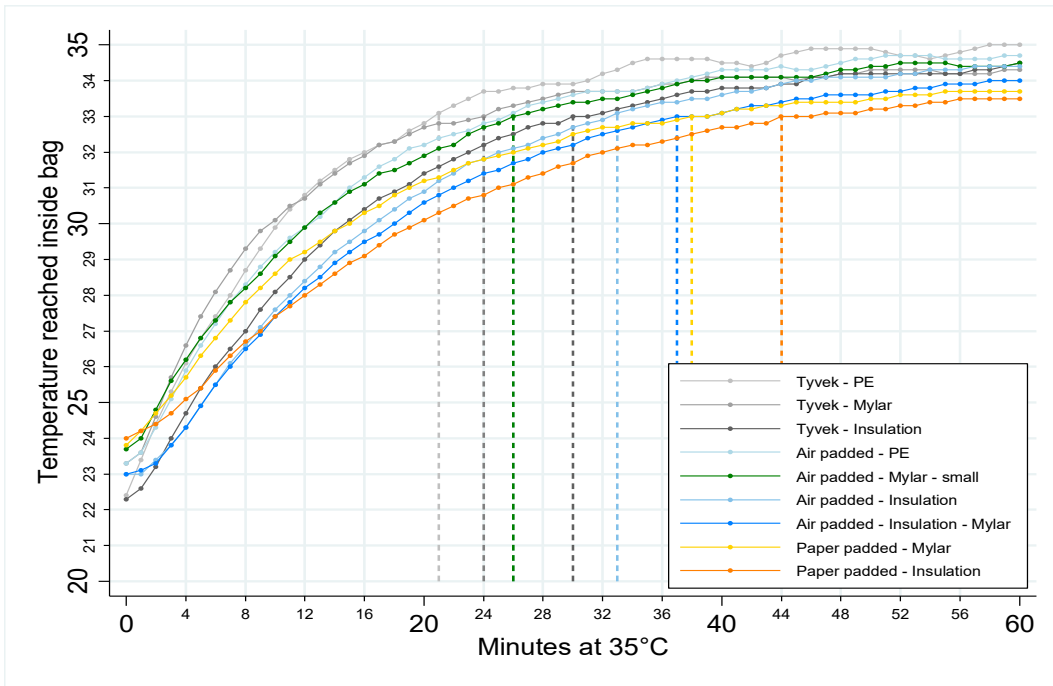
Figure 2: Envelope testing for temperature protection. Various envelope/bag combinations were exposed to 35°C. Temperature inside the bags reached a threshold of 33°C after 21 to 44 minutes (dashed vertical lines).

Long-term temperature control during shipment: During shipment to the biobank, the DBS samples are exposed to varying – unknown – temperatures. However, the exposure to high temperature has been shown to influence the subsequently measured biomarker level, if DBS will be analyzed for thermally labile markers (A. Börsch-Supan, Weiss et al., 2020; Crimmins, Zhang et al., 2020). We used long-term temperature trackers alongside the DBS samples during shipment to learn more about the temperature levels the samples are actually exposed to and their association to the outside temperature at the time of collection of the blood sample. The trackers are small plastic strips (Fig. 3; TimeStrip Label 30 and 38°C; Timestrip UK Ltd, Cambridge, UK, and Shockwatch WarmMark® Long-Run indicator, Hanwell Solutions Ltd. UK, for 31°C) with an activating mechanism to be initiated by the interviewer when preparing the blood sample for shipment.
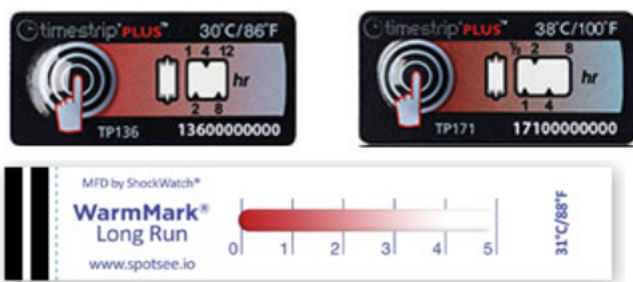

Figure 3

Timestrip® indicators 30 and 38°C not activated.

WarmMark®Long Run indicator activated; lines 1 to 5 indicate exposure times of 12, 30, 60, 110, and 168 hours.

The tracking strips indicated the surpassing of a certain temperature threshold and its duration by the spread of colored indicator. This information was recorded at receipt of the

sample by the biobank staff. The local outside temperature during the interview and blood taking was estimated by the interviewer and entered into the CAPI.

- During the pretest of Wave 6, we used two different temperature strips indicating the exposure to 30°C, respectively 38°C, or higher temperatures. The duration of the exposure was measured in steps of one hour for up to 12 (or 8) hours overall.
- During the main data collection, we tracked the temperature during shipment in some Israeli samples. In this case, we used a long-run tracker, indicating the exposure to 31°C or higher for 0, 12, 30, 60, 110 (2.5 days), or 168 (7 days) hours overall.

Only few samples reached the threshold of 38°C during pretest (11 out of 223 samples with linkable interview data). For the 30°C and 31°C, we created dummies to indicate whether the respective threshold was reached at all and calculated a logistic regression of this dummy on the estimated outside temperature, controlling for shipment time. The correlations between the outside temperature and the dummies were both significant. The predictive margins of the 30°C and 31°C dummies over the estimated outside temperature (controlled for shipment time) are shown in Figs. 4 a and 4b.
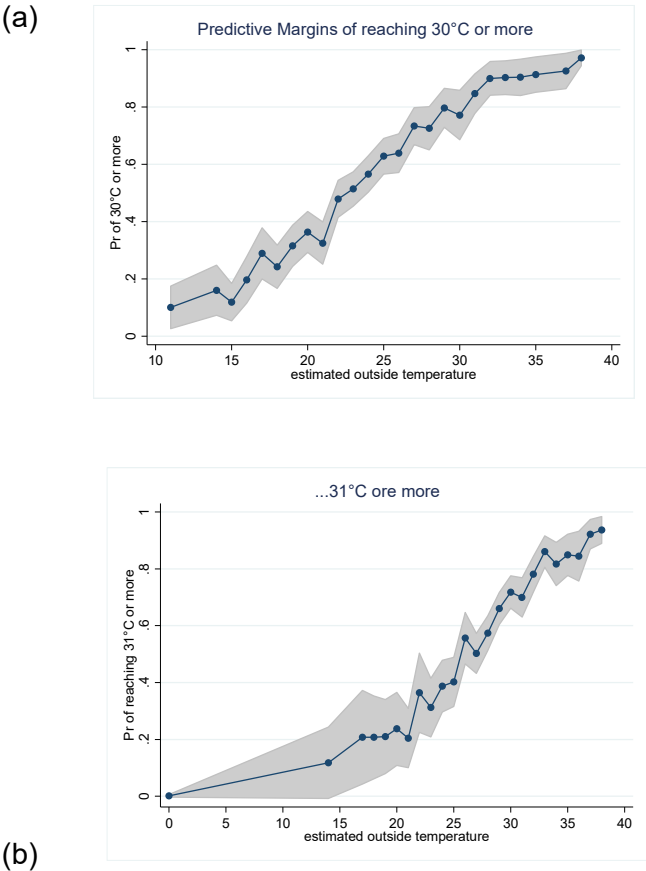
(a)

(b)

Figure 4: Predictive margins of reaching a temperature threshold after logistic regression of the according dummy on the estimated outside temperature and controlling for shipment time. (a) threshold at 30°C, Wave 6 pretest samples, N=288; **(b)** threshold at 31°C, Wave 6 Israeli main survey samples, N=299.

We conclude that the outside-temperature estimation by the interviewer during blood collection provides valuable information on the temperature pattern the sample is exposed to during the entire shipment time.

Desiccant: We studied product comparisons of silica gel vs. molecular sieve. (e.g., https://www.sorbentsystems.com/desiccants_charts.html#table1 https://www.multisorb.com/blog/pharmaceuticals/molecular-sieve-vs-silica-gel-whats-the-difference/). Molecular sieve adsorbs water from the surrounding faster and more aggressively than silica gel, though its equilibrium capacity for water is slightly lower than silica gel. On the other hand, molecular sieve does not release water at higher temperature as easy as silica gel. We calculated that 2g of molecular sieve in a sachet per DBS card will be fine for a dried DBS sample containing up to five blood spots.

2.1.5 The DBS module in the CAPI instrument

To conduct the blood sampling, SHARE set up a short questionnaire module within the CAPI instrument, the BS module (for the English version see: http://www.share-project.org/fileadmin/pdf_questionnaire_wave_6/Generic_main_qnn_6_3_13.pdf) closely following the DBS collection module used in the Health and Retirement Study (HRS[4]) (Crimmins, Guyer et al., 2008). The module instructions guided the interviewers through the DBS collection: from introducing the purpose of the DBS collection to the consent gaining and finally a possibility for the interviewer to report problems that may have occurred during the blood collection.

The BS module was routed via a preloaded variable; it only appeared for DBS-eligible respondents (see **2.2.1**). As a reminder, the interviewer instruction at the beginning of the BS module denoted it as "non-proxy module": In case the respective interview was a proxy[5] interview, no blood was collected, and the interviewer skipped the entire module.

The first item to be read out was a short introduction with two versions: (1) for countries that announced the DBS collection in their advance letters (see **2.1.1**) and (2) for all other countries. For the actual blood sampling, the CAPI referred the interviewer to the printout step-by-step instruction, eliminating the need to handle the laptop during the blood collection while wearing protective gloves. After the blood sampling, this printed instruction lead him/her back to the CAPI to enter the barcode number of the blood sample twice. A programmed soft-check ensured the entry of identical numbers to avoid typing errors. Then, the regular interview continued with the next modules. Finally, two items at the very end of

---

[4] HRS is the SHARE "sister" study in the United States of America

[5] In an interview a proxy respondent answers in lieu of the person, from whom information is being sought; this could be e.g., a partner, spouse, confidant.

the regular SHARE interview of DBS-eligible respondents were not part of the actual BS module, but routed the same way: Item one asked the interviewer to estimate the current outside temperature. This information was used to get information on the temperature the sample was exposed to during local handling and start of shipment (Weiss & Börsch-Supan, Chapter 37, in: Börsch-Supan, A et al. (Eds.), 2019). The second item referred the interviewers again to the printed step-by-step instruction, where they were guided through the preparation of the sample for shipment. Having this latter item not directly at the end of the BS module, but at the end of the regular SHARE interview (with other modules in between), helped to ensure the drying time for the blood sample.

2.1.6 Training the SHARE interviewers to collect DBS

While survey agencies and their interviewers were already experienced from previous SHARE waves in taking physical performance measurements, the collection of DBS was a novelty. With the exception of six countries (BE, CH, DE, DK, IT, PT), which had participated in a logistics test run in the pretest of SHARE Wave 5, most survey agencies in SHARE Wave 6 had not collected DBS before.

