Indicators and Biomarkers of NCDs:

Evaluating the Health Benefits of Clean Cooking Adoption

Technical Meeting Report

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Acronyms

AAP	Ambient air pollution	IAQ	Indoor air quality
ABP	Ambient blood pressure	IARC	International Agency for Research on Cancer
ACE	Angiotensin-converting enzyme	IER	Integrated exposure response
ALRI	Acute lower respiratory infections	IL	Interleukin
BAL	Bronchial alveolar lavage	LBW	Low birth weight
BOND	Biomarkers of Nutrition for Development	LDH	Lactate dehydrogenase
BP	Blood pressure	LF	Lung function
BS	Biomass smoke	LICs	Low income countries
BSE	Biomass smoke extract	MI	Myocardial infarction
CAL	Chronic airflow limitation	MICs	Middle income countries
CHD	Coronary heart disease.	MMP	Matrix metalloproteinases
CHS	Child Health Study	NCD	Non communicable disease
CIMT	Carotid intima-media thickness test	NIH	National Institutes of Health
CO	Carbon monoxide	NO	Nitric Oxide
COHb	Carboxyhaemoglobin	OH-PAH	Hydroxylated polycyclic aromatic hydrocarbons
COPD	Chronic obstructive pulmonary disease	OR	Odds ratio
CRP	C- reactive protein	OxLDL	Oxidized low density lipoprotein
CVD	Cardiovascular disease	PFT	Pulmonary function tests
CXR	Chest x-ray	PM	Particulate matter
DBP	Diastolic blood pressure	qPCR	Quantitative real-time polymerase chain reaction
DBS	Dried blood spots	RCT	Randomized control trial
DLCO	Carbon monoxide diffusing capacity.	RNA	Ribonucleic acid
ELISA	Enzyme-linked immunosorbent assay	SAA	Serum amyloid A
FEV_1	Forced expired volume in one second	SBP	Systolic blood pressure
FVC	Forced vital capacity	SHS	Second hand smoke
GACC	Global Alliance for Clean Cookstoves	TAAs	Tumor associated antigens
GBD	Global burden of disease.	TNF-a	Tumor necrosis factor-alpha
GOLD	Global Initiative for Chronic Obstructive Lung Disease	TS	Tobacco smoke
HAP	Household air pollution	TSE	Tobacco smoke extract
HbA1c	Glycosylated haemoglobin A1c	VOCs	Volatile organic compounds
HICs	High income countries	WS	Wood smoke
HTN	Hypertension		

Report prepared for the Global Alliance for Clean Cookstoves by Kirstie Jagoe, Independent Consultant.

Overview and Objectives

According to the 2010 global burden of disease (GBD) comparative risk assessment household air pollution (HAP) is among the top global health risk factors, attributable to child pneumonia, chronic obstructive pulmonary disease (COPD), lung cancer and cardiovascular disease (CVD) (Lim 2012)[1]. It is clear that HAP is a major modifiable risk factor for non-communicable diseases (NCDs) among the poor in developing countries. Unclear at the moment, however, is the extent to which adopting clean cooking can reduce this burden, and over what time horizon.

While the link between HAP and chronic lung disease and lung cancer is well established; direct evidence for CVD is still limited. A small number of studies have measured the impact of exposure to HAP on CVD and risk factors for CVD in developing countries, and provide evidence of the negative cardiovascular (CV) outcomes attributable to exposure to HAP, as measured by changes to biomarkers, CVD risk factors, and disease rates. However, research questions and evaluation challenges remain. As a result, the CVD burden attributable to HAP is currently based on risk estimates extrapolated from the other major sources of combustion-source pollution, namely ambient air pollution (AAP), tobacco smoke (TS) and second-hand smoke (SHS).

In December 2014, The Global Alliance for Clean Cookstoves (the Alliance) in coordination with National Institutes of Health (NIH), convened a one-day workshop to bring together experts from the fields of AAP, tobacco smoking and respiratory and CV public health. The purpose of the workshop was to draw on existing knowledge and experience to highlight potential indicators and biomarkers of NCDs that might be appropriate to use in HAP settings. The following questions were considered:

- What are the mechanisms by which a shift to clean cooking can cause measureable impacts on indicators of adult chronic respiratory and CVD?
- Where are the 'clean' technologies being scaled up? Will scale up result in exposure reductions substantial enough to reduce the risk of chronic respiratory and CVD?
- What are the right 'indicators' that will demonstrate health benefits over a relatively short time frame? What are the challenges of field-based epidemiology in terms of feasibility?
- What can be learned from epidemiology of other combustion-source pollution?
- How should competing risk factors, i.e. other sources of air pollution (AP), population susceptibility, and other behavioral risk factors be addressed?

This report provides a brief summary of presentations, as well as key points raised in discussion at the meeting. Links to detailed presentations are also provided. It should be noted, however, that the report should not be seen as an endorsement of the slides presented, nor of the validity of all points raised during the discussion. Rather, the report is intended to capture the key points raised during the workshop, in order to help guide the development of a strategic, feasible research agenda necessary to evaluate the health benefits of clean cooking adoption over a relatively short time frame.

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[1] Lim et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet , Volume 380 , Issue 9859 , 2224 - 2260

Presentations

Session 1: Effects on NCDs: Evidence and Gaps

1.1. Global variability in NCDs

Dr MyLinh Duong, Mc Master University

Presentation Summary

The PURE (Prospective Urban Rural Epidemiology) study aims to examine the societal and environmental determinants of risk factors and disease burden on a global level using standardized methodology. Data is being collected at multiple levels (community, environment, household and individual) within a region. Household level metrics include cooking methods and indicators for HAP. Individual level parameters include levels of physical activity, blood and urine tests, pulmonary function test (PFTs) and blood pressure (BP) measurements.

The 4-year follow-up data is currently being analyzed and presented. Overall, a 10-year follow-up is planned. HAP monitoring and measures of personal exposure are to be included in the next phase of the study.

CVD: Evidence shows significant differences in **event rates, type of events and fatality** by countries of different development. Case fatality increases as income levels decrease, with the highest disease burden in low-income countries (LICs), particularly in rural areas.

CVD burden, measured using the INTERHEART Risk Score[2], showed a reverse pattern in risk factor burden, where a low **risk factor** burden is associated with high **disease** burden. This could be due to poor management and recognition of the known, traditional risk factors or due to unrecognized risk factors, not being considered in risk assessment. Risk factor management, e.g. treatment of hypertension (HTN) is poor globally but significantly worse in rural areas of LICs.

Respiratory: The baseline prevalence of COPD and chronic airflow limitation (CAL) is being measured using the Global Initiative of Chronic Obstructive Lung Disease (GOLD) criterion for COPD (a post bronchodilator $FEV_1/FVC < 0.70$, where FEV_1 = forced expiratory volume in 1 second and FVC = forced vital capacity). Some headline results include:

- The lung function (LF) data taken from non-smokers showed large differences in population baselines. Follow-up data is currently being analyzed to explore for explanatory differences in ambient conditions.
- The prevalence of CAL varies globally, with particularly high prevalence in Africa and South Asia and within women where reported smoking rates are low, suggesting that risk factors other than TS are significant in these regions.
- AAP data taken from satellite readings showed much higher concentrations of particulate matter less than 2.5 micrometers in diameter ($PM_{2.5}$) in LICs compared to HICs.

[2] The INTERHEART Risk Score is a non-laboratory risk stratification tool used in the INTERHEART case-control study conducted in over 52 countries.

- Linking this to LF showed a linear relationship even at higher doses of $PM_{2.5}$ in terms of FEV_1/ FVC[3] ratio decrease with increases in $PM_{2.5}$ concentrations.
- Data from China and India showed much more pronounced declines in FEV_1 with increments of PM.

Discussion points raised

Due to a big difference in demographic age structure of each country, which could minimize the differences to the other risk factors of interest, it might be beneficial to remove age from INTERHEART risk score and provide age-adjusted results.

- INTERHEART risk score is validated against outcomes at regional levels of high, middle and low-income countries, as well as globally.

Caution needs to be taken when comparing baseline LF values across different countries, ethnicity or race and relating them to normal values, which are typically Caucasian North American. The apparent difference in LF could relate more to factors determined by ethnicity and/or race rather than real differences in lung health.

- PURE assumes they are genetic differences but plans to carry out further analysis to explore if the LF values are predictive of outcome or not.

