Minimally invasive collection of plasma in the field: Evaluation and early findings on adult health from Malawi

Beth J. Soldo[1,2]

Philip Anglewicz[2],

Iliana V. Kohler[2]

Hans-Peter Kohler[2]

University of Pennsylvania

Introduction

Collecting biomarkers inevitably involves balancing the ease and cost of collection with specimen stability and assay reliability. These concerns are exacerbated in developing country with poor infrastructures for health care and transportation. These obstacles impede our understanding of population health in resource-poor countries, which are transitioning from an acute disease regime to one increasingly dominated by chronic conditions (Murray and Lopez 1997; Murray et al. 2003).

To date the primary option for obtaining biomarkers in developing countries was dried blood spots (DBS). Thom McDade pioneered techniques for collecting and storing small blood samples and developing assays for important components, such as hsCRP to assess level of inflammation (McDade et al. 2004 and 2006). The infrastructure for doing DBS assays is not sufficiently developed, however, to process the large volume of DBS required in surveys representative of heterogeneous populations. The protocol for DBS collection in the 2006 wave of the Health and Retirement Study (HRS) required collection of 6 DBS (3 circles on two cards). The quality of the blood spots, however, progressively decayed with subsequent spots[3].

In this paper we describe a new approach to collecting biologic materials in Malawi, one of the poorest countries in sub-Saharan Africa with high levels of HIV/AIDS and other infectious diseases, including malaria. Our objective in collecting these materials is to assess adult health using indicators of activity in

[1] Corresponding author and Director, Population Aging Research Center, University of Pennsylvania, 3718 Locust Walk, Philadelphia, PA 19104; E-mail: *bsoldo@pop.upenn.edu*

[2] Population Studies Center, University of Pennsylvania

[3] The number respondents who were able to fill all 6 circles was reduced from the maximum yield of 6941 who provided an informed consent.

three basic biologic systems: immune, metabolic (lipids and glucose), and renal (or clearance). While there has been considerable research on the health of individuals infected with HIV (see, for example, de Maat and Kluft 2001), very few studies have examined the health of adults sharing the same environments. Gurven et al. (2008) and others have shown that environmental and life circumstances such as those found in Malawi provide considerable exposure to endemic parasites and associated infections. We anticipate that adults not infected with HIV in Malawi nonetheless will have an elevated pathogen burden (hsCRP), evidence of malnutrition (albumin, total protein); high levels of renal or clearance problems (creatinine, total protein, urea); low levels of diabetes and cardiovascular disease. Compared with data from the National Health and Examination Study (NHANES) we also anticipate higher age-specific levels of the biomarkers we collect, indicating a faster pace of aging consistent with a hostile epidemiologic environment. We also compare our results with published data on the Tsimine of Bolivia (Gurven et al. 2008).

Study Background

The Malawi Diffusion and Ideational Change Project (MDICP) is a longitudinal research project based in rural Malawi. The primary goals of research under MDICP research is to examine the role of social interactions on attitudes related to contraceptive use, family planning, HIV/AIDS knowledge and risk behavior; and identify mechanisms used by individuals to cope with mortality due to AIDS.

MDICP data collection takes place in three sites in rural Malawi, each representing one of the three regions of the country: Balaka (southern region), Mchinji (central), and Rumphi (north). The first wave of MDICP data collection took place in 1998, at which time MDICP completed interviews for 1,541 of 1,790 ever-married women between 14-49 years old and 1,065 of 1,520 for their husbands. In 2001, the first follow-up wave collected data for the same respondents, and respondents who were not found in 1998 as well as new spouses for respondents who married again between 1998 and 2001[4].

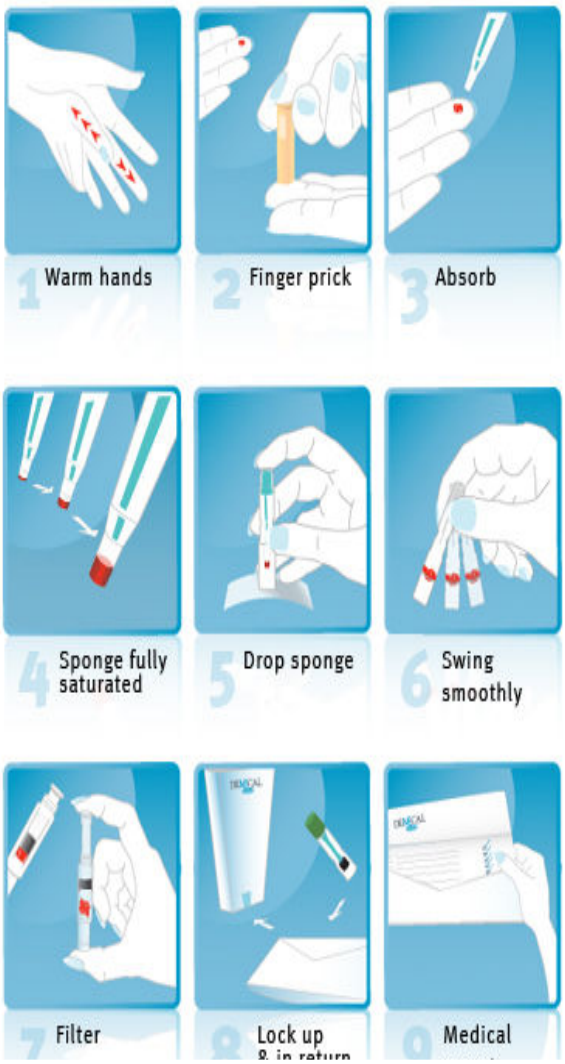
In 2004, MDICP returned to the field to re-interview panel respondents and added two new data collection components. A sample of approximately 500 married and never-married adolescents aged 15-28 were added for each data collection site[5]. With this addition and the induction of never-married adolescents into the MDICP sample (the 1998 sample was restricted to ever-married men and women), the samples in each district are representative of their