All interviewers of the countries participating in SHARE Wave 6 main survey had to be trained extensively and in their national languages. SHARE works with the hierarchical "train-the-trainer" (TTT) scheme: First, members from the SHARE country teams and their survey agencies were trained and prepared in a central TTT workshop in Munich using English language. The TTT agenda served as a template for the subsequent national training sessions now conducted by the respective national survey agencies and in the languages of the interviewers.

The training session for the DBS collection was part of the general interviewer training for SHARE Wave 6. It provided the interviewers with background knowledge about the DBS collection, the markers to be analyzed as well as biosafety concerns and data privacy rules. They were trained to answer DBS-specific questions, dispel doubts and explain procedural and medical facts. The process of consent gaining was carefully scripted and required more skills on part of the interviewer than for other survey items (Korbmacher, 2014). Being well prepared and well trained played an important role for successfully gaining consent from the respondents.

A central part of the DBS training consisted of a thorough hands-on training. The collection kit was introduced followed by a mock interview with blood taking. Every interviewer had to perform DBS collection under supervision. Mistakes or missing steps had to be retrained. Once the interviewers could successfully perform the blood-taking procedure, they were certified. Only certified interviewers were allowed to collect DBS samples for SHARE. Moreover, interviewers were trained for particular situations like advising participants taking

blood thinners or helping participants, who do not bleed easily and were prepared to support respondents, who might feel dizzy from finger pricking. All interviewers were provided with printed DBS interviewer instructions as a reference for self-training or as preparation for questions arising during the fieldwork.

Attention was given to the interviewers' own attitude towards the blood collection and feeling comfortable in performing it as well. To address this, we worked closely together with the German survey agency. German interviewers were questioned after the pretest DBS training to suggest improvements. We considered their suggestions for the main survey training. Most of those interviewers, who had stated to feel somewhat worried with the idea of collecting blood samples before the training session, told us afterwards to feel better, if not good, when thinking of the upcoming DBS collection. Interviewers, who were still worried, were not assigned to interview DBS-eligible respondents.

2.2 Collecting DBS

2.2.1. Eligibility for DBS sampling

In the countries participating in the DBS collection, all respondents in panel households were asked for consent to the blood sampling, i.e. all respondents, who had previously participated in any wave of SHARE and their partners (for them independently of a previous participation) No blood was taken from respondents, who refused to participate or were not able to give consent themselves. Proxy respondents and respondents, who mentioned a medical reason preventing blood taking (such as bleeding disorder), were also excluded from the DBS collection. In France, DBS were collected only in four districts. From 884 DBS-eligible respondents living in these districts, 569 respondents consented to the blood collection. Some respondents from other districts, thus actually not DBS-eligible, had also been asked by interviewers working in DBS-eligible and non-eligible districts and nine of these respondents consented to the DBS collection as well. These samples were included yielding 578 French DBS samples altogether.

2.2.2 Blood collection from the respondent and dispatch of the DBS card to the biobank

Starting the DBS collection, the interviewer provided a printed information leaflet, explained the procedure, asked for consent, but expressed that participation is voluntary. Upon agreement of the respondent, written consent was collected by having the respondent sign the consent form.

Then, the interviewer prepared the blood collection: An activated warming pad was handed to the respondent. Warming the hands increases the blood flow into the fingers, thereby

increasing the chance to collect a sufficient amount of blood. The date of the blood collection was noted on the DBS card, which was labelled with a unique barcode sticker.

While wearing protective gloves, the interviewer disinfected the puncture site on a finger - preferably the ring finger on the non-dominant hand - and used a semi-automatic lancet for pricking. Self-pricking by the respondent[6] or pricking by another person was an alternative option. The first drop of blood was wiped off to eliminate remaining alcohol from the disinfection. The next blood drops were collected by letting them fall onto the absorbent filter paper of the SHARE DBS card (into pre-printed circles). The filter card was left to dry until the end of the regular SHARE interview, but at least 15 minutes. At the end of the interview, a CAPI item reminded the interviewer to pack the DBS card, which was slipped into a small polyethylene (PE) bag together with desiccant; the bag was zip-closed and placed into the Tyvek® envelope.

Another sticker with the same barcode number was attached to the consent form; the barcode number was entered into the CAPI instrument. The signed consent form was sent to the national survey agency and the DBS card to the SHARE biobank in Odense, DK. The barcode number ensures that the CAPI data, the consent form and the collected DBS can be matched, while guaranteeing the respondents' anonymity. All three data sources (DBS card, consent form, CAPI interview) have to be available for the sample to be considered "complete" and to be considered for laboratory analyses.

2.2.3 Monitoring fieldwork in SHARE Wave 6 - in particular DBS collection

The SHARE Wave 6 data collection lasted from January until November 2015, and fieldwork was actively managed by SHARE Central through monitoring interviewer activity e.g., contact and cooperation rate, response/retention rate as well as consent for and quality of DBS samples. Reports were mailed bi-weekly to all survey agencies and country teams and served as feedback of the interviewer performance (Malter & Sand, 2017). Within this frame of the general participation monitoring, we reported the number of collected DBS samples in each country as part of the bi-weekly reports together with the country-specific consent rate, which was calculated by the DBS team in Munich (see Section **3**). The survey agencies were explicitly requested to get in touch with under-performing interviewers as SHARE was not only striving for a high participation rate, but also wanted to ensure the collection of high-quality DBS samples and enough blood spots for later marker analyses. Our goal was to detect sampling mistakes in a timely manner and correct them by reminding or retraining respective interviewers. The monitoring of sample quality and quantity involved the staff at

---

[6] see **2.1.1**; for the French respondents

the SHARE biobank, who checked all DBS samples arriving in Odense for visible shortcomings ([Friedel & Weiss, Chapter 6.3.2, in: Malter & Börsch-Supan, A (Eds.) 2017](#)). The biobank staff reported the list of received DBS samples (identified by the attached barcode number) together with quality information collected for each incoming sample to the biomarker team in Munich in a bi-weekly manner as well.

On arrival at the biobank, the following information was recorded for each incoming sample:

- Every possible damage to the Tyvek® mailing envelope containing the PE bag with the DBS filter card (Fig. 6)

- Information on proper humidity protection during shipment:
    - The status of the small PE bag containing the DBS card and desiccant and if it had been properly zip-closed
    - The presence of a desiccant and whether it had been taken out of its own humidity protection, the aluminum pouch

- In case a temperature indicator had been added (only during pretest and in Israel during the main survey), the reported duration of the temperature threshold exceedance was noted. This gave us some insight into temperature exposure of the DBS cards during shipment (see **2.1.4**).

- Information on DBS quality, quantity and any visible damage to the card or blood spot(s), namely
    - Number and quality of blood spots (size, smeared blood or overlapping spots) on the DBS filter card (Fig. 8), providing information on the amount of blood available for analyses for each barcoded sample. In some cases, this information could be used to disentangle erroneously doubled barcode numbers (retrieving incomplete samples; see **4.1**).
    - Imprints of the blood spots on the filter-paper flap covering the blood-spot circles, a sign that the card was closed while the spots were still wet. This indicated a too short drying time before packing the DBS card for shipment.
    - The dates of blood taking (written on the DBS card) and of final freezer storage; these were used to calculate shipment times per sample and country-specific averages (Fig. 8).
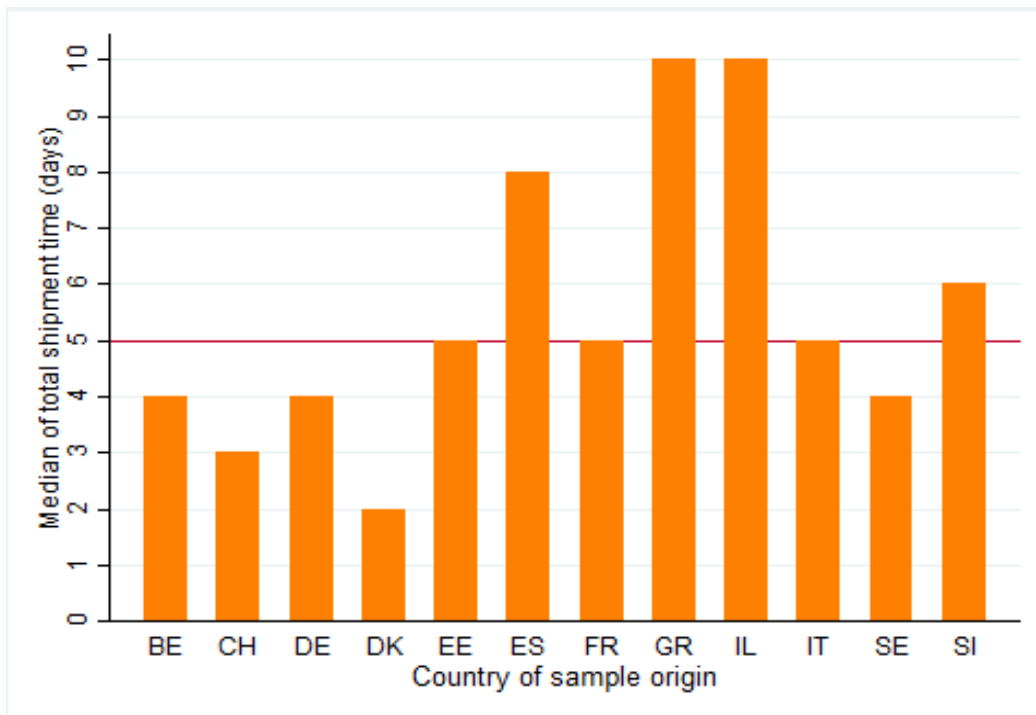
Figure 5: Median shipment time of DBS samples. The Y-axis shows the time (in days), which elapsed between the date the blood was taken (date on the DBS cards) and the date the card had arrived at the biobank in Odense. The horizontal line shows the median shipment time of five days for all countries.

Figure 5 shows the time (in days) which had elapsed between blood sampling (date written on the DBS cards) and arrival of the sample at the biobank in Odense. Yet, shipping time is a result of interviewer performance (how quickly did the interviewer deliver the DBS card to the postal office) and the functioning of the national postal systems (opening hours of postal offices, availability of post-boxes and frequency of being emptied, postal strikes, international delivery). For the summer months, interviewers were instructed to deliver the envelopes with the blood sample directly to a post office instead of dropping them into heat- or sun-exposed mailboxes.

As mentioned in Sections **2.1.4** and **2.1.5**, the interviewers were asked to type the current local outside temperature at the time of the blood-taking into the CAPI. To check the usability of this information and to gain some insight into the temperature exposure of the DBS card during shipment, we had equipped the SHARE Wave 6 pretest DBS kits with temperature indicators (TimeStrip Label 30 and 38°C; Timestrip UK ltd, Cambridge, UK) to be activated by the interviewers and then added to the PE-bag with the DBS card before shipment to the biobank; the Israeli country team acquired temperature indicators during the SHARE Wave 6 main survey DBS collection for temperature monitoring of the DBS samples starting in June 2015 (Shockwatch WarmMark® Long-Run indicator showing temperature exposure of ≥31°C for up to 168 hrs.) Both kinds of strips had to be activated by the interviewers. The temperature-checking experiments are described and evaluated in **2.1.4** above.