1.2: Risks for respiratory NCDs from exposure to HAP: an overview of the evidence

Dr. Nigel Bruce, University of Liverpool

Presentation summary

The life course approach is a useful model to explore the HAP-associated respiratory disease risks throughout life and to consider how they might be inter-related.

Birth weight: There is consistent evidence of approximately 40% increased risk of low birth weight with exposure to HAP. There is supportive analogous evidence from studies of active smoking and a smaller effect seen in second-hand smoking. Evidence from AAP is less conclusive.

Impact of HAP on lung growth: There are two key phases in lung growth, the first 3 years of life when new alveoli develop and then in later childhood when lung growth by expansion occurs. After comparing the annual lung growth in children from most vs. least polluted communities, the 12 Community California study (Kulkani et al, (2006))[4] concluded that early exposure to air AP results in clinically significant FEV_1 deficits as children reach adulthood.

Asthma: There is currently no consistent evidence on the impact of HAP on asthma. A recent systematic review (Wong et al., 2013) looking at 'cooking with biomass and asthma' revealed limited and inconsistent results.

[3] FEV_1 is the volume of air that has been exhaled at the end of the first second of forced expiration. FVC is forced vital capacity: the determination of the vital capacity from a maximally forced expiratory effort. The ratio represents the proportion of a person's vital capacity that they are able to expire in the first second of expiration. Normal values are approximately 80%.
[4] Please see associated power point presentations for full references.

COPD: Evidence is available on range of important steps of disease progression and outcomes for COPD. Several systematic reviews all showed that women's risk of COPD is higher than men's, which is mostly likely to be the product of exposure to biomass. Analogous evidence from tobacco is very powerful.

The small amount of HAP or exposure reductions data available shows evidence of reduction in risk (measured by reduction in annual decline in FEV_1) with cleaner cooking technology intervention. An intervention-based cohort study in Guangzhou, China (Zhou 2014) showed a duration effect trend for COPD seen in study groups with ventilation or clean fuels.

The integrated exposure-response (IER) curve for COPD is currently not well-defined. The effect estimates are higher than is expected from this model and do not fit curve very well. In contrast to lung cancer, the curve is flat at high exposures and steeper at lower exposures.

Restriction vs obstruction: Recent evidence from the BOLD study suggests that the higher mortality from COPD in LICs could be due to restriction rather than obstruction, which has important implications when choosing outcomes to demonstrate impacts of HAP. Further clarification is required but analysis of restrictive as well as obstructive components might be required when looking at effects of biomass on LF.

Lung Cancer: In 2010, the International Agency for Research on Cancer (IARC) monograph classed emissions from coal as a group 1 carcinogen and showed higher risk of lung cancer for women than men. A recent systematic review (Bruce (submitted)) which aimed to determine causal inference for biomass and lung cancer, showed relatively small effects. When adjusted for clean fuel use, a large significant effect was seen, with a much larger effect in LICs.

The IER for lung cancer is the most linear of the 4 functions (ischemic heart disease (IHD), CVD (stroke), COPD, and lung cancer). Exposure response (ER) evidence from five published studies showed inconsistent analysis and findings. A re-analysis of published data by IARC provided a stronger case of causal link between biomass fuels and lung cancer, with clear evidence of an ER function relationship in men but not women, an additional IARC review is required for conclusive evidence.

Further study is needed to understand and synthesize overall impacts across lifespan but practical and time frame issues requires that the focus is on key periods for disease markers. Intervention studies are starting to show disease effects and LF decline but the role of longer-term cohorts needs to be considered moving forward.

<u>Discussion points raised</u>

Predictive values for lung function:

- Although the BOLD study had strict quality assurance for the LF measurements, it remains unclear which reference values to use when defining normal lung function or restrictive or obstructive lung disease in LICs.
    - A comparison of LF within the child health community shows that once barriers to growth (such as malnutrition/ disease) are removed, children grow at the same rate all over the world. This suggests that the differences in LF seen across populations may not be due to underlying genetic factors but may be a function of prior exposures and does not require cultural adjustment.
- Spirometry from the PURE study shows regional differences that reflect race and income level patterns. For the populations in middle income countries (MICs) and LICs to drop their FEV_1/FVC ratio to <0.70 and thereby meet the GOLD criteria for COPD, they need very severe airways

obstruction. The PURE team are looking to establishing normal reference values for these LICs using 5% lower limit of normality.

- The PURE study data suggests that looking at composition of what is inhaled might influence the type of defect (obstructive vs restrictive) found.
    - Differences in patterns of LF were seen depending on source of AP. A restrictive defect was identified with increases in levels of outdoor $PM_{2.5}$, yet a more obstructive pattern was seen when looking at HAP using solid fuel as a marker.

Particle categorization

Studies are needed to provide better understanding of particles generated by multiple sources so to avoid separately categorizing overlapping exposures e.g. treating second hand smoke (SHS) in homes and workplaces as though separate agents.

1.3: Cardiovascular effects of HAP: Evidence and gaps

Dr. Sola Olopade, University of Chicago

Presentation Summary

Although the CV implications of exposure to biomass are not as well understood as respiratory effects, there are several studies that have shown that biomass fuel use increases CVD risk as measured by biomarkers.

Dutta et al (2011) showed significant changes in several biomarkers after exposure to biomass smoke in Indian households.

- Levels of oxidized low-density lipoprotein (OxLDL) were higher in biomass users.
- HTN (pre or clinical HTN) was positively associated with both PM_{10} and $PM_{2.5}$ levels in cooking areas after correcting for all potential confounders. This is significant as HTN is considered one of the most important risk factors for CVD.
- Platelet aggregation, a marker of prothrombolitic effect of exposure and development of arthrosclerosis, was heightened by biomass exposure.

A cross-sectional study in Peru (Painschab et al 2013) used carotid ultrasound to show that biomass fuel use compromises carotid lumen measured by the carotid intima-media thickness test (CIMT: reduction of carotid artery diameter). There were also significant differences in prevalence of carotid atherosclerotic plaques.

Very few intervention studies have been conducted. Alexander at al (2014) showed that post intervention reduction in indoor PM levels, correlated with systolic blood pressure (SBP) reduction after stove installation in Bolivia, suggesting that a reduction in exposures can potentially modulate CV risk, particularly in LMICs.

Mechanisms: Why does exposure to PM increase risk of CVD?

The role of cytokines, particularly Interleukin 6 (IL-6) and their relationship with IHD is key when trying to understand the mechanisms involved in the increased risk of CVD due to exposure to PM.

- Mutlu et al (2007) showed in laboratory tests that acute exposure to PM promotes thrombogenesis and compromises vascular flow.
- IL-6 is known to play an important role in hemostasis and can induce a thrombophilic state.

- Exposure to PM increases IL-6 levels in bronchial alveolar lavage (BAL) fluid. Such elevated levels are consistent with the notion that IL-6 is **necessary** for prothrombotic state induced by PM (Mutlu 2007).
- Exposure to PM elevates catecholamines (adrenaline elevated in HTN). Blocking with β-adrenergic blockade prevents the prothrombotic effect of PM (Chiarella 2014).

Biomarkers for CVD risk

- IL-6, C-reactive protein (CRP), and oxLDL appear to be useful biomarkers for HAP-mediated cardiovascular risk.
    - Elevated levels of IL-6 are correlated with risk factors for coronary artery disease (CAD).
    - IL-6 also induces acute phase reactants including CRP, which is a surrogate marker of IL-6 related effect.
    - Both IL-6 and CRP are independently associated with increased relative risk for mortality from CV disease.

Larger studies are required to better define the role of biomarkers in risk assessment as well as developing biomarkers for early screening and risk stratification.

Discussion points raised

Additional studies measuring LF, CVD and HAP: There are additional studies that can and will provide further evidence on the impact of HAP on CVD.

- The RESPIRE randomized control trial (RCT) in Guatemala (McCracken et al 2011) showed reduced occurrence of nonspecific ST-segment depression and improvements in BP after chimney stove installation.
- The Child Health Study in California recently analysed the impact of reduction in pollution levels on LF and pulmonary symptoms. The results will be published soon.

Measuring acute vs chronic effects of PM:

- The studies by Dutta (2011) and Painschab (2013) examine chronic effects from long-term stove use. In studies of chronic exposure, population level changes for levels of biomarkers associated with changes in chronic exposure levels can be seen.
- Even with low-level exposure the body responds so can look markers of oxidative stress. However this response does require good nutrition and good anti-oxidant defence.

The laboratory work by Mutlu explored the acute effects of PM.