[4] For more details on the 1998 and 2001 sampling strategy, see Watkins et al, 2003.
[5] A description of sampling strategy for the 2004 adolescent sample can be found at: http://www.malawi.pop.upenn.edu/Level%203/Malawi/docs/Sampling3.pdf

respective population. In 2004 and subsequent waves, the MDICP collected HIV biomarkers from all consenting MDICP respondents (Bignami-Van Assche et al. 2004). In 2004, HIV prevalence was 6.7 percent for the entire sample with some regional variation in prevalence: 4.5 percent in the Northern region, 8.2 percent in Balaka in the South (the area of the current biomarker collection), and 7.5 in the Central. The 2006 data indicate an overall HIV prevalence of 7.2% (Obare et al. 2006).

MDICP conducted a fifth wave of data in the summer of 2008. In this wave MIDICP expanded its focus on HIV/AIDS research to investigate not only the individual reactions to HIV infection, but also the consequences of AIDS mortality on rural individuals, families, and households. In addition to testing for HIV, MIDICP-5 collects new biomarkers for 50% of all respondents and parents in Balaka, the MDICP site with the highest HIV prevalence.

Figure 1 Demecal™ sample collection



Research Design of Current study

To collect blood for the current biomarker study we used an innovative blood sampling system. Demecal™ kits require but 1-2 drops of blood harvested from a finger stick. These kits were designed for patient's home use, but in Malawi we trained and certified field staff to perform the requisite procedures. The distinctive feature of this system is that the blood is pressed through a patented filter that separates out plasma and cells. Unlike a clinic based procedure for obtaining blood plasma, the Demecal™ system does not require the use of a centrifuge.

While the reliability of the test kits has been demonstrated by Demecal™ in the Netherlands and Japan, they were developed for individual use, not field use in difficult environments. Malawi is the first field test of the applicability of the kits for collecting measures of population health and their adaptability to extreme conditions in tropical zones. Our results indicate the reproducibility

of biomarkers obtained from the Demecal™ system.

We evaluate the overall health of approximately 1000 persons participating in MDICP panel study. All respondents live in rural communities in the Balaka region of southern Malawi. These respondents were previously evaluated for both HIV and STDs, but no other health assessments have been made. At the time of collecting Demecal™ biomarkers, we also administered a brief personal questionnaire to gather information on the respondent's living environment, including source of drinking water, type of sanitation, prior malaria exposure, and use of sleeping nets. We also ask participants when they last ate, what they had (particularly, carbohydrates), and about how much they consumed, if now pregnant, or recently had an infection.

We report on the collection of biomarkers to evaluate their overall health and well-being using a brief face-to-face interview and conventional biomarkers such as: hsCRP, an indicator of inflammation; cholesterol, LDL, HDL, triglycerides (a lipids panel); circulating glucose; urea, albumin, creatinine, total protein, uric acid, (collectively a measure of renal function and clearance); and, circulating glucose; if the blood glucose level is elevated, we also conduct the HbA1c test, a 3-month average of the extent to which sugar molecules attach to hemoglobin. We report on the stability and analytic performance of biomarkers that were collected using an innovative blood sampling system.

Field Procedures

MDICP tested for the above biomarkers by collecting plasma samples from approximately half of adolescents, adults, and parents in the Balaka MDICP sample, i.e. a 50% random sample of participants. Biomarkers were collected on about 1000 cases, including the approximately 110 cases that tested positive for HIV in a previous wave of MDICP. These samples were collected using the kit that allows for collecting plasma, as illustrated in Figure 1 above. The Demecal™ system requires only a single drop of blood[6]. A lancet is used to puncture the finger tip [2]. A sponge device is used for absorbing the drop of blood [3]. After the sponge turns completely red [4], it is dropped into the container with the buffer fluid [5]. A gentle swinging motion for 40 seconds [6] is necessary to release the. A filter is used to separate the red blood cells from the plasma [7], rather than the used in clinical labs by centrifuge.