The described sample-quality checks provided us with information about the total number of blood spots per card as well as the number of blood spots filling the pre-printed circles (full-size blood spots) and they mentioned smeared (by touching the card) and overlapping DBS (multiple drops on the same spot). In Figure 6 we show examples of DBS samples of various qualities.
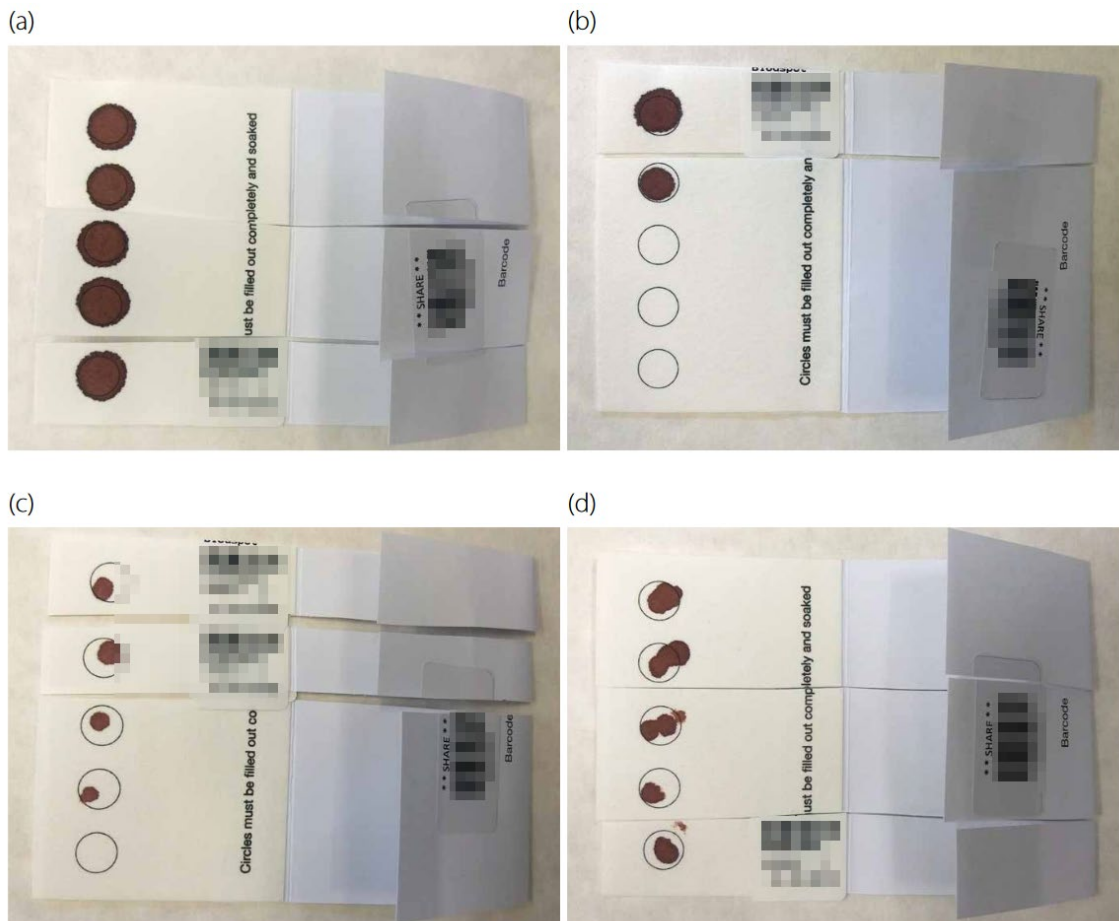
Figure 6: DBS filter cards received at the Biobank. Filter cards arrived with different amounts of blood/number of blood-filled pre-printed circles and in varying quality. (a) This card shows spots of best quality; the amount will allow a large number of analyses. (b) Fine quality but only two spots. (c) Minimal amounts of blood (all spots >> one filled circle). (d) More blood than on the card (c) but the card was touched in circles 1, 2, 3 and drops are overlapping in circles 3, 4, 5; this card is unsuitable for marker analyses except for HbA1c (see **4.2**). After arrival and ahead of freezer storage, cards were cut into fractions. Different fractions were labelled with the same barcode and stored for security reasons in separate freezers. In addition, for punching and analyses the necessary amount of blood (one or two fractions) were used without thawing the entire DBS sample.

3 Participation and consent rates

We calculated two different consent rates (per country and per interviewer):

$$consent\ rate_1 = \frac{N_{consent\ given}}{N_{DBS\ eligible} - N_{prevented\ by\ medical\ reasons}} \qquad (1)$$

$$consent\ rate_2 = \frac{N_{consent\ given}}{N_{DBS\ eligible}} \qquad (2)$$

Consent rate$_1$ is based on eligible DBS respondents, who were able to give consent on their own, but *excludes* those, who mentioned medical reasons preventing them from participating in blood taking. Consent rate$_2$ *adds* those respondents in the denominator, who abstained from participation due to medical reasons. Consent rate$_1$ is therefore always higher than consent rate$_2$ (see orange vs. dark red bars in Fig. 7). We instructed interviewers not to cajole respondents into DBS participation, who had refused due to medical reasons; therefore, consent rate$_1$ may reflect the success of interviewers in consent gaining more precisely. However, consent rate$_2$ is the effective rate of consent among eligible interviews and, hence, the outcome of interest for the project. Not gaining consent from all respondents always bears the risk of biases caused by systematic differences between consenting and non-consenting respondents (Sakshaug, Couper, & Ofstedal, 2010).

The cooperation of all countries participating in the DBS collection is illustrated in Fig. 7. For each country, consent rate$_1$ (orange bars) as well as consent rate$_2$ (dark red bars) are shown. The horizontal line is the mean of the dark bars over all countries with a consent rate$_2$ of 72%. This overall consent rate was very high showing that respondents' and interviewers' acceptance of this new measurement was very good. More than 80% consent was reached by BE, CH, DK, EE, SE, and SI when adjusting for medical reasons which prevented the participation. The calculation of the French consent rates is based on the small, selected DBS-eligible sample of French respondents[7].

The final consent rates in Fig. 7, however, do not describe the large variation between interviewers. This is depicted in the box-plot diagrams of Fig. 8. Some countries have little variation while others predominantly in southern Europe have very large variation in interviewer-specific consent rates. The small variation among the Danish interviewers for example may come from their experience as most of them had collected DBS previously to the SHARE blood collection. Their confidence with the procedure may have helped to confer a positive attitude to the respondents.

---

[7] As described in paragraph **2.2.1**, French panel households in only four districts were eligible for DBS collection. Nevertheless, a certain number of non-eligible respondents were asked and gave consent. The calculation of consent rates in France is based on the actually eligible subsample, while e.g., French numbers of available DBS samples refer to all received DBS cards.
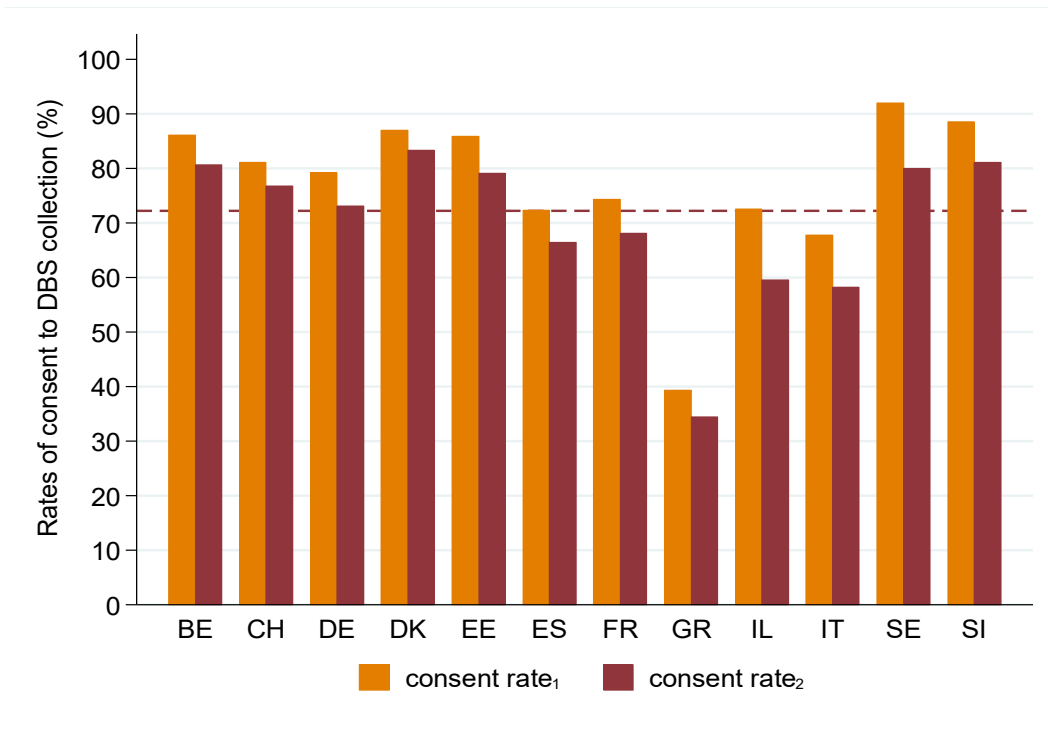
Figure 7: Final rates of consent to DBS taking in the participating countries. Orange bars: consent rate$_1$, see equation (1); dark red bars: consent rate$_2$, see equation (2); dashed line: overall consent rate of 72%.
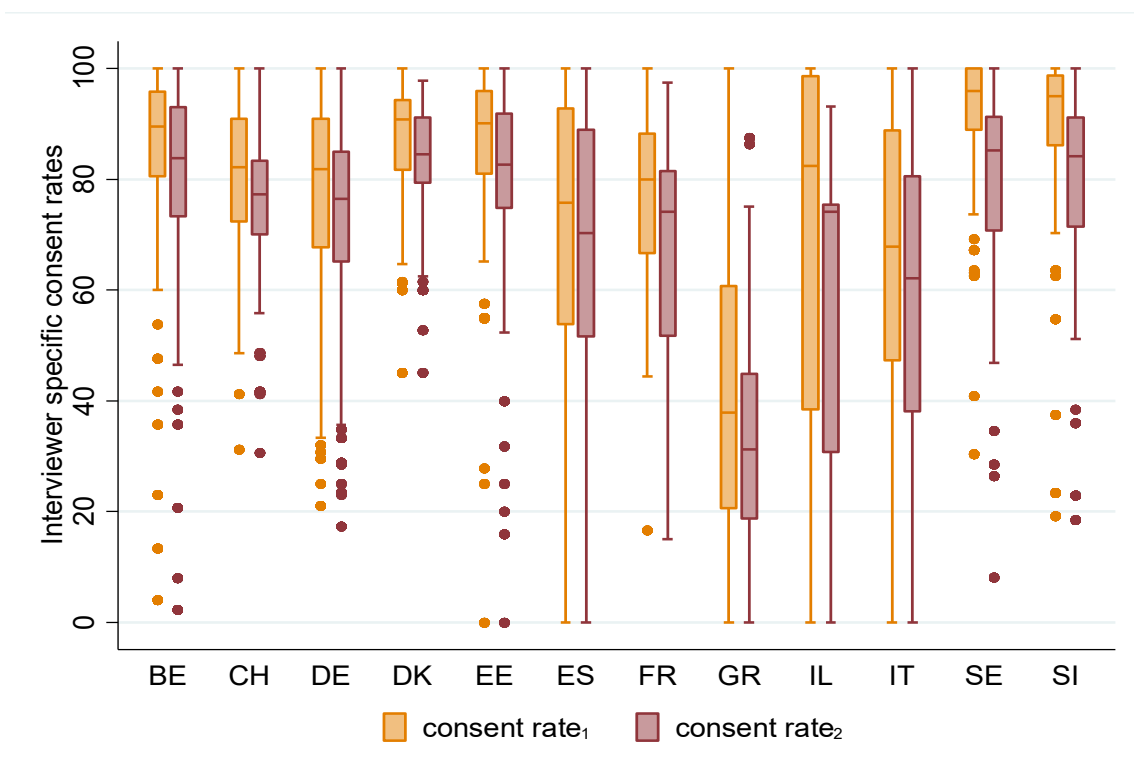
Figure 8: Box-plot diagram of consent to DBS taking in the participating countries. The diagram illustrates the strong involvement of interviewer performance on consent giving. Variation of consent per interviewer (interviewer performance) in southern Europe is much larger than in the North.