- It is difficult to translate acute effects seen in the laboratory to human studies when people have used the same stove for a long time.
- However, the evidence suggests that changes in oxidative stress, inflammatory and haemostasis markers in relation to acute changes in day-to-day AP can be measured.
- Studies looking at acute effects of exposure to differing levels of diesel exhaust, have explored changes in heart rate (HR) and ST segment depression

Extrapolating AAP data: Caution is required when extrapolating the mechanisms and results from AAP in through HAP.

- They are not only a different source but also made up from very different components and the opportunity for photochemistry is very different for HAP as it is for AAP.

- Further understanding of this issue is hopefully going to be provided by a current Alliance-funded study in Nigeria, which is aiming to relate pregnancy outcomes to indoor and ambient exposure using personal exposure monitoring in conjunction with GPS data

Is the specificity of C - reactive protein (CRP) high enough or should the focus be more on biomarkers related only to endothelial inflammation?

- Studies comparing rural exclusive biomass fuel use to urban no biomass use, have found that, once traditional risk factors had been controlled for, the average CRP was lower in rural setting where there is higher levels of biomass use.
- A one-year cohort intervention study in Nigeria aiming to identify effective biomarkers found little correlation between CRP and obstructive lung disease. However, this might have been due to the very small sample size and further study is needed.

Session 2: Notes from the Field

2.1: Identifying biomarkers of household air pollution exposure and cardiovascular risk in China

Dr. Jill Baumgartner, McGill University

Presentation Summary

HAP from cooking with wood and coal is associated with elevated BP in women. Certain components of AP such as black carbon are more strongly associated with elevated BP than other more commonly used measures. A large pooling of Asian cohorts showed a reduced risk of stroke and heart disease with a reduction in BP, which suggests there could be possible CV benefits of population-based energy interventions. However, the linear dose-response relationship suggests exposures need to be very low to see a large health benefit

There are two ongoing studies in China looking at HAP and CV risk:

- They are providing environmental exposure and health data in four regions and at multiple time points.
- In addition to providing an opportunity for potential biomarker discovery they are also valuable evaluations of future interventions.
- One key aim is to have impacts on sustainability agenda in energy and housing, as well as NCD prevention and clinical guideline development.

The IGNITE Study is a 5-year, non-randomized intervention evaluation study measuring personal exposure and several intermediary vascular and biological indicators and covariates that are associated with CV risk. Integrated $PM_{2.5}$ and black carbon exposure is measured every 6 months. Initial pilot data shows that women in high exposure groups had higher brachial and central SBP, augmentation index and pulse wave velocity (both markers of arterial stiffness). Women in the high exposure group also had shorter telomere length, which is associated with CV risk. The study demonstrated the feasibility of measuring markers of vascular function, and collecting such biomarkers as part of a field study.

The second study is a large pilot study that has re-enrolled the living participants from the China INTERMAP study, plus some additional younger subjects. Multiple surveys and measurements of fuel use, diet, alcohol, weight etc plus two 24-hour urine collections were carried out in 1990's. Hypothesis-driven and agnostic approaches will be used to identify urinary metabolites or other markers of environmental

risk that can discriminate between populations and different subjects, and assess independent relationships of these metabolites (or groups of metabolites) to clinical symptoms such as raised BP, arterial stiffness. The ultimate goal is to identify markers of exposure and CVD that can be used in larger intervention studies. Data is not yet available, but similar work in China shows that metabolic profiles could distinguish between populations and between individuals with different CV risks.

Discussion points raised

Urinary biomarkers: The optimal urinary biomarkers to be examined in the study are currently being determined. The better-known markers i.e. oxidative stress measures will be used but an agnostic approach is being used to look at metabolites and metabolic profiles.

Intervention adoption rate:
Adoption and use of the intervention stove is a definite challenge for any stove intervention study. The study worked to develop an effective intervention that meets people's needs. Over the three-year study, the stove has under gone several rounds of testing (both emissions and consumer acceptance) with many subsequent adaptations. The intervention stove is a tier 4 equivalent, locally-made semi-gasifier pellet stove for cooking and heating. The aim is to develop an effective intervention that will work in multiple locations with the possibility to scale up local manufacture.

2.2. Biomarker feasibility en el campo: lessons learned from Honduras and Nicaragua

Dr. Maggie Clark, Colorado State University

Presentation Summary

Colorado State University (CSU) has implemented multiple stove impact studies involving the use of biomarkers in Honduras. The criteria applied when choosing biomarkers of inflammation includes:

1) Needs to be an important risk factor for CVD.
2) Should have an established relationship with AAP.
3) Should be able to change meaningfully over a short period of time.
4) Can easily be measured in rural areas of developing countries.

Dried blood spots (DBS): Finger-stick collection of blood is a potentially promising approach for the following reasons;

- It is less invasive than venous samples
- There is minimal post-collection processing
- It has been used successfully in remote rural settings.
- DBS spots can be used to measure biomarkers such as CRP, serum amyloid A (SAA), IL1-β, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α).
- It is possible to measure 100s of markers of inflammation in a single dried blood spot.
- Pilot data to validate the use of DBS in rural Honduras showed that most variation was between, rather than within person.

CRP seems to be the most promising outcome at this point for the following reasons:

- Although it is not part of the biological mechanism, it is a good indicator of risk as there is an established relationship with CVD risk.

- Evidence from the AAP literature shows that elevated PM and carbon monoxide (CO) is associated with increased CRP.

Preliminary data on metabolomics in DBS is promising and is to be followed up with sensitivity analyses. The CSU team is also working to match the metabolites with existing libraries.

Other applications for blood spot measures worthy of consideration include Haemoglobin A1c (HbA1c) and blood lipids (as end points and as part of metabolic syndrome). The monitoring equipment is lightweight and provides instant readings.

Telomere Length: Telomeres are a potential biomarker of cumulative burden of oxidative stress.

- It is a field appropriate method where a cheek swab is taken with a toothbrush, after which the sample can be left for >30 days at room temperature before being analyzed.
- The recent controversy on utility is mostly due to the variation on how it is measured. The most frequently used method to analyze telomere is the quantitative real-time polymerase chain reaction (qPCR); however, while this provides average telomere length over a population of cells rather than single cells, the Colorado team is currently exploring methods that can provide data on frequency and distribution of lengths per cell.

Exhaled Nitric Oxide (NO): Exhaled NO is an established measure of airway inflammation, but some field-testing issues such as electrical interference with monitors (NIOX MINO) mean its application is limited. A new design of monitor is expected to be ready for field-testing in February 2015.

<u>Discussion points raised</u>

Use and applicability of biomarkers:

- Exhaled NO appears to be well-correlated with allergic responses such as those seen in asthma but less so non-allergic conditions. Studies that measured NO before and after heavy exposure to biomass cooking smoke showed a minimal increase.
- It is not expected that telomere length will improve (lengthen) after cookstove intervention, but rather exhibit a decreased rate of shortening.
- The Women's Health Initiative team are working on extracting HbA1c from DBS.
- Carbon monoxide diffusing capacity (DLCO) could be a means of measuring CO levels. It has been used in high altitude regions of Kenya, but accessing the required gases can be a real impediment to its applicability.

Multiple endpoints:

- Collating, understanding and optimizing the large amount of data being collected across the air pollution sector is a challenge. This could be an area where the Alliance can facilitate cross-validation of shared databases and some integrated frameworks i.e. pathway models and tools to bring this together in as systematic way.
- Collaborative consortia arrangements where teams conduct pre-specified analysis on-site and then share the summary statistics to be pooled could address such issues. This would also overcome the issue of not being able to take biological samples out of country, but does raise the issue of calibration, which can impact on the type of pooled analysis carried out.
- Multiple measurements can raise an analytical issue related to multiple comparisons when looking at exploratory research.

- Carefully selected markers, which have been chosen because there is an indication to believe there is pulmonary and/or CV risks do not need to be adjusted for in multiple comparisons.
- There is a stronger case for adjustment with metabolomics because of their high number but a case could be made for not doing full adjustment if there are clear pathways of interest and the associated markers are known.

2.3. Biomarkers: Potential lead in cookstove-health studies

Dr. Anindita Dutta

Presentation Summary

Inflammation and oxidative stress are the main pathways leading to disease development.
Biomarkers to reflect CV pathway include

- Alveolar macrophages (AM) in sputum,
- TNFα, IL-8 and IL-6, reactive oxygen species (ROS) in sputum and blood
- CRP, platelet P-selectin (CD62P), oxLDL measured in blood samples.