Because the administration of such tests require personnel trained in biomarker specimen collection and HIV/STD counseling, the MDICP recruit a team of 50 nurses and HIV testing counselors from hospitals in Malawi to collect

[6] More information about the Demecal kit can be found at http://www.demecal.nl/.

the biomarkers. These counselors will undergo a one-week training period in the use of the Demecal™ kits prior to beginning biomarker collection in Balaka. This training includes the importance of protecting the "cold chain" from the time of specimen collection to the placement in a -20° freezer, as well as protecting respondent confidentiality and privacy.

Each counselor was signed to collect biomarkers from particular respondents by the Biomarker Coordinator. Using village guides, the counselor visited the home of the assigned a respondent, obtain consent (either by signature or thumbprint) for biomarker collection, and then collect the plasma using the Demecal™ kit. To preserve the respondent's confidentiality, the specimens was marked by a special identification number, that only the MDICP Biomarker Coordinator can link the plasma sample with the respondent or his/her personal information. The counselor will place the label with this identification number on the biomarker sample. After successfully collecting and labeling the plasma sample, the counselor returns to the biomarker coordinator, who will store all plasma samples in a cooler.

Upon returning from the field each day, the biomarker coordinator will check all samples to verify that they were collected and labeled properly, and then all plasma samples will be placed in a -20° freezer. At the end of each week, all biomarker samples were cross-checked with field records, and sent via DHL from Malawi to the Demecal™ laboratory in the Netherlands for testing. En route to the Netherland, the samples will be packed in a special cooler provided by Demecal™ that is designed for transporting frozen blood samples. The biomarkers were sent with a list of identification numbers, so that the entry of test results by Demecal™ will retain the confidentiality of all MDICP respondents.

After the biomarker processing is completed by Demecal™ (on average about 2 weeks after arriving in Amsterdam), the results are sent to MDICP. Upon receiving the test results, MDICP presents an information session for all participating villages in which potential health concerns associated. Individuals may meet in private with a health care counselors to discuss their individual results. The MDICP also worked with local health clinics to address health issues identified by the biomarker tests. Demecal™ also prepared a database with the assayed values and individual IDs that will be mapped to the extant database maintained by the MDICP of data from prior waves.

Schedule. The test kits were delivered directly to Malawi in September. A test-run of all field procedures for about 25 cases has been completed, including the freezing protocol in Malawi, the transport protocol, and the assays themselves. In this trial we are interested in how well the integrity of the plasma

samples was maintained and the reliability of the assays themselves. The actual field work will commence in late October and be completed by Mid-December. We expect to receive results 2 weeks after all specimens are received by Demecal™ in Amsterdam.

Analysis

Once the biomarker data are merged with demographic background variables, analyses will begin. Because of the interest in combining survey data and population biomarkers, we will analyze the quality of the assays as well as related problems in field procedures, if any. We expect these results will be of interest to the HRS, sponsored by NIA, as well as other field studies in the U.S., Africa, and Latin America.

The main focus of the paper will be assessing the overall health of the sample population, contrasting those who are known to have HIV with those who tested positive in the summer of 2008. Assuming that those who are HIV-positive will anchor the poorest end of a "health continuum", we seek to identify two subgroups within the HIV-negative participants: "more or less healthy" and at poor health.

We expect those who are known to have HIV/AIDS to have appreciably highest level of inflammation as indexed by hsCRP. Drain et al. (2007) show that CRP is an independent predictor of disease progression and mortality among those with active AIDS. But CRP is a generic measure of inflammation that may signal opportunistic infections secondary to HIV/AIDS or a recent malaria or current or recent diarrheal episode. We also expect to see low concentration of albumin in those with recent acute diseases, especially falciparum malaria (Nuchsong et al. 2007), and normal levels of total protein and blood glucose in HIV-negative participants who were not recently exposed to acute infectious diseases (Adeosun et al. 2007). Elevated total protein is a risk factor for pneumonia or dehydration; if low, total protein may be an indicator of malnutrition in the non-HIV group. Results from the lipids panel indicates degree of risk with chronic disease, for example, elevated triglycerides in the presence of low levels of HDL (the good cholesterol) is a strong predictor of ischemic stroke. We anticipate low risks for heart disease, diabetes, and stroke in the non-HIV/AIDS subgroup. But to the extent that lipids and circulating glucose or hbA1c glucose indicate risk for cardiovascular disease we anticipate those affected to have a younger age structure than comparable populations in the U.S. or in the Bolivian Tsimine.

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