4 DBS samples

Overall, ca. 27,200 DBS cards were collected during the main survey of SHARE Wave 6. However, not all of these samples were complete and could be used for final analysis. Three major criteria had to be fulfilled for a SHARE DBS sample to be counted as complete: 1) the consent for blood taking had to be given in writing and the signed consent form was received at the national survey agency; 2) the DBS card was received at the biobank; and 3) a CAPI interview was available; and all three data sources had to be linkable by the same barcode. After fieldwork had finished in all countries, the DBS samples were scrutinized for completeness. Incomplete samples were excluded from laboratory analyses.

4.1 Data cleaning: excluding incomplete, but retrieving apparently incomplete samples

Only samples fulfilling the three criteria for "complete" were assigned for laboratory analysis. Some samples, though, were incomplete only at first glance because they were not linkable to the respective SHARE interview. In many of those cases, this was due to incorrectly entered barcode numbers: the typing errors could have happened when 1) entering the number into the CAPI instrument at the time of the interview or 2) in the lists of DBS samples when the cards were received at the biobank or 3) in the national consent list, when the forms were received at the survey agency. Many of these cases could be solved (and hence, samples could be completed) by considering available information from all data sources. For example, we identified obviously twisted digits or impossible digits like an "8" where the highest possible digit would have been a "3" ([M. Börsch-Supan, Friedel, & Weiss, Chapter 6.4, in: Malter & Börsch-Supan, A (Eds.), 2017](#)).

Another mostly correctable source of mistakes was the double use of a barcode number. This happened if an interviewer used stickers from one barcode sheet (with the same barcode numbers) for more than one respondent. Many of these cases could be retrieved by comparing the interview date on the card with the interview date in the CAPI and/or by comparing the number of blood spots actually on a card (and documented in the biobank list) with the number of collected spots stated in the CAPI.

4.2 Quality of the collected samples – recordable shortcomings and invisible damage

Table 1 shows the numbers of received, complete and usable DBS samples per country. In addition to being complete, a SHARE DBS sample had to meet certain quality requirements. Earlier, in **2.2.3**, we mention the quality features and the amount of blood (number and size of spots), which the biobank team recorded for each individual DBS card arriving in Odense. A vast majority of 26,705 samples were complete according to the three

criteria, but 354 of those had no blood[8], leaving 26,351 complete samples with blood (columns 2 and 3).

We excluded further cards of minor quality with exclusively too small or overlapping or smeared blood spots, which are responsible for the 2528 samples difference between DBS numbers in column 3 and 4 in Table 1); thus, 23,823 DBS samples were left (column 4), which had at least one good blood spot and were usable for analyses.

The card of a single SHARE respondent could have contained varying numbers of blood spots of same or different size, optimally five completely filled pre-printed circles. On many cards, less blood was collected and a substantial share of the usable DBS cards had limited amount of blood (≤ one full circle, see Fig. 6). 452 of those 2528 samples could be used, nevertheless. Overlapping or small spots may be analyzed for HbA1c as this is the ratio of glycosylated versus total hemoglobin and, thereby, independent of the volume contained in a sample (which is not clear in smeared or overlapping spots). Therefore, a total of 24,275 samples were prepared (punched; see **5.2.1**) for analyses.

Several of the visible shortcomings, which were recorded on arrival at the biobank, lead to reduced amount of blood on the DBS card and, therefore, in some cases limited the analyses of markers, in worst cases even excluded entire DBS samples due to smearing, overlapping drops, touched cards, too small spots, too short drying times, forgotten desiccant (see **2.2.3**). They could have been avoidable with better care exercised by the interviewer, though, the field situation and the interplay with the respondent may have forged the result.

Much harder to control, respectively, not recordable, is the "invisible" impact of the shortcomings and the damage caused to the blood markers with an impact on the later to-be-measured marker values in the DBS sample. However, the monitoring by the biobank staff identified possible sources for these kinds of invisible damage: forgotten desiccant, which means a missing humidity protection; long shipment times indicating extended exposure to, e.g., high temperatures in summer. The same is true for environmental conditions, like high outside temperatures and humidity while taking the sample and even more during shipment, as well as unreliability of postal service leading to long shipment times.

---

[8] Complete samples with no blood resulted, when the interviewer attempted to take blood by pricking the finger but the respondent would not bleed. This happened significantly more often with increasing age of the respondent. This is a no-blood result in contrary to a refusal, since consent was given.

	Total number of DBS samples collected	Number of complete DBS samples	Number of complete DBS samples with blood	Complete DBS samples with at least one good-quality blood spot
Belgium	3690	3604	3484	3166
Switzerland	2132	2095	2051	1953
Germany	3147	3124	3095	2872
Denmark	2861	2842	2833	2715
Estonia	3683	3670	3652	3285
Spain	1827	1771	1754	1462
France	578	552	507	429
Greece	807	736	735	580
Israel	1073	1030	1030	821
Italy	2175	2153	2121	1839
Sweden	3013	2907	2893	2600
Slovenia	2242	2221	2196	2101
Total	**27 228**	**26 705**	**26 351**	**23 823**

Table 1: **Number of DBS samples collected by country**. Column 1**: total number of DBS samples received at the biobank;** column 2***: number of samples fulfilling the three criteria for completeness;** column 3**: number of cards received in Odense carrying any amount of blood;** column 4**: number of samples with at least one good-quality blood spot for analyses** (as reported by the biobank staff at receipt of the sample). **Source:** SHARE data release 7.0.0 and internal data on DBS samples

* Including 355 cards with no blood; respondent had consented to blood taking, but interviewer did not manage to collect blood from this person – see footnote 8.

5 Analyzing DBS samples for blood markers of age-related conditions

SHARE had chosen to assay the DBS samples for 18 blood biomarkers (M. Börsch-Supan & Andersen-Ranberg, Chapter 6.1, in: Malter & Börsch-Supan, A (Eds.), 2017). Priority in the selection process was given to their relevance in evidencing typical diseases and conditions at older age, such as CVD, diabetes and cognitive decline. Additional criteria were the existence of suitable assays (i.e. reagents and procedures to detect the biomarker) and the

availability of comparative population values from (inter)national health registers (e.g. the Robert-Koch-Institute in Germany, Eurohealth or WHO) and other population surveys, including HRS and its sister studies. Selection was also guided by the ability to share the same DBS extract across multiple analyses, thereby limiting the amount of blood needed. Two laboratories, the Staten Serum Institute (SSI) in Copenhagen, Denmark, and the Department of Laboratory Medicine, University of Washington (UW) in Seattle, USA, were entrusted with the DBS analyses. Both laboratories have the expertise to analyze the DBS samples for various blood markers and are able to handle such huge amounts of samples. The SHARE DBS samples were apportioned among the two laboratories into two sets of markers according to the laboratories' expertise in analyzing certain markers (see **5.2.1**). Admittedly, splitting DBS for analyses of different blood markers in two different labs on two continents posed a challenge.

5.1 The markers and aging

5.1.1 Routine blood markers - analyses at the Department of Laboratory Medicine in Seattle

The first set contains seven markers used in routine blood analyses. For these markers, reference values from venous blood samples are well established.

Glycosylated hemoglobin (HbA1c or A1c) is a marker for diabetes. An excess of sugar molecules in the blood irreversibly bind to hemoglobin. The so-formed A1c signals longstanding and chronically high levels of blood sugar (e.g., Florkowski, 2013).

High density lipoprotein (HDL) cholesterol, (total) cholesterol (TC) and triglycerides (TG) are molecules of the lipid panel and important players in lipid metabolism, such as serving as building blocks for cells and transport molecules for lipids. Imbalances in lipid metabolism lead to various diseases of the cardiovascular system (e.g., North & Sinclair, 2012; Upadhyay, 2015).

Cystatin C (CysC) is a marker for kidney function. CysC, though a measure for the clearance of degradation products from blood, also signals risk of CVD. Those with elevated CysC levels have been shown to be at highest risk for CVD, even when kidney dysfunction is mild; those with the highest levels of CysC are older and have hypertension, dyslipidemia, high BMI, and higher levels of C-reactive protein (e.g., Levin & Lan, 2016; Sarnak, Katz et al., 2008; Yaffe, Lindquist et al., 2008).

C-reactive protein (CRP) marks the general level of inflammation in the body caused by acute infections or chronic diseases. Inflammatory processes are involved in CVD, diabetes,

chronic kidney disease, obesity, and cognitive decline (e.g., Lassale, Batty et al., 2019; Tang, Fung et al., 2017).

Total hemoglobin (tHb) is a marker of anemia that indicates a decrease in red blood cells or hemoglobin, thereby lowering the ability to carry oxygen in the blood. Anemia may arise from loss of blood, pathological removal of blood cells, diseases of the hematopoietic system, chronic inflammatory diseases, kidney disease, wasting diseases (e.g. cancers) and more (e.g., Goodnough & Schrier, 2014).