Many of theses biomarkers can be easily measured using enzyme-linked immunosorbent assay (ELISA) and have been shown to have markedly elevated levels in biomass users compared to people user clean fuels i.e. LPG. There are many other biomarkers that need further exploration, preferably using a cohort study design.

Discussion points raised

None

2.4. Ambulatory blood pressure in GRAPHS

Dr. Darby Jack, Colombia University

Presentation Summary

The Ghana Randomized Air Pollution and Health Study (GRAPHS) is a cluster RCT hypothesizing that the use of improved cook stoves before 3rd trimester of pregnancy will lead to a significant increase in average birth weight in newborns and a significant reduction in the rate of physician-assessed severe pneumonia during the first 12 months of life.

The 'Lung Development' component of the trial uses the conceptual model that fetal exposure can lead to altered fetal pulmonary phenotype and increases the risk for COPD later in life. A single occlusion LF test is conducted when the baby is 1 month old to detect altered pulmonary phenotype. A series of biomarkers in cord blood are being examined to understand confounders and mechanisms by which HAP alters fetal development, predisposing the infant to low birth weight and acute lower respiratory infections (ALRI). AAP literature suggests that oxidant imbalance is driving the response, but more exploratory hypothesis are also being considered.

The study is also investigating ambulatory blood pressure (ABP) as a marker of CV risk. ABP is the most accurate method to measure BP in people for the following reasons:

- It removes the 'white coat affect',
- Has been shown empirically to be a better predictor for CVD compared to alternative methods
- It gives additional parameters i.e. awake /asleep BP.
- It has the ability to assess how BP reacts to short-term changes in exposure.
- Using international standards 87% of the ABP sessions in the feasibility study were valid.

Discussion points raised

The project intends to continue with co-located ABP and exposure monitors.

The LF test carried out at 1 month old and is only feasible up until 1 year old, after which it becomes difficult to measure LF until aged 6.

- The data from the first 50 infant LF tests were of good quality.
- This method appears to be suitable to apply in the field but there is currently no independent validation available.
- Using a portable device the test momentarily occludes the airway in a sleeping baby and then measures the response to that occlusion.
- 6 yrs old is the youngest age it is possible to obtain good spirometry data.

Session 3: Anticipating Future Health Benefits: Measuring biomarkers and indicators of NCDs

3.1. Biomarkers of exposure for HAP exposure assessment – our experience with urinary bio monitoring of polycyclic aromatic hydrocarbon metabolites

Dr. Luke Naeher, University of Georgia

Presentation Summary

Measuring exposure to HAP has several unique challenges;

- The remote, rural nature of many of the study sites limits the exposure monitoring methods that can be used.
- The introduction of cleaner cooking (LPG/electrical stove) requires that very low levels of exposure need to be measured accurately to validate the health effects. Confounding or background exposures could reduce utility at lower exposure levels since there are other potential exposure sources.
- Challenges are caused by the inherent characteristics of HAP exposure and personal behavior, which includes variability in fuels and emitted constituents, variability (spatial and temporal) in exposure and variability in absorption.
- Monitoring requires full cooperation and compliance from the community and participants.
- It is expensive and time-consuming.

Biomarkers for HAP exposure could resolve some of the issues related to more traditional HAP exposure assessment methods. A small number of field studies have assessed HAP exposure by measuring biomarkers in:

- Exhaled breath: e.g. exhaled carbon monoxide
- Blood e.g. carboxyhaemoglobin (COHb),

- Urine e.g. benzene metabolites, levoglucosan, methoxyphenol and hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs).

The University of Georgia team has conducted a series of studies where the impact of intervention stoves on personal exposures to HAP was tested with urinary biomarkers.

- A study conducted in rural Peru measured exposure to $PM_{2.5}$ and carbon monoxide (CO), using urinary OH-PAHs. Urinary excretion of PAHs was significantly reduced after 3-weeks use of new chimney stoves (Li et al. 2011).
- At a separate location in rural Peru an intervention study showed a 20% reduction in urinary naphthalene metabolites (2-NAP) 3-10 months after introduction of new stoves. This occurred even though measurement of 48-hr CO showed no difference between intervention and control households, suggesting that the biomarkers were able to capture the nuances in personal exposure better then the CO tube data (Hartinger et al. 2011).
- A peri-urban/ urban study in Peru, looking at low exposures in households using LPG showed that even with such low exposures 1-NAP and 2-NAP are strong biomarkers markers for wood smoke exposure (Adetona et al., 2013).
- Cross-shift changes in creatinine adjusted urinary OH-PAHs were assessed in firefighters and showed that 1-NAP and 2-NAP were the most responsive to wood smoke exposure (Adetona et al., in prep)

Implications for applicability of certain metabolites as biomarkers

- In both studies, post intervention levels were still quite high for the 2-NAP, even when the smoke had been reduced in the homes (as seen in study 1). 2-NAP is probably the most promising of the OH-PAH biomarkers.
- The metabolite 1-PYR didn't show much change pre- and post-stove intervention, even with a reduction in measured exposures. This could be affected by residuals left in the homes such as soot, but suggests that this might not be a good biomarker for wood smoke.
- Urinary OH-PAHs are a strong biomarker for exposure, as they are responsive to HAP/wood smoke exposure levels.
    - They change following a change in exposure.
    - Compared to levoglucosan and methoxyphenols – two other urinary woodsmoke biomarkers – OH PAHs might be better biomarkers based on several factors such as sensitivity, specificity, robustness and stability, analytical method.

Challenges to consider when using urinary biomarkers include:

- The feasibility of sample collection and storage must be considered, in addition to the many challenges associated with extreme terrain and poor access when choosing the exposure assessment method.
- The presence of other sources can confound outcomes. Biomarkers by design capture all routes of exposure and not just air.
- Metabolites of PAHs broken down in body and excreted in urine are very short-lived (rise in first 24 hrs post exposure then and disappear 24 hrs later), which creates challenges in measurement.

<u>Discussion points raised</u>

- Sensitively and specify of the OH-PAHs have not yet been quantified.

- OH-PAHs in urine have a short half-life in addition to different variability within individuals and within a 24-hr period. Therefore, if logistically feasible a 24-hr urine sample is recommended.
    - After pooling 10 post exposure 24-hr urine samples, Simpson et al found a strong correlation with PM, CO and levoglucosan, whereas 10 spot samples had a poor correlation.

3.2: Household air pollution: biomarkers of exposure and the search for predictors of respiratory disease

Dr. William Martin, Ohio State University

Presentation Summary

Biomarker overview

- There are several definitions of biomarkers, but the key aspect is that they provide 'quantifiable signs of health and disease'.
- The value of biomarkers as surrogate endpoint requires that it is **clinically relevant** and valid. However, it is hard to find biomarkers that truly predict clinical outcomes; approved biomarkers are always "provisional" as surrogate endpoints.
- If the biomarker can fit into the clinical paradigm, it has the advantage of not requiring the long-term (10yr +) process of validation.

According to recent work by Rylance et al (2013), effective biomarkers of HAP will:

1) Improve epidemiological accuracy in association studies with health effects.
2) Reduce cost and complexity of monitoring interventions- field ready/less expensive.
3) Provide data for education of the public and policy makers about risk.
4) Inform clinicians and public health community about human HAP exposures that are not well-characterized

There is a suite of standard clinical biomarkers of respiratory diseases available. Examples are provided below with comments on their applicability to the HAP sector:

- PFT: This is a standard test, using spirometry but sometimes difficult to do well in field.
- Chest radiography, e.g., chest x-ray (CXR), computerized tomography (CT) scan, magnetic resonance imaging (MRI): These are standard biomarkers but difficult to do in field. CXR is possible with physician support and was used successfully in the RESPIRE study in Guatemala, but others are not feasible.
- Blood tests, e.g., lactate dehydrogenase (LDH) and angiotensin-converting enzyme (ACE): These not very specific.
- Fraction of exhaled nitric oxide (FeNO): This remains work in progress for asthma and how to translate this into field practice not clear.

Desirable characteristics of biomarkers include:

- Simple,
- Inexpensive,
- Point of care,

- Reproducible. This aspect is currently the least considered, e.g. Rad-57 to measure COHb is simple non-invasive but to achieve within and between person reproducibility need respiratory therapist to help guide this.

The sensitively of the biomarker needs to be considered as move down the dose-response curve with lower emissions from cleaner stoves

Next steps: Can we learn from others who have promoted use of biomarkers in other fields?