5.1.2 Markers indicating risk of cardiovascular disease and cognitive decline – analyses at Staten Serum Institut in Copenhagen

The second set of biomarkers is more innovative and the introduction of cytokine analyses from DBS to assess chronic inflammatory status, CVD and cognitive status in a survey of aging is new (Koelman, Pivovarova-Ramich et al., 2019; Rea, Gibson et al., 2018). Five markers in this set are cytokines, small blood-based proteins prominently involved in inflammatory processes; three are (neuronal) growth factors and two apolipoproteins. Cytokines are not routinely analyzed in a blood count, but in research they offer the opportunity to more closely investigate low-level chronic inflammation, such as in atherosclerosis, to fine-tune the search for the risk of CVD among the survey respondents beyond blood-fat values (such as cholesterol), and to biologically back up the results of objective cognitive testing in the survey questionnaire. The selection and importance of the 10 markers are described by Borbye-Lorenzen and Börsch-Supan (2019).

The five proteins *IL-8, IL-16, IL-18, IL-12/23p40, and MCP-1* indicate inflammation occurring in the body; all molecules are pro-inflammatory markers but act in different tissues. Elevated blood levels of either one or several of these cytokines could better inform us about the type of inflammation and its degree; they may confirm the report of, for example, CVD or atherosclerosis by a respondent or point to yet undetected inflammation, cancer and/or (onset of) cognitive impairment (e.g., Brosseron, Krauthausen et al., 2014; Saleem, Herrmann et al., 2015; Scarabino, Peconi et al., 2020).

Vascular epithelial growth factor (VEGF) and epidermal growth factor (EGF) are proteins involved in angiogenesis and normal and pathologic cell and tissue growth. They are needed in healing (e.g., in blood vessels) but may also indicate proliferating tissue in the case of cancer; studies suggest that angiogenesis (VEGF) may be involved in the onset process of cognitive impairment (L. Huang, Jia, & Liu, 2013; Lange, Storkebaum et al., 2016).

Brain-derived neurotrophic factor (BDNF) is another growth factor. BDNF acts on neurons of the central and peripheral nervous system, supports the survival of neurons and encourages growth of neuronal tissue. BDNF is involved in learning and memory. Its level increases with

exercise and social embeddedness. In turn, the BDNF blood level is lowered in cognitive decline (e.g., Nascimento, Pereira et al., 2015; Salinas, 2016). Other reports relate BDNF to the progression of human cardiovascular disease (e.g., Bahls, Könemann et al., 2019).

Apolipoprotein E (ApoE) belongs to a class of proteins involved in the metabolism of fats in the body and is a component of the lipid panel. ApoE mediates the cholesterol metabolism, transports cholesterol to neurons and is the principal cholesterol carrier to/in the brain. ApoE is important in Alzheimer's and cardiovascular diseases. Carrying the *ApoE-e4* gene variant (allele) influences the lipid/cholesterol metabolism and increases the risk for dementia also lowering the age of onset (e.g., Mooijaart, Berbée et al., 2006; Rasmussen, Tybjærg-Hansen et al., 2015; Safieh, Korczyn, & Michaelson, 2019).

Clusterin (APOJ) has the general vital function in the organism to clear misfolded proteins or cell debris; through this function, clusterin is involved in aging and many diseases related to oxidative stress, including neurodegenerative and inflammatory diseases and cancers (e.g., Baralla, Sotgiu et al., 2015; Hsu, Lee et al., 2017; Jongbloed, van Dijk et al., 2015).

Vitamin D (VitD) is essential for several biological processes. Being deficient is associated with mortality and diseases, among them CVD but also functional loss from lower muscle function and muscle mass (sarcopenia), affecting postural stability. Additionally, osteoporosis can be caused by a low VitD level because it is essential for the absorption of calcium from the diet (e.g., Polly & Tan, 2014).

5.2 Analyses of the SHARE DBS samples

5.2.1 Planning blood-biomarker analyses and preparing the samples

For the analyses of a single marker only a small amount of a blood, less than one full-size DBS filling the pre-printed circle, is needed. This requires that the original DBS collected during the survey has to be further divided, which is done by punching small discs (the punches) with a diameter of 3.2 mm or 1/8 inch from the blood spots (see Fig. 9, and M. Börsch-Supan, Friedel, & Weiss, Chapter 6.4, in: Malter & Börsch-Supan, A (Eds.), 2017). The punching is followed by extraction of the disk with a solvent to yield a liquid sample. Given the differences in quantity and quality between the individual DBS samples collected from the respondents during fieldwork (see Fig. 6), we were aware that we would not be able to perform analyses for all 18 markers from all DBS samples as good-quality DBS of sufficient size (not too small diameter) are required. We spent considerable time carefully planning how to gain as much blood-marker information as possible from all cards, and at the same time cover the list of markers which we intended to analyze with as many samples as possible each. Fortunately, multiple analytes can be measured from a single punch due to

highly sensitive detection methods. DBS-LC-MS/MS (liquid chromatography–mass spectrometry), for example, is such a method, but it also has its limitations in assay sensitivity due to the small sample size, and sample quality is often a concern (Wenkui Li & Tse, 2010); in addition, immunoassays are very sensitive and specific. This method was employed for the cytokine analyses.

For the analyses of all 18 markers described above, five 3.2-mm punches from each DBS sample, not necessarily from one spot, are required (Table 2 and 3; Fig. 9). The 18 markers were divided into three marker groups A, B; C. Markers in a group can be extracted together from the punched blood disc(s) as listed in Table 2; then the analyses for the individual marker follows from aliquots of the extract.

Marker Group	Individual Markers	Number of 3.2 mm discs needed for analyses
A-markers	- HbA1c - total Hemoglobin (tHb) - HDL-Cholesterol	**2**
- or - **HbA1c-only** **(in case of limited material)**	- HbA1c	**1** (can be taken from an overlapping or smeared blood spot)
B-markers	- total Cholesterol (TC) - Triglycerides (TRG) - C-reactive protein (CRP) - Cystatin C (CysC)	**1**
C-markers	IL-16, IL-8, IL-12/23, IL-18, MCP1; VEGF, EGF, BDNF, Clusterin, ApoE4-protein.	**2**
Vitamin D	Vitamin D	Remaining extract frozen; not yet analyzed

Table 2: Markers/marker groups analyzed from SHARE DBS. A- and B-marker were analyzed at UW in Seattle; C-marker and Vitamin-D analyses were assigned to SSI in Copenhagen.

The assays of the seven "routine blood markers" at UW required three punches: two for the analyses of the markers HbA1c, HDL and THb (A-marker group) and only one for TC, TRG, CRP, and CysC (B-marker group). Analyses were conducted according to published techniques: HbA1c (Egier, Keys et al., 2011), tHb (Frenchik, Tsonev, & McFaul, 2004), TC (Lakshmy, Gupta et al., 2010; Lakshmy, Mathur et al., 2012), HDL (Arranz-Peña, Tasende-Mata, & Martin-Gil, 1998; Y.-C. Huang, Kao, & Tsai, 1997), TRG / TG (Lakshmy, Gupta et al., 2010; Quraishi, Lakshmy et al., 2006), high-sensitivity CRP (Brindle, Fujita et al., 2010; McDade, Burhop, & Dohnal, 2004), and CysC (Vogl, 2014). For the analyses of multiple

markers from the extracts of only one or two punches, UW has developed SHARE-specific (in-house) assays based on the above-mentioned references.

Possible # of punches per DBS sample	Punch 1	Punch 2	Punch 3	Punch 4	Punch 5
5 and more	C	C	A	A	B
4	C	C	A*	B	
3	A	A	B		
2	A*	B			
1	B or A* (depending on spot quality)				

Table 3: Blood amount in a DBS sample and assignment for marker analyses. With large enough samples (first row), the five punches necessary for the analyses of 18 markers could be made. Limited amount of blood i.e., few or one DBS on a card and/or blood spots of small diameter also limit the number of punches. A choice for the markers to be analyzed has to be made. Punching was conducted according to the scheme presented in this table. Therefore, not every DBS sample could be analyzed for all markers.

The analyses of the second marker group at SSI with the cytokines and vitamin D required further two punches for the simultaneous analysis of 10 markers with a multiplex immunoassay; vitamin D will be analyzed in a separate assay from the extract.

As cytokines and growth factors usually circulate in the body at very low concentrations, detecting them requires a highly sensitive method such as immunoassays. Modern multiplex approaches analyze multiple markers from very small amounts of blood, such as DBS (Borbye-Lorenzen & Börsch-Supan, 2019; Skogstrand, 2012; Skogstrand, Thorsen et al., 2005).
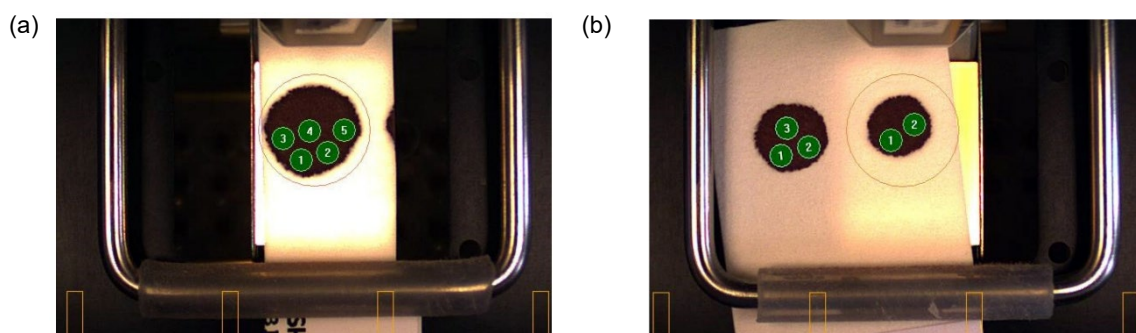
Figure 9: DBS cards in the puncher. Cards are turned over for aiming at the punch spot to place the punch at a fully soaked section of the DBS. While punching the DBS samples, the semi-automated robot punching system (Panthera Puncher 9, Perkin Elmer, Waltham, Ma, USA) used at SSI assigned the punch location and took a photo of the blood spot. This provided us with precise information of size and quality of all punched DBS samples. (a): Some, yet few, spots were large enough to gain all five punches needed for the SHARE assays from a single DBS. More frequently the punches for a DBS sample had to be taken from two or more (smaller) blood spots.

The subdivision – the punching – of the DBS samples (blood amount on an individual DBS filter card) was carried out at SSI in Copenhagen. In general: before the punching starts with a semi-automated robot punching system (Panthera Puncher 9, Perkin Elmer), the DBS cards have to be hand-sorted for the punching of the respective marker groups (see Table 3). Then, the sample is registered in the system by scanning the respondent-specific barcode. The card is manually positioned in the puncher; the 3.2mm disc is cut by the robot from the flipside[9], and the punch drops into the well of a standard 96-deep-well microtiter plate (Fig. 10). Each well of a microtiter plate contains one, respectively two punches out of the sample of a different respondent; the barcode scanning of the robot creates an electronic plate map and facilitates the later linkage of the analyses result to the interview data of a respondent. For the analyses of the A-markers, for example, two discs are cut and the wells contain two punches. Then the B-/C-marker punch(es) are cut into other plates.
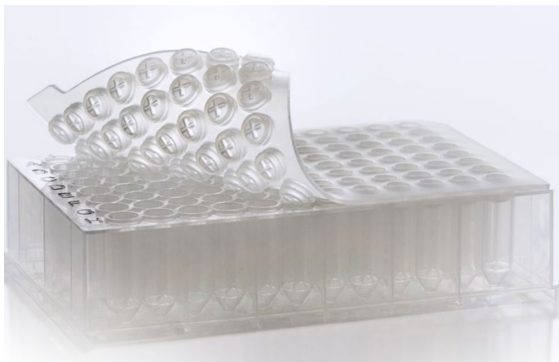

Figure 10: 96-well microtiter plate with cover.

The procedure is repeated until 72 or 76 wells of a plate are filled (UW uses 24 and SSI 20 wells for controls with known marker levels). Now the plate receives an identifying number and is sealed with a cover to prevent the discs from falling out or getting mixed during handling and future transport of the sample-punches to the laboratories.
The plates were frozen at – 20°C until shipment of the A- and B-marker plates from Copenhagen to Seattle or in-house analyses of the C-markers at SSI.
The punching of all ca. 24,000 SHARE Wave 6 main survey DBS samples lasted from November 2016 till May 2018.

---

[9] After falling on the DBS card, the blood drop spreads laterally and vertically (soaking) in the paper. At the fringes of blood spot the vertical spread, the soaking, may not be as complete as in the middle, i.e., the diameter on the flipside may be slightly smaller than on top. Therefore, the filter card is turned and the punch is cut from the flipside to create a homogenously soaked punch.

5.2.2 Analyzing the SHARE DBS samples

A random subsample of ca. 8000 samples was assayed in Seattle between October 2017 and May 2018 for the A- and B-markers listed in Table 2. At the time of writing, May 2020, analyses of the remaining A- and B-marker samples, ca. 16,000, has started in Seattle. Analyses for all SHARE samples, which had been punched for C-markers (ca. 16,000), started at SSI in June 2018 and were finished end of August the same year. The C-markers were analyzed in a multiplex (10plex) immunoassay. For this purpose, a custom-designed SHARE analysis kit was assembled by SSI and the company MesoScale Diagnostics, Maryland, USA.

5.3 Adjusting for varying field conditions

In awareness of the influence of field conditions and sample quality on the DBS analyte values, in 2018, we performed controlled laboratory experiments at UW by systematically exposing DBS to adverse fieldwork conditions. DBS and venous blood was collected from 20 volunteers; 3420 outcomes were used to create an equation to convert analyte values obtained from DBS samples into values we would have obtained had it been feasible to collect the donors' venous plasma or wet blood samples by standard analytical methods ([A. Börsch-Supan, Weiss et al., 2020](#)). We applied the obtained equation on a dataset from a small field experiment conducted during the SHARE Wave 6 pretest in Poland: nurses collected DBS samples and venous blood samples from 37 respondents and we observed a good convergence to the respondent-matched venous blood sample values; the experiment is described in ([Weiss, Börsch-Supan et al., Chapter 38, in: Börsch-Supan, A et al. (Eds.), 2019](#)).

A comparable experiment investigating fieldwork condition effects was performed in Odense in 2019 with 45 patients from Odense University Hospital Falls Clinic and 15 healthy controls. For the C-markers we aim to adjust the values for field influences; as no reference values are available for those markers, it is not necessary to convert the DBS values to venous blood values.

6 Summary and Outlook

SHARE has collected 27,213 DBS samples from respondents in 12 countries as part of its sixth wave. This was the largest DBS sampling from a representative adult population in Europe. In addition, a small field validation experiment was performed in Poland in 2016, collecting DBS and venous blood from 37 respondents by a nurse. A total of 23,823 samples fulfilled the SHARE quality standards for assaying. 8068 samples are already analyzed for

HbA1c, tHb, TC, HDL, TRG, CRP, and CysC. The analyses of the remaining ca. 16,000 samples have been initiated at UW. After completion of all analyses, the raw data will be converted according to the validation experiment described above.

In 2018, Staten Serum Institut in Copenhagen analyzed 15,977 samples for the cytokines IL-8, IL-16, IL-18, IL-12/23p40, and MCP-1, the growth factors BDNF (neuronal), VEGF and EGF, and the apolipoproteins APOe4 and APOJ. The necessary correction for field conditions will be made once the adjustment experiment performed in Odense is evaluated.

SHARE data is free for users with proof of a scientific affiliation; this also applies to biomarker and biomeasure data. The DBS data will be available after completion of the analyses and data conversion. SHARE will publish on its home page (www.share-project.org) once the DBS data will be available.

References

Arranz-Peña, M.-L., Tasende-Mata, J., & Martin-Gil, F. J. (1998). Comparison of two homogeneous assays with a precipitation method and an ultracentrifugation method for the measurement of HDL-cholesterol. *Clinical chemistry, 44*(12), 2499-2505.

Bahls, M., Könemann, S., Markus, M. R., Wenzel, K., Friedrich, N., Nauck, M., Völzke, H., Steveling, A., Janowitz, D., & Grabe, H.-J. (2019). Brain-derived neurotrophic factor is related with adverse cardiac remodeling and high NTproBNP. *Scientific reports, 9*(1), 1-9.

Baralla, A., Sotgiu, E., Deiana, M., Pasella, S., Pinna, S., Mannu, A., Canu, E., Sotgiu, G., Ganau, A., & Zinellu, A. (2015). Plasma clusterin and lipid profile: a link with aging and cardiovascular diseases in a population with a consistent number of centenarians. *PLoS One, 10*(6), e0128029.

Bergmann, M., Scherpenzeel, A., & Börsch-Supan, A. (Eds.). (2019). *SHARE Wave 7 Methodology: Panel innovations and life histories*. Munich: Munich Center for the Economics of Aging and the Max Planck Institute for Social Law and Social Policy.

Blane, D., Netuveli, G., & Bartley, M. (2007). Does quality of life at older ages vary with socio-economic position? *Sociology, 41*(4), 717-726.

Boekholdt, S. M., & Stroes, E. S. (2012). The interleukin-6 pathway and atherosclerosis. *The Lancet, 379*(9822), 1176-1178.

Borbye-Lorenzen, N., & Börsch-Supan, M. (2019). Identification of Cytokine and Lipoprotein Markers for Analyses in SHARE Wave 6 Dried Blood Spots. In A. Börsch-Supan, J. Bristle, K. Andersen-Ranberg, A. Brugiavini, F. Jusot, H. Litwin, & G. Weber (Eds.), *Health and Socio–economic Status Over the Life Course: First results from SHARE Waves 6 and 7* (pp. 375-384). Berlin: De Gruyter.

Börsch-Supan, A. (2013). Myths, scientific evidence and economic policy in an aging world. *The Journal of the Economics of Ageing, 1*, 3-15.

Börsch-Supan, A., Brandt, M., Hank, K., & Schröder, M. (Eds.). (2011). *The Individual and the Welfare State: Life Histories in Europe*. Heidelberg: Springer Science & Business Media.

Börsch-Supan, A., Brandt, M., Hunkler, C., Kneip, T., Korbmacher, J., Malter, F., Schaan, B.,

Stuck, S., & Zuber, S. (2013). Data resource profile: the Survey of Health, Ageing and Retirement in Europe (SHARE). *International journal of epidemiology, 42*(4), 992-1001.

Börsch-Supan, A., Brandt, M., & Schröder, M. (2013). SHARELIFE—One century of life histories in Europe. *Advances in Life Course Research, 18*(1), 1-4.

Börsch-Supan, A., Bristle, J., Andersen-Ranberg, K., Brugiavini, A., Jusot, F., Litwin, H., & Weber, G. (Eds.). (2019). *Health and Socio-Economic Status over the Life Course. First Results from SHARE Waves 6 and 7*. Berlin: De Gruyter.

Börsch-Supan, A., Brugiavini, A., Jürges, H., Kaypteyn, A., Mackenbach, J., Siegrist, J., & Weber, G. (Eds.). (2008). *First results from the Survey of Health, Ageing and Retirement in Europe (2004-2007) Starting the longitudinal dimension*. Mannheim: Mannheim Research Institute for the Economics of Aging (MEA).

Börsch-Supan, A., Brugiavini, A., Jürges, H., Mackenbach, J., Siegrist, J., & Weber, G. (Eds.). (2005). *Health, ageing and retirement in Europe – First results from the Survey of Health, Ageing and Retirement in Europe*. Mannheim: Mannheim Research Institute for the Economics of Aging (MEA).

Börsch-Supan, A., & Jürges, H. (Eds.). (2005). *The Survey of Health, Aging, and Retirement in Europe—Methodology*. Mannheim: Mannheim Research Institute for the Economics of Aging (MEA).

Börsch-Supan, A., Kneip, T., Litwin, H., Myck, M., & Weber, G. (Eds.). (2015). *Ageing in Europe -Supporting Policies for an Inclusive Society*. Berlin: De Gruyter.

Börsch-Supan, A., Weiss, L. M., Börsch-Supan, M., Potter, A., Cofferen, J., & Kerschner, E. (2020). *Dried Blood Spot Collection, sample quality and fieldwork conditions: structured validations for conversion into standard values*. Submitted for publication.

Börsch-Supan, M., & Andersen-Ranberg, K. (2017). Background - why collect Dried Blood Spot (DBS) samples. In F. Malter & A. Börsch-Supan (Eds.), *SHARE Wave 6: Panel Innovations and collecting Dried Blood Spots* (pp. 163-168). Munich: Munich Center for the Economics of Aging (MEA).

Börsch-Supan, M., Friedel, S., & Weiss, L. M. (2017). Analysis of the samples – status quo and further tasks. In F. Malter & A. Börsch-Supan (Eds.), *SHARE Wave 6: Panel Innovations and collecting Dried Blood Spots* (pp. 182-187). Munich: Munich Center for the Economics of Aging (MEA).

Börsch-Supan, A., Weiss, L., Börsch-Supan, M., Potter, A., Cofferen, J., & Kerschner, E. (2019). *Comparing Health in Europe based on Dried Blood Spot Samples*. Paper presented at the NIA-sponsored Biomarker Network Meeting, Austin, TX, USA.

Bound, J. (1991). Self-Reported versus Objective Measures of Health in Retirement Models. *Journal of Human Resources, 16*(1), 106-138.

Brindle, E., Fujita, M., Shofer, J., & O'Connor, K. A. (2010). Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *Journal of immunological methods, 362*(1-2), 112-120.