- The Biomarkers of Nutrition for Development (BOND) program has many cross similarities with HAP and child development making it a good model to consider.
- Use of government related foundations e.g. Foundation for National Institutes of Health (FNIH) is a way to access funds and work with government.
- US FDA public-private partnerships or consortia e.g. COPD Biomarker Qualification Consortia involves a multi disciplinary team (investigators, industry, NGOs) working together to align the process to produce a comparable, pool of studies. There is value in considering this approach early in the process to achieve congruity of study approaches going forward

<u>Discussion points raised</u>

There might not be a genetic component to the variations in lung size.

- Variation might be a product of generations of exposure to various levels of air pollution and what we perceive as normal LF reflects these exposures.
- Establishing normal values for LF is difficult. However, is an absolute standard for comparison important in this situation?
    - In an intervention study, randomized groups are going to be comparable with respect to the baseline value. Before and after studies the within person comparisons remove this issue and even for observational studies there are methods to control for confounders such as this.

Validation studies are very important.

- Statistical methods are available to apply validation data to correct for exposure/outcome measurement error. Nothing is perfectly valid or reproducible; it is a matter of degrees.
- Funders such as NIH like studies to use validated, proven, biomarkers than can produce comparable data. Comparable robust results are required to influence policy.

Other considerations when identifying appropriate respiratory biomarkers include:

- Studies have shown no association between changes in ambient CO and COHb.
- Measurement of DLCO is worth considering, as there is standardized approach, but gas distribution (access and costs of transportation) can be a barrier to use.
- Field studies of lung health related to World Trade Centre dust found parallel reductions in FEV_1 and FVC, suggesting restrictive lung patterns. The study also noticed small particles ($PM_{2.5}$) deposited in distal lung. DLCO is **not** is good measure of distal lung function. Impulse oscillometry was used as a biomarker in the field on 1000 participants and appears to be worth considering for biomass studies.

3.3 Biomarkers and indicators of COPD: Evidence of change

Dr John Balmes, University of California, San Francisco and Berkeley

Presentation Summary

Biomass Smoke and COPD: There have been multiple studies conducted in several different countries evaluating the relationship between biomass smoke exposure on COPD. Notably, the RESPIRE RCT provided an opportunity to study respiratory health outcomes in mothers and their babies using. The results showed small but consistent effect across multiple symptoms. There was no difference found in the rate of decline of LF between intervention and control households. However, the 18-month measurement period was most likely too short to detect changes in LF. Exposure-response analysis showed an association between exhaled CO and FEV_1.

Romieu I et al (2009) also conducted LF testing during a study in Mexico looking at the impacts of installing an improved biomass stove. The intention-to-treat (ITT) analysis showed no effect in FEV_1, but in women who reported using the stove there was a lower decline in FEV_1 (-31 ml) vs open fire use (-61 ml) over 1-year follow-up. Again, these results indicate that a 1-year timeframe may not be a long enough follow up to see an effect.

Mechanisms: Several studies have looked at disease mechanisms after exposure to wood smoke (WS) and comparing it to TS exposure.

- Montano M et al., (2004) examined BAL fluid and macrophages in patients exposed to WS and TS, and from control subjects. Elastolytic activity increased significantly in WS and TS cells, in comparison to control subjects, with a greater effect in WS exposed people than TS.
- Biopsies comparing lungs exposed to wood verses TS found more airway fibrosis and less emphysema in wood verses TS (work by Perez-Padilla)
- Krimmer et al. (2013) exposed human pulmonary epithelial cells to tobacco smoke extract (TSE) or biomass smoke extract (BSE) and looked at fibronectin, which is released from fibroblasts. BSE had similar effects to CSE had the capacity to directly induce fibrosis, suggesting that the mechanisms by which biomass and TS exposure cause lung damage may be similar.
- The RESPIRE study looked at a sub-group to compare personal CO concentrations with gene expression in sputum cells. Higher 48-hr personal CO concentrations were associated with higher gene expression for inflammatory cytokines associated with COPD susceptibility reaching statistical significance for matrix metalloproteinases- 9 (MMP-9) and MMP-12 when comparing open fire subjects to chimney stove (Guarnieri et al., 2014)

Potential Biomarkers of Susceptibility/ Early Effect: A suite of potential biomarkers that may be appropriate for use in HAP studies are listed below, along with points of consideration regarding appropriateness:

- Accelerated decline of LF: currently is the best marker, but requires training.
- Airway inflammation and oxidative stress:
    - Blood samples: Peripheral blood markers of systemic inflammation and oxidative stress are starting to become more useful.
    - Induced sputum: has potential but not easily done on scale, also gaining sample often has social-cultural challenges.
    - Exhaled breath condensate: not yet adequately studied.
    - Exhaled NO: problematic when as studying COPD, as apposed to asthma.

- Genetic variants/epigenetic modification: might play role but still to be determined how useful they are in global population study.

Blood biomarkers might have some utility with regard to BS and COPD. Montano et al (2014) found that plasma levels of inflammatory cytokines (MMP-1, MMP-7, MMP-9, and MMP-9/TIMP-1) and CRP were higher in BS exposed women and tobacco smokers compared to non-smokers. Inverse correlations between the plasma concentrations of the same inflammatory cytokines and CRP and FEV_1 were observed. CRP was higher in BS and TS compared to non-smokers.

Lung function testing using spirometry is simple, reproducible, and inexpensive.

- Ramirez-Venegas A, et al (2014) used annual spirometry measurements to examine mean rate of decline in LF in people with COPD associated with biomass or tobacco over a 15-year follow-up period. The mean rate of decline was significantly lower for the BS exposure group than for the TS group. The study also found that women with BS-related COPD had no different survival outcomes than women with TS-related COPD.

Research Needs: Below is a high-level list that highlights the research needs in the field of HAP and COPD.

- Better RCTs of "clean" stoves in order to show an impact.
- Longer duration studies to monitor LF effect in relation to biomass (longer than 12-18 months).
- More exposure-response data for COPD and HAP.

Discussion points raised

- Peripheral markers of systematic inflammation are logistically feasible to measure in the field. The challenge is the ability to disaggregate the independent associations between peripheral markers with exposure, respiratory disease and CVD.
    - This is not easy, as COPD and CVD are so closely linked. FEV_1 is a good marker of survival for CVD. Even with statistical analysis, it hard to disaggregate.
- How often should spirometry be measured? How should the approach be standardized?
    - Pre- and post bronchodilator measurements should always be conducted. It would be useful to reach consensus on the duration between intervention and measurements.
- 'Smoking' can result in different levels of exposure depending on the intensity (frequency and amount) of smoking, type of tobacco, use of filters etc.

3.4: Susceptibility to woodsmoke

Dr Yohannes Tesfaigzi, Lovelace Respiratory Research Institute

Presentation Summary

Lovelace Smoker's Cohort study is following 2400 participants with high pack years and early COPD for over 12 years. Results show that there is an additive effect of exposure to current TS & WS on LF. Participants exposed to WS or pollution have reduced risk for chronic bronchitis if they have the p53Pro variant.

Discussion points raised

None

3.5 Early detection and impact of treatment of lung cancer

Dr William Rom, NYU School of Medicine

Presentation Summary

IARC has declared particulate air pollution a class 1 carcinogen. The available suite of biomarkers for lung cancer and their applications are described below:

- **Blood tests:** Measures of plasma osteopontin in screened lung cancers showed that the rate of increase differentiates lung cancers from controls.
- **Proteomics:** Modified single-stranded DNA aptamers that bind specific proteins was used to screen sera from patients with cancer and smoking controls. A 12-protein panel was identified that discriminates cancer-bearing subjects from controls with 89% sensitivity and 83% specificity in a separate validation cohort.
- **Integrated Diagnostics:** Mass spectroscopic proteomic analysis of plasma proteins has identified 375 proteins that can be used (along with nodule size) to help differentiate benign from malignant nodules. This assay is commercially available but expensive.
- **Autoantibodies to Tumor Associated Antigens (TAAs):** Comparison of expression levels of TAAs was used to identify a sub-set with discriminatory ability. A preliminary panel of 6 TAAs including c-Myc, Cyclin A, CDK2, were found to have good sensitivity and specificity in distinguishing cancer patients from smoking controls. To date 40 candidate TAAs have been identified and studies are on-going to optimize a potential screening panel.
- **Airway Genomics:** Ribonucleic acid (RNA) expression profiling of bronchial epithelial cells was used to identify an 80-gene expression predictor set to discriminate individuals with cancer and controls with non-malignant disease. Validation studies are under way. This test is commercially available.
- **Serum genomics:** RNA expression profiling of peripheral blood mononuclear cells has also been used to construct a 29-gene classifier set to distinguish cancer patients from high-risk smoking controls. Appropriate reduction in expression was observed after lung cancer resection suggesting this profile also has temporal sensitivity.
- **Breath Markers of Lung Cancer**: Volatile organic compounds (VOCs) in breath sample highlight many compounds altered in lung cancer, making this a potential early detection biomarker. An instrument that can provide data at site is being developed.