Brindle, E., O'Connor, K. A., & Garrett, D. A. (2014). Applications of dried blood spots in general human health studies. In L. Wenkui & M. S. Lee (Eds.), *Dried blood spots: Applications and techniques* (Vol. 1, pp. 114-129). Hoboken, NJ, USA: John Wiley & Sons, Inc.

Brosseron, F., Krauthausen, M., Kummer, M., & Heneka, M. T. (2014). Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. *Molecular neurobiology, 50*(2), 534-544.

Brüünsgaard, H., & Pedersen, B. K. (2003). Age-related inflammatory cytokines and disease. *Immunology and Allergy Clinics, 23*(1), 15-39.

Chang, A. Y., Skirbekk, V. F., Tyrovolas, S., Kassebaum, N. J., & Dieleman, J. L. (2019). Measuring population ageing: an analysis of the global burden of disease study 2017. *The Lancet Public Health, 4*(3), e159-e167.

Conti, G., & Heckman, J. J. (2013). The developmental approach to child and adult health. *Pediatrics, 131*(Supplement 2), 133-141.

Crimmins, E. M., Guyer, H., Langa, K., Ofstedal, M. B., Wallace, R., & Weir, D. (2008). Documentation of Biomarkers in the Health and Retirement Study. *Ann Arbor: Survey Research Center*.

Crimmins, E. M., Kim, J. K., McCreath, H., Faul, J., Weir, D., & Seeman, T. (2014). Validation of blood-based assays using dried blood spots for use in large population studies. *Biodemography and social biology, 60*(1), 38-48.

Crimmins, E. M., Zhang, Y. S., Kim, J. K., Frochen, S., Kang, H., Shim, H., Ailshire, J., Potter, A., Cofferen, J., & Faul, J. (2020). Dried blood spots: Effects of less than optimal collection, shipping time, heat, and humidity. *American Journal of Human Biology*, e23390.

Daskalopoulou, C., Stubbs, B., Kralj, C., Koukounari, A., Prince, M., & Prina, A. M. (2017). Physical activity and healthy ageing: A systematic review and meta-analysis of longitudinal cohort studies. *Ageing research reviews, 38*, 6-17.

Deaton, A. (2002). Policy implications of the gradient of health and wealth. *Health affairs, 21*(2), 13-30.

Doiron, D., Fiebig, D. G., Johar, M., & Suziedelyte, A. (2015). Does self-assessed health measure health? *Applied Economics, 47*(2), 180-194.

Dregan, A., Stewart, R., & Gulliford, M. C. (2013). Cardiovascular risk factors and cognitive decline in adults aged 50 and over: a population-based cohort study. *Age and Ageing, 42*(3), 338-345.

Dugravot, A., Fayosse, A., Dumurgier, J., Bouillon, K., Rayana, T. B., Schnitzler, A., Kivimaki, M., Sabia, S., & Singh-Manoux, A. (2020). Social inequalities in multimorbidity, frailty, disability, and transitions to mortality: a 24-year follow-up of the Whitehall II cohort study. *The Lancet Public Health, 5*(1), e42-e50.

Dwyer-Lindgren, L., Bertozzi-Villa, A., Stubbs, R. W., Morozoff, C., Mackenbach, J. P., van Lenthe, F. J., Mokdad, A. H., & Murray, C. J. (2017). Inequalities in life expectancy among US counties, 1980 to 2014: temporal trends and key drivers. *JAMA internal medicine, 177*(7), 1003-1011.

Egier, D. A., Keys, J. L., Hall, S. K., & McQueen, M. J. (2011). Measurement of hemoglobin A1c from filter papers for population-based studies. *Clinical chemistry, 57*(4), 577-585.

European Commission. (2015). *The 2015 Ageing Report*. Retrieved from Brussels: https://ec.europa.eu/economy_finance/publications/european_economy/2015/pdf/ee3_en.pdf

Eyles, D., Anderson, C., Ko, P., Jones, A., Thomas, A., Burne, T., Mortensen, P. B., Nørgaard-Pedersen, B., Hougaard, D. M., & McGrath, J. (2009). A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots. *Clinica chimica acta, 403*(1-2), 145-151.

Fagundes, C. P., Bennett, J. M., Derry, H. M., & Kiecolt-Glaser, J. K. (2011). Relationships and inflammation across the lifespan: Social developmental pathways to disease. *Social and personality psychology compass, 5*(11), 891-903.

Florkowski, C. (2013). HbA1c as a diagnostic test for diabetes mellitus–reviewing the evidence. *The Clinical Biochemist Reviews, 34*(2), 75.

Frenchik, M. D., Tsonev, L. I., & McFaul, S. J. (2004). A microplate assay for the determination of hemoglobin concentration. *Clinica chimica acta, 1*(339), 199-201.

Friedel, S., & Weiss, L. (2017). Fieldwork monitoring. In F. Malter & A. Börsch-Supan (Eds.), *SHARE Wave 6: Panel Innovations and collecting Dried Blood Spots* (pp. 177-179). Munich: Munich Center for the Economics of Aging (MEA).

Goodnough, L. T., & Schrier, S. L. (2014). Evaluation and management of anemia in the elderly. *American journal of hematology, 89*(1), 88-96.

Gruenewald, T. L., Seeman, T. E., Ryff, C. D., Karlamangla, A. S., & Singer, B. H. (2006). Combinations of biomarkers predictive of later life mortality. *Proceedings of the National Academy of Sciences, 103*(38), 14158-14163.

Health, S. N. I. o. P. (2006). *Healthy Ageing - A Challenge for Europe*. Retrieved from http://www.healthyageing.eu/sites/www.healthyageing.eu/files/resources/Healthy%20Ageing%20-%20A%20Challenge%20for%20Europe.pdf

Hsu, J.-L., Lee, W.-J., Liao, Y.-C., Wang, S.-J., & Fuh, J.-L. (2017). The clinical significance of plasma clusterin and Aβ in the longitudinal follow-up of patients with Alzheimer's disease. *Alzheimer's Research & Therapy, 9*(1), 91.

Huang, L., Jia, J., & Liu, R. (2013). Decreased serum levels of the angiogenic factors VEGF

and TGF-β1 in Alzheimer's disease and amnestic mild cognitive impairment. *Neuroscience Letters, 550*, 60-63.

Huang, Y.-C., Kao, J.-T., & Tsai, K.-S. (1997). Evaluation of two homogeneous methods for measuring high-density lipoprotein cholesterol. *Clinical chemistry, 43*(6), 1048-1055.

Jongbloed, W., van Dijk, K. D., Mulder, S. D., van de Berg, W. D., Blankenstein, M. A., van der Flier, W., & Veerhuis, R. (2015). Clusterin levels in plasma predict cognitive decline and progression to Alzheimer's disease. *Journal of Alzheimer's Disease, 46*(4), 1103-1110.

Jürges, H. (2007). True health vs response styles: exploring cross-country differences in self-reported health. *Health economics, 16*(2), 163-178.

Jylhä, M. (2009). What is self-rated health and why does it predict mortality? Towards a unified conceptual model. *Social science & medicine, 69*(3), 307-316.

Kalyani, R. R., Golden, S. H., & Cefalu, W. T. (2017). Diabetes and aging: unique considerations and goals of care. *Diabetes Care, 40*(4), 440-443.

Kivimäki, M., Batty, G. D., Pentti, J., Shipley, M. J., Sipilä, P. N., Nyberg, S. T., Suominen, S. B., Oksanen, T., Stenholm, S., Virtanen, M., Marmot, M. G., Singh-Manoux, A., Brunner, E. J., Lindbohm, J. V., Ferrie, J. E., & Vahtera, J. (2020). Association between socioeconomic status and the development of mental and physical health conditions in adulthood: a multi-cohort study. *The Lancet Public Health, 5*(3), e140-e149.

Koelman, L., Pivovarova-Ramich, O., Pfeiffer, A. F., Grune, T., & Aleksandrova, K. (2019). Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation. *Immunity & Ageing, 16*(1), 11.

Korbmacher, J. M. (2014). Interviewer effects on respondents' willingness to provide blood samples in SHARE. *SHARE Working Paper Series, 20*.

Lakshmy, R., Gupta, R., Prabhakaran, D., Snehi, U., & Reddy, K. S. (2010). Utility of Dried Blood Spots for Measurement of Cholesterol and Triglycerides in a Surveillance study. *Journal of Diabetes Science and Technology, 4*(2), 258-262.

Lakshmy, R., Mathur, P., Gupta, R., Shah, B., Anand, K., Mohan, V., Desai, N. G., Mahanta, J., Joshi, P. P., & Thankappan, K. R. (2012). Measurement of cholesterol and triglycerides from a dried blood spot in an Indian Council of Medical Research–World Health Organization multicentric survey on risk factors for noncommunicable diseases in India. *Journal of clinical lipidology, 6*(1), 33-41.

Lange, C., Storkebaum, E., De Almodóvar, C. R., Dewerchin, M., & Carmeliet, P. (2016). Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nature Reviews Neurology, 12*(8), 439-454.

Lassale, C., Batty, G. D., Steptoe, A., Cadar, D., Akbaraly, T. N., Kivimäki, M., & Zaninotto, P. (2019). Association of 10-Year C-reactive Protein Trajectories With Markers of Healthy Aging: Findings from the English Longitudinal Study of Aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 74*(2), 195-203.

Levin, A., & Lan, J. H. (2016). Cystatin C and cardiovascular Disease: Causality, Association, and Clinical Implications of Knowing the Difference. *Journal of the American College of Cardiology, 68*(9), 946-948.

Lewer, D., Jayatunga, W., Aldridge, R. W., Edge, C., Marmot, M., Story, A., & Hayward, A. (2020). Premature mortality attributable to socioeconomic inequality in England between 2003 and 2018: an observational study. *The Lancet Public Health, 5*(1), e33-e41.

Li, W., & Huang, E. (2016). An update on type 2 diabetes mellitus as a risk factor for dementia. *Journal of Alzheimer's Disease, 53*(2), 393-402.

Li, W., & Lee, M. S. (2014). *Dried blood spots: Applications and Techniques*. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Li, W., & Tse, F. L. (2010). Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. *Biomedical Chromatography, 24*(1), 49-65.

Mackenbach, J. P. (2012). The persistence of health inequalities in modern welfare states: the explanation of a paradox. *Social science & medicine, 75*(4), 761-769.

Malter, F., & Börsch-Supan, A. (Eds.). (2013). *SHARE Wave 4: Innovations & Methodology*. Munich: Munich Center for the Economics of Aging (MEA).

Malter, F., & Börsch-Supan, A. (Eds.). (2015). *SHARE Wave 5: Innovations & Methodology*. Munich: Munich Center for the Economics of Aging (MEA).

Malter, F., & Börsch-Supan, A. (Eds.). (2017). *SHARE Wave 6: Panel innovations and collecting Dried Blood Spots*. Munich: Munich Center for the Economics of Aging (MEA).

Malter, F., & Sand, G. (2017). Fieldwork monitoring and survey participation in sixth wave of SHARE. In F. Malter & A. Börsch-Supan (Eds.), *SHARE Wave 6: Panel innovations and collecting Dried Blood Spots*. Munich: Munich Center for the Economics of Aging (MEA).

Marmot, M. (2002). The influence of income on health: views of an epidemiologist. *Health affairs, 21*(2), 31-46.

Marques, A., Peralta, M., Sarmento, H., Martins, J., & González Valeiro, M. (2018). Associations between vigorous physical activity and chronic diseases in older adults: a study in 13 European countries. *European Journal of Public Health, 28*(5), 950-955.

Mathers, C. D., Stevens, G. A., Boerma, T., White, R. A., & Tobias, M. I. (2015). Causes of international increases in older age life expectancy. *The Lancet, 385*(9967), 540-548.

McDade, T. W., Burhop, J., & Dohnal, J. (2004). High-Sensitivity Enzyme Immunoassay for C-Reactive Protein in Dried Blood Spots. *Clinical chemistry, 50*(3), 652-654.

McDade, T. W., Williams, S., & Snodgrass, J. J. (2007). What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography, 44*(4), 899-925.

Mei, J. V., Alexander, J. R., Adam, B. W., & Hannon, W. H. (2001). Use of filter paper for the collection and analysis of human whole blood specimens. *The Journal of nutrition, 131*(5), 1631S-1636S.

Mooijaart, S. P., Berbée, J. F., Van Heemst, D., Havekes, L. M., De Craen, A. J., Slagboom, P. E., Rensen, P. C., & Westendorp, R. G. (2006). ApoE plasma levels and risk of cardiovascular mortality in old age. *PLoS Med, 3*(6), e176.

Nascimento, C. M. C., Pereira, J. R., Pires de Andrade, L., Garuffi, M., Ayan, C., Kerr, D. S., Talib, L. L., Cominetti, M. R., & Stella, F. (2015). Physical exercise improves peripheral BDNF levels and cognitive functions in mild cognitive impairment elderly with different bdnf Val66Met genotypes. *Journal of Alzheimer's Disease, 43*(1), 81-91.

North, B. J., & Sinclair, D. A. (2012). The intersection between aging and cardiovascular disease. *Circulation research, 110*(8), 1097-1108.

Palmefors, H., DuttaRoy, S., Rundqvist, B., & Börjesson, M. (2014). The effect of physical activity or exercise on key biomarkers in atherosclerosis–a systematic review. *Atherosclerosis, 235*(1), 150-161.

Pedersen, L., Andersen-Ranberg, K., Hollergaard, M., & Nybo, M. (2017). Quantification of multiple elements in dried blood spot samples. *Clinical biochemistry, 50*(12), 703-709.

Polly, P., & Tan, T. C. (2014). The role of vitamin D in skeletal and cardiac muscle function. *Frontiers in Physiology, 5*, 145.

Power, C., & Kuh, D. (2006). Life course development of unequal health. In J. Siegrist & M. Marmot (Eds.), *Social Inequalities in Health: New evidence and policy implications* (pp. 77-115). London: Oxford University Press.

Prince, M. J., Wu, F., Guo, Y., Robledo, L. M. G., O'Donnell, M., Sullivan, R., & Yusuf, S. (2015). The burden of disease in older people and implications for health policy and practice. *The Lancet, 385*(9967), 549-562.

Quraishi, R., Lakshmy, R., Prabhakaran, D., Mukhopadhyay, A. K., & Jailkhani, B. (2006). Use of filter paper stored dried blood for measurement of triglycerides. *Lipids in health and disease, 5*(1), 20-22.

Rasmussen, K. L., Tybjærg-Hansen, A., Nordestgaard, B. G., & Frikke-Schmidt, R. (2015). Plasma Levels of Apolipoprotein E and Risk of Dementia in the General Population. *Annals of neurology, 77*(2), 301-311.

Rea, I. M., Gibson, D. S., McGilligan, V., McNerlan, S. E., Alexander, H. D., & Ross, O. A. (2018). Age and age-related diseases: role of inflammation triggers and cytokines. *Frontiers in immunology, 9*, 586.

Ridker, P. M. (2007). Inflammatory biomarkers and risks of myocardial infarction, stroke,

diabetes, and total mortality: implications for longevity. *Nutrition reviews, 65*(suppl_3), S253-S259.

Safieh, M., Korczyn, A. D., & Michaelson, D. M. (2019). ApoE4: an emerging therapeutic target for Alzheimer's disease. *BMC medicine, 17*(1), 1-17.

Sakshaug, J. W., Couper, M. P., & Ofstedal, M. B. (2010). Characteristics of Physical Measurement Consent in a Population-Based Survey of Older Adults. *Medical care, 48*(1), 64-71.

Saleem, M., Herrmann, N., Swardfager, W., Eisen, R., & Lanctot, K. L. (2015). Inflammatory Markers in Mild Cognitive Impairment: A Meta-Analysis. *Journal of Alzheimer's Disease, 47*(3), 669-679.

Salinas, J. (2016). Social Relationships, Serum Brain-Derived Neurotrophic Factor, and the Risk for Stroke and Dementia: The Framingham Heart Study (P1. 098). *Neurology, 86*(16 Supplement).

Sarnak, M. J., Katz, R., Fried, L. F., Siscovick, D., Kestenbaum, B., Seliger, S., Rifkin, D., Tracy, R., Newman, A. B., & Shlipak, M. G. (2008). Cystatin C and aging success. *Archives of internal medicine, 168*(2), 147-153.

Scarabino, D., Peconi, M., Broggio, E., Gambina, G., Maggi, E., Armeli, F., Mantuano, E., Morello, M., Corbo, R., & Businaro, R. (2020). Relationship between proinflammatory cytokines (Il-1beta, Il-18) and leukocyte telomere length in mild cognitive impairment and Alzheimer's disease. *Experimental Gerontology, 136*, 110945.

Schmidutz, D. (2016). Synopsis of Policy-Rules for Collecting Biomarkers in Social Surveys – Field Report on the Collection of Dried Blood Spot Samples in SHARE. *Deliverable D6.10 of the SERISS project funded under the European Union's Horizon 2020 Research and Innovation Programme GA No: 654221*.

Schmidutz, D., Ryan, L., Müller Gjesdal, A., & De Smedt, K. (2013). Report about New IPR Challenges: Identifying Ethics and Legal Challenges of SSH Research. *Deliverable D6.1 of the DASISH project funded under the European Union's Seventh Framework Programme GA No: 283646*.

Schmidutz, D., & Weiss, L. (2017). Preparing the Inclusion of Dried Blood Spots in SHARE - legal, ethical and organisational aspects. In F. Malter & A. Börsch-Supan (Eds.), *SHARE Wave 6: Panel innovations and collecting Dried Blood Spots*. Munich: Munich Center for the Economics of Aging.

Sen, A. (2002). Health: perception versus observation - Self reported morbidity has severe limitations and can be extremely misleading. *British Medical Journal, 324*, 860-861.

Skogstrand, K. (2012). Multiplex assays of inflammatory markers, a description of methods and discussion of precautions–Our experience through the last ten years. *Methods, 56*(2), 204-212.

Skogstrand, K., Thorsen, P., Nørgaard-Pedersen, B., Schendel, D. E., Sørensen, L. C., & Hougaard, D. M. (2005). Simultaneous Measurement of 25 Inflammatory Markers and Neurotrophins in Neonatal Dried Blood Spots by Immunoassay with xMAP Technology. *Clinical chemistry, 51*(10), 1854-1866.

Skoog, I., Lernfelt, B., Landahl, S., Palmertz, B., Andreasson, L.-A., Nilsson, L., Persson, G., Odén, A., & Svanborg, A. (1996). 15-year longitudinal study of blood pressure and dementia. *The Lancet, 347*(9009), 1141-1145.

Smith, J. P. (2003). Unraveling the SES-Health Connection. *The Institute for Fiscal Studies: Working Paper WP04/02*.

Stampfer, M. (2006). Cardiovascular disease and Alzheimer's disease: common links. *Journal of internal medicine, 260*(3), 211-223.

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A Meta-Analysis of Cytokines in Alzheimer's Disease. *Biological psychiatry, 68*(10), 930-941.

Tang, Y., Fung, E., Xu, A., & Lan, H. Y. (2017). C-reactive protein and ageing. *Clinical and Experimental Pharmacology and Physiology, 44*, 9-14.

Upadhyay, R. K. (2015). Emerging Risk Biomarkers in Cardiovascular Diseases and Disorders. *Journal of lipids, 2015*. doi:10.1155/2015/971453

Vogl, P. T. (2014). *Measurement of Cystatin C in Dried Blood Spot Specimens.* (Master of

Science), University of Washington, Seattle.

Weiss, L. M., & Börsch-Supan, A. (2019). Influence of fieldwork conditions and sample quality on DBS values. In A. Börsch-Supan, J. Bristle, K. Andersen-Ranberg, A. Brugiavini, F. Jusot, H. Litwin, & G. Weber (Eds.), *Health and socio-economic status over the life course: First results from SHARE Waves 6 and 7* (pp. 359-366). Berlin: De Gruyter.

Weiss, L. M., Börsch-Supan, M., Myck, M., Nocoń, K., Oczkowska, M., Topór-Mądry, R., Andersen-Ranberg, K., & Börsch-Supan, A. (2019). Blood Collection in the Field - Results and Lessons from The Polish Test Study. In A. Börsch-Supan, J. Bristle, K. Andersen-Ranberg, A. Brugiavini, F. Jusot, H. Litwin, & G. Weber (Eds.), *Health and Socio-economic Status Over the Life Course: First results from SHARE Waves 6 and 7* (pp. 367-374). Berlin: De Gruyter.

Weiss, L. M., Sakshaug, J. W., & Börsch-Supan, A. (2018). Collection of Biomeasures in a Cross-National Setting: Experiences in SHARE. In T. P. Johnson, B. E. Pennel, I. Stoop, & B. Dorer (Eds.), *Advances in Comparative Survey Methods: Multinational, Multiregional, and Multicultural Contexts (3MC)* (pp. 623-641). New York: John Wiley & Sons, Inc.

Williams, S. R., & McDade, T. W. (2009). The Use of Dried Blood Spot Sampling in the National Social Life, Health, and Aging Project. *Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 64*(suppl_1), i131-i136.

World Health Organization. (2018). *World health statistics 2018: monitoring health for the SDGs, sustainable development goals*. Retrieved from Geneva:

Yaffe, K., Lindquist, K., Shlipak, M. G., Simonsick, E., Fried, L., Rosano, C., Satterfield, S., Atkinson, H., Windham, B. G., & Kurella-Tamura, M. (2008). Cystatin C as a Marker of Cognitive Function in Elders: Findings from the Health ABC Study. *Annals of neurology, 63*(6), 798-802.