Discussion points raised

As low dose CT is not good for identifying incident lung cancers such as squamous cell, small cells and aggressive adeno-carcinomas. Biomarkers that will identify incident tumours are needed. Recent developments include micro RNAs and non-coding RNAs to identify early signalling pathways, but this continues to be an important area for research.

3.5: Choosing study outcomes that reflect cardiovascular disease: From 'biomarkers' to burden of disease

Dr Joel Kaufman, University of Washington and Dr Greg Wellenius, Brown University

Presentation Summary

What outcomes make sense to study in rigorous way as we move from thinking about biomarkers to outcomes? Which can be seen as a true linkage to the burden of disease?

It is not feasible to measure the outcome actually interested in (i.e. CV death, stoke in the population of interest), so clinical risk prediction is required using clinically relevant and valid surrogate endpoints.

Framework for Choosing "Subclinical" Outcomes to Study

1. What clinical outcomes are of interest?

As with the GBD this can be based on data from TS, SHS and AP exposure.

- Short-term increases in pollutant concentration are associated with triggering acute CV events
- Long-term concentration gradients associated with increased risk of CV events.

2. What pathways to these clinical outcomes can be meaningfully examined?

- There is a paradigm where air pollution exposure is *somehow* related to ischemic events, arrhythmia events or congestive heart failure: whether it is through acute or long-term exposure, it leads to progression to disease. Studies to date have looked for particle translocation, systemic oxidative stress and inflammation, and autonomic nervous system imbalance. However, in a study looking at cookstove transition, finding the initiation of any of these processes does not explain the relationship between exposure and burden of disease.
- It is of more value to look further down the final common pathway to thrombosis, atherosclerosis, altered cardiac structure and function. There is no evidence to suggest that the HAP exposures are mediating CV disease through any processes different from the processes of all other kinds of CV disease events.
- When thinking about how to connect these together and what to study, consider:
    - What will be of value and what won't depends on hypothesized process or downstream effects of interest.
    - Long-term exposure and acute vs chronic events.
    - When looking at large-scale intervention, use a few carefully selected outcomes.

3. What measures provide meaningful insight into likely contribution to burden of disease?

- Insight into mechanism is of secondary interest, as many of the mechanisms considered will have adequate biological plausibility.
    - It is not necessary to prove biological plausibility if the measures are informative and highly predictive.
    - Risk prediction is more important than mechanism.
- Time-course of effect is challenging to conceptualize, particularly when studying something that changes within a person over months to years.
    - Sometimes challenging to identify kind of change that occurs in a relatively short time that can be plausibly related to event prediction.

What Makes an Ideal Test?

- Can be measured repeatedly in individual:
    - Shown by good reproducibility, low test-test variability, and operator independent.
- Could be hypothesized to change in response to risk factor modification within months or years.

- There are examples of rapidly changing markers such as BP and HRV, which are open to the effect of confounding by time of day, stress levels etc, less quickly changing markers such as inflammatory responses, and slowly changing such as arthrosclerosis.
- In the slow changing outcomes that reflect a lifetime of exposure, do we believe that an intervention within the last month/years has affected that outcome?

- Clearly predictive of clinical CVD events/mortality where a change in measurement predicts subsequent events.
  - Is there evidence that a **change in risk factor** is related to subsequent event? Would a wider audience accept the evidence?
  - Evidence is augmented if the marker;
    - Already has a proven record of success
    - Is previously associated with a CV risk factor
    - On the disease pathway.
  - Focused on CVD prediction vs elucidating disease mechanism(s)
- Can be feasibly measured in resource-poor settings.
- Previously shown to be associated with ambient air pollution could be an advantage.

Markers of Cardiovascular Disease

If we want to affect policy and influence burden of disease, ideally we should measure incident of myocardial infarction (MI), stroke, and cardiac death but there are several challenges associated with this approach that makes it unfeasible.

- We need to consider what are the risk factors for the risk factors?
- The markers of choice are very different if the goal is on prediction of CVD events/mortality or elucidation of mechanism.

There is a suite of tests available to measure CVD risk factors. These are presented below with a brief discussion on each:

- **Blood pressure** is a major modifiable risk factor for premature CVD and is associated with many CV outcomes. It is a relatively simple test, but variability has been seen in outcomes among studies that have shown reduction in HAP (see papers by McCracken (2007) and Clark (2013).
- **Retinal Arteriolar/Venular Diameter** is a marker of micro vascular structure, associated with MI, stroke, CVD mortality. It is a non-invasive, highly reproducible test, which has been associated with AAP, but its utility in the field remains questionable.
- **Tests of Cardiovascular Function**: There are two types of CV function tests**;**

  1) Vascular tests such as flow mediated dilation, which is frequently used in the AAP sector. Reproducibility is an issue with this test.

  2) ECG-based measures such as ST-segment changes and HRV tend to indicate more about mechanism than about CV risk. They are not very good predictors of CV risk, except in some very specific populations.
- **Markers of Atherosclerosis:** Ankle brachial index is an easy to measure indicator, which is predictive of CVD events. CIMT is measured using an ultra sound scan. Reproducibly can be an issue, but it is strong predictor of CVD events.
- **Biochemical Assays:** There are many biochemical assays available, but they provide more indication about mechanism rather than predictors of CV event. CRP is an exception. Additionally, a clear correlation has been seen between HBA1c and CV risk, suggesting it has clinical significance and could be explored in HAP field.

Example of using key questions to consider blood pressure vs ideal test

- Is it meaningful to measure repeatedly in an individual? Yes, but varies a great deal within person. It is difficult to establish if the metrics are due to an acute or persistent long-term response, making time course important.
- Is it responsive to risk factor modification within months or years? Yes, it can be effectively treated.
- Is it predictive of clinical CVD or mortality? Yes
- Is it measureable in resource-poor settings? Yes
- Is it previously associated with AP? Yes, for short term, although less evidence for long-term exposures.

Study population

- When identifying the most appropriate population to study, consideration needs to be given to levels of exposure, life stage and pre-existing disease in the population of interest.
- Incidence of risk and outcomes in the population will impact sample sizes. Prior studies have mostly focused on young, adult women without overt disease and with a low risk for CVD events and mortality. This requires very large sample sizes for some study design. Using the 'at risk' group's i.e. postmenopausal women and men means a very different study population.
- Some markers are useful in the general population and others are better for diseased/high risk populations.

Discussion points raised

Policy Needs

- The causal inference in GBD assumes that a great proportion of the burden associated with HAP is attributed to CVD. If this is established, what are the policy needs to make the next strategic steps?
- Consideration of the target audience is key when considering different indicators. To make CVD a high policy priority, research is needed on disease outcomes as opposed to intermediate markers in order to make the case.
- To influence policy, it recommended that a study incorporates not just one indicator/ biomarker but rather a suite of indicators.

Study Design Considerations

Observational studies

- The most compelling studies in AAP literature have been large observational studies with adequate control of confounding. These worked to move the research from ecological studies to semi-ecological /individual level studies to establish that an exposure was strongly associated with an outcome.
- To do something comparable in the HAP arena would require a very large study with good control of confounding, as the 'at risk population' has a low rate of disease and risk for CVD events/mortality limiting the number of cases that can be detected within the study duration.
- There are other exposures besides air, which are producing underlying inflammatory responses. It is unlikely that an observational study can adjust for this and isolate the effect HAP is having.
- How low do the exposure levels need to be in order to see a CV health effect? Large observational epidemiological studies exploring the association between surrogate measures of exposure and, for example, MI rates, does not get us to where we need to be.

- The reductions necessary to achieve a health effect might be different in different diseases/ adult vs child.
- If evidence suggests that exposure to HAP causes CVD can it be assumed that with an effective intervention the disease/exposure patterns will reverse along the same pathway as they occurred? Work by Levy et al suggests that we do not know if this is the case.

Confounding

The Identification, measuring and control of confounding is challenging. Some key issues and consideration raised by the group are outlined below:

- Confounding is problematic, as people with clean cook stove technology in an observational setting are often different to people who have biomass stoves (for example differences in urban/rural/ soc-economic risk factor).
- How do you deal with confounding variables when they are so tightly correlated?
- Large samples sizes are required to properly assess the impact confounders are having.
- The issue of residual confounding requires consideration.

Cohort Studies

- It is worth considering leveraging existing longitudinal/cohort studies to explore some of the HAP and CVD questions.
- There are several other epidemiological studies that could serve to guide study design and research questions. Examples include:
  - The PURE study (see presentation 1.1. for more detail)
  - Cross-sectional study in China with has reported association with CVD and diabetes biomass v clean fuel.
  - A retrospective cohort study in Bangladesh (which has some issues with inadequate adjustment).
- A next step could be to identify what studies are currently on-going, as well as conducting a critical analysis of epidemiological evidence.
- Is the duration of a cohort study too long to wait before action can be taken?
  - Is it possible to follow some of the mechanistic pathways and then blend them with other approaches to allow these questions to be answered in a shorter time frame?

Extrapolation from AAP data

How feasible is it to extrapolate risks and associations from patterns seen in AAP data?

- If we believe AAP is causally associated with CV risk, it reasonable to think HAP (which is orders of magnitude higher) is also causing these outcomes.
  - Reasonable hypothesis but still needs to be tested
  - In many region HAP contributes to approximately 1/3 of AAP.
- Not all air pollution is the same, even within the same locations.
  - Photo-chemistry is very different between indoor generated particles even if toxic.
  - Yet, work in China looking at chemical composition of people exposed to biomass smoke found that it is not that different from a population in urban china. There was higher exposure to primary organic pollutants than in urban setting, but the same chemical components were present.
- Particles do differ, but SHS is biomass produced in an indoor environment, which results in fairly acute exposure, so it might serve as a model of what you could potentially learn from HAP.

Classification of biomarkers

Without clinical benchmarks, does grouping the biomarkers into those related to mechanism and biochemical measures verses biomarkers of CV risk differentiate these two sets of biomarkers?

- Is saying mechanism does not predict risk an over simplification?
    - Some biomarkers address both mechanism and risk (e.g. left ventricular hypertrophy (LVH) reveals something about mechanisms, but it also informs about risk prediction).

Session 4: Study Design Considerations

4.1 General Design and Analytic Considerations

Dr Kiros Berhane, University of Southern California

Presentation Summary

There are many different conceptual models to choose from. General Considerations when choosing one include:

- Objective of study will influence your study design.
- Type and nature of exposures/outcomes.
- Cost and practicality.

There are several cost-effective methods to obtaining biomarkers, for example the two-phase case control study design.

Main analytic issues include:

1. Proper model set up
2. Appropriate functional forms for relationships, including lag structures
3. Account for potential uncertainties in exposure and/or biomarker data. This is important in poor resource settings.
4. Account for mediation, when appropriate
5. Need to synthesize across exposures and outcome biomarkers in a meaningful way
6. Account for measurement error in exposure and outcome (biomarker or otherwise)

The Children's Health Study (CHS) in Californian is a good example for a model that could be used in cookstove studies.

Data on multi-pollutants and multi-biomarkers means that multi-co linearity is often an issue. It is a challenge to identify what is a real signal from what is 'noise'. It is possible to use purely statistical approaches or pathway driven approaches, which is more advisable, using analysis-modeling approaches to synthesize evidence.

4.2 Recurring issues on design and analysis

Dr Donna Spiegelman, Harvard School of Public Health

Presentation Summary

Exposure measurement error:

It is worth considering conducting validation studies to provide surrogates for exposure to PM.

- The ideal situation would be to know personal exposure to total PM from cooking sources separate from other indoor sources. The more usual situation is having data on numerous characteristics of the household and duration of certain activities, which can be used to provide a surrogate for exposure to $PM_{2.5}$ from cooking sources.
- Validation studies are then needed to look at personal exposure to actual outcome of interest in relation to all the various surrogates.
- An unexposed group is not vital, as we can still infer the exposure-response relationship as long as there is a range of exposures.
- Several analytical assumptions need to be applied to the model when taking into account multiple surrogates to produce a single odds ratio (OR), adjusted for confounding variable.

Methods for pooling and consortia:
Pooling data within the HAP sector using methods similar as those used for meta-analysis, could be an efficient way to provide robust evidence. Some considerations when pooling data include;

- It is important to include all studies in a pooled analysis and not just use a convenience sample.
- To yield a robust assessment strict inclusion criteria are required, with a clear-cut off.

Discussion points raised

How do you determine appropriate size of validation study relative to whole sample?

- The lower the correlation between the true exposure and surrogate the larger sample size needed. Often it is the case that the more validation you have the better correction you can do- however this will be determined by the budget available.

Session 5: Lessons from Other Combustion Risk Factors

5.1: What can we expect from ending exposure to biomass smoke? The example of tobacco smoke

Dr Jonathan Samet, USC Institute of Global Health

Presentation Summary

Removal of powerful risk factors should have measurable consequences. A chain of accountability/effect, which guides concepts of accountability and causal inference, is a useful tool when exploring the potential impact of reduced exposure. Such a tool can be used to start to address questions such as:

- What changes will occur in human health in response to certain actions related to air quality?
- What is the effect of increasing vs decreasing exposure?
- Does the risk associated in higher exposure go back down the same pathway when that exposure is removed/reduced?
- Is the response as expected? Finding some comparability and achieving expectations helps increase the certainty on outcomes from interventions.

Key lessons from active and passive smoking include:

- There are 7000 different toxic compounds in TS, many of which have useful biomarkers and variety of indictors.
- The many mechanisms on how smoking causes disease are well established.

- Patterns seen with biomass smoke fit well with TS for disease mechanisms.

Time course

Temporal dynamics are a critical consideration and tobacco cessation provides some insights into short and medium term phenomena.

- There is fairly extensive epidemiologic data documenting the time course of change of risk of major disease after cessations of smoking.
    - Some are fairly immediate i.e. if women stops smoking after first two trimesters the baby weight come back up substantially.
- In COPD change in symptoms post cessation occurs relatively quickly in quitters as well as those who modified their rate of smoking.
    - Airway inflammation decreases during the first month after cessation.
    - Data shows that the progressive decline in LF seen in smokers is halted and returned to the same path as that seen in those who have never smoked.
- In first six months after smoking cessation there is a 50% reduction in decline of acute CV events. This decline continues over several decades.
- Long-term tracking of consequences is needed.

What evidence is needed to influence policy at this stage?

A key question to consider moving forward is if more research is currently needed.

- If the causal associations that underlie the GBD estimates are true then perhaps there is enough information to take action.
- Evidence and understanding is sufficient on many of the mechanisms and further insight on this might not increase required evidence.

Discussion points raised

One key difference with cookstoves compared to tobacco is that different data is needed to influence changes in economic and public health policy. The burden is on the clean cooking sector to provide robust indisputable evidence, as many of the clean stove programs are potentially going to require substantial funds.

The progression towards a phase of intervention is often slowed by funders requesting evidence on relationship between HAP and disease outcomes including their mechanisms before directing funds towards intervention evaluation.

1998-2014 Committee of National Academy on Research Priorities for Airborne Particulate Matter drew up a framework to identify key uncertainties and linked them to decision-making and what evidence was needed. It might be beneficial for the HAP sector to use a similar model to organize how emissions from cookstoves damage health, identify where critical uncertainties lie and what research is need to address these.

5.2 Lessons from ambient air pollution: Evidence from the Beijing Olympics

Dr Jim Zhang, Duke University

Presentation Summary

The Beijing Heart Study aimed to mechanistically explain the impact of exposure to particles on CVD risk. Relative changes in pollutant concentrations were monitored pre, during and post the Olympic period and were tracked with relative changes in pulmonary, autonomic function, haemostasis and systemic biomarkers. The rate at which the biomarkers responded and the pathway that is responding to pollution changes was also examined.

A brief summary of the results is outlined below:

- Biomarkers responded at varying rates:
    - Biomarkers related to pulmonary inflammation and oxidative stresses were the early responders.
    - Hemostasis and systemic inflammation more gradual responders (over a 2-3 day period.)
    - Autonomic biomarkers showed no response, which could be due to young healthy study population.
- Biomarkers of cardio-respiratory risk factors improved during a 7-week intervention that resulted in an overall air quality improvement.
    - Biomarker changes were associated with individual pollutant concentrations changes within a few days.
    - Maximal changes occurred with different lag days (1-4 days) for different biomarkers.

Discussion points raised

- Pollution levels and composition will change; making it difficult to identify which pollution is related to which biomarker.
- The limited effect on SBP could possibly due to the fact that it was a young population and perhaps the change in pollution levels was not sufficient to create an impact.

5.3 Toxicant and carcinogen metabolite biomarkers: Applications in studies of tobacco use and wok cooking

Dr Stephen Hecht, University of Minnesota

Presentation Summary

Urinary Tobacco Carcinogen and Toxicant Biomarkers:

A panel of quantitative biomarkers is available to assess exposure to toxicants and carcinogens in tobacco products. The high-level characteristics of these biomarkers are provided below.

- All are shown to decrease significantly upon smoking cessation and are significantly elevated in cigarette smokers.
- Tobacco-specific biomarkers can also identify exposures of non-smokers to cigarette smoke or other tobacco products.
- They can be used to provide reliable and objective exposure data in large field studies and can identify toxicant and carcinogen exposures from cooking.
- Certain biomarkers in this panel such as cotinine, NNAL, NNN, and PheT are related to risk of lung or esophageal cancer, based on prospective epidemiologic studies.

Wok studies

There is consistent elevation of biomarkers of acrolein and crotonaldehyde exposure in non-smoking women who do regular wok cooking. Although neither is a strong carcinogen, both are respiratory irritants for which there is evidence for involvement in lung cancer etiology.

Discussion points raised

Is there a dose/response for carcinogenicity?

- For benzene there is one suggestive with animal data, but no human data is currently available.
- There is dose/ response data available for smoking as a whole but less so for individual constituents.

Summary

This section is not a comprehensive summary of the entire meeting. Rather, it briefly summarizes some of the concluding remarks and sentiments of the meeting participants.

Potential differences in the composition of different combustion sources should be evaluated. Available evidence suggests that combustion source pollution, namely AAP, TS, and HAP, share similar characteristics. In many biomass dependent regions, where HAP contributes to approximately one-third of AAP, studies have found similar chemical components in biomass smoke and AAP. At the same time, it remains unclear exactly how variability in source characteristics and transport/transformation, including photochemistry, for different sources of combustion pollution could result in different pollutant mixtures with different health effects. SHS, produced within indoor, including household environments, may be the most similar source of combustion source pollution to HAP.

Direct evidence linking HAP and CVD is needed. There is clear and direct evidence linking combustion source pollution, including HAP, AAP, SHS and active smoking, with chronic respiratory disease. While it is a reasonable and biologically plausible to interpolate HAP risk based on evidence from AAP and TS, direct evidence is needed.

The priority is to measure the impact of clean cooking adoption using the best possible predictors of chronic disease. Ideally, to influence policy and ultimately burden of disease, impacts on COPD, lung cancer, incident MI, stroke, and cardiac death should be measured, but there are several challenges associated with this approach that make it unfeasible. The goal, therefore, is to be able to measure the impact of scaling up clean cooking on reliable predictors of chronic respiratory and cardiovascular disease as accurately as possible.

There are established pathways for chronic respiratory and CVD, and no evidence to suggest that HAP exposures would mediate disease through different processes. At the same time, particularly for CVD, where direct evidence remains limited, the markers of choice are very different if the goal is prediction of CVD events/mortality or elucidation of mechanism. While some biomarkers address both mechanism and risk, most inform the mechanistic pathway. However, as many of the mechanisms considered already have adequate biological plausibility, more consideration needs to be given to establishing the 'risk factors for the risk factors'.

A suite of biomarkers have been used successfully and repeatedly in the field to predict risk of CV and respiratory disease. Blood pressure is an example of this. However, consideration needs to be given to

several factors when identifying the appropriate biomarker, such as study population, outcome of interest, whether the biomarker is responsive to risk factor modification within a relative short time period, feasibility in study setting etc.

For example, there are currently no established reference values to define normal LF or restrictive and/or obstructive lung disease that can be applied with confidence to populations in low income regions. It is unclear if there is genetic component to the variation in lung capacity seen across these regions that needs adjusting for or if the regional variations are due to poor lung health that requires no adjustment. It is clear further research is needed to clarify if separate reference values are required and if so what they should be. Until such data is available caution needs to be taken when comparing baseline LF values across different countries, ethnicity or race and relating them to normal values, which are typically Caucasian North American.

Taking into account these considerations, several questions remain on what level of intervention is required to generate an impact, what changes can be expected with effective interventions and how to unequivocally interpret these changes.

While disaggregating the independent contribution of exposure reduction will remain a challenge, the burden is on the clean cooking sector to provide robust, indisputable evidence of the impact of scaling up clean cooking on appropriate indicators and predictors of adult chronic disease.

Several design challenges must be addressed when assessing the impacts of exposure to HAP on adult chronic disease. These include:

- The use of observational study designs will require huge sample sizes and long study durations, since the 'at risk population' has a low rate of disease and risk for chronic disease events/mortality.
- There are other exposures besides air, which are producing underlying inflammatory responses. It is unlikely that an observational study can adjust for this and identify the effect HAP is having.
- It is unknown how low exposure levels need to be in order to see effects
- The identification, measurement, and control of covariates is particularly challenging in HAP studies where confounding variables are often tightly correlated.
- Action needs to be taken before the results of a chronic disease cohort would be available.

As demonstrably clean cooking technologies are taken to scale, this is an opportune moment to fill in critical gaps in the evidence base, so the public health benefits of adopting clean cooking can be more accurately assessed. There appear to be many potential opportunities to evaluate potential indicators and biomarkers for chronic respiratory and cardiovascular disease. Several of these opportunities could leverage work in progress.

- There is a need for validation/replication and use of integrative models.
- The development of a database of studies and interventions in progress would foster collaboration and replication.
- It would be useful to facilitate harmonization of methods and approaches to characterizing technologies being evaluated (clear descriptors of clean cooking technologies (stoves, ventilation, and fuels), including available lab- and field- based estimates of performance, intensity of adoption and exposure assessment.

In addition, as mentioned above, more direct evidence for HAP and CVD, as well as additional data to better connect the dots across combustion risk factors is needed. Addressing these gaps over the next

few years will inform the design of larger randomized control trials, as well as applied public health evaluations to be conducted in the near future.

The Global Alliance for Clean Cookstoves would like to thank all participants for their invaluable contributions to this informative and stimulating meeting.

Annex

1.1 Agenda

Breakfast and Informal Introductions	
8:15	Welcome and Overview *Mehta, Rosenthal*
Effects on NCDs: Evidence and Gaps	
8:30	Global variability in NCDs *Duong*
8:45	Risks for respiratory NCDs from exposure to HAP: an overview of the evidence *Bruce*
9:15	Cardiovascular effects of HAP: Evidence and gaps *Olopade*
9:45	Break
Notes from Field	
10:00	Identifying biomarkers of household air pollution exposure and cardiovascular risk in China *Baumgartner*
10:15	Biomarker feasibility en el campo: lessons learned from Honduras and Nicaragua *Clark*
10:30	Biomarkers: Potential lead in cookstove-health study *Dutta*
10:45	Ambulatory blood pressure in GRAPHS *Jack*
Anticipating Future Health Benefits: Measuring biomarkers and indicators of NCDs	
11:00	Biomarkers of exposure for HAP exposure assessment – our experience with urinary biomonitoring of polycyclic aromatic hydrocarbon metabolites *Naeher*
11:30	Household air pollution: biomarkers of exposure and the search for predictors of respiratory disease *Martin*
12:00	Biomarkers and indicators of COPD: Evidence of change *Balmes*
12:20	Susceptibility to Woodsmoke *Tesfaigzi*
12:25	Early detection and impact of treatment of lung cancer *Rom*
12:45	Lunch
13:30	Choosing study outcomes that reflect cardiovascular disease: From 'biomarkers' to burden of disease *Wellenius, Kaufman*
Study Design Considerations	
15:00	General Design and Analytic Considerations *Berhane*
15:15	Recurring issues on design and analysis *Spiegelman*
Lessons from Other Combustion Risk Factors	
15:30	Lessons from ambient air pollution: Evidence from the Beijing Olympics *Zhang*
16:00	What can we expect from ending exposure to biomass smoke? The example of tobacco smoke *Samet*
16:30	Toxicant and carcinogen metabolite biomarkers: Applications in studies of tobacco use and wok cooking *Hecht*
17:00	Summary and Next Steps *Mehta*

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