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Conference Summary

Biomarkers of tobacco exposure or harm: Application to clinical and epidemiological studies

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Adverse outcomes from tobacco use may take decades to develop. Biomarkers are measures that can be used in the early stages of tobacco use to assess exposure to tobacco toxins or to predict adverse health outcomes with which they are associated. Examples of biomarkers include specific chemical components of tobacco or their metabolites; early biochemical, histological, or physiological effects; and early health effects. Mechanistically relevant and quantitatively valid biomarkers are essential for assessing the ultimate impact of new products, treatments, preventive measures, and public health policies on tobacco-related disease. The tobacco industry's recent introduction of a variety of new tobacco products or devices with implied claims of reduced health risks highlights the need to develop methods for assessing their potential for benefit or harm. A wide variety of biomarkers for tobacco exposure or harm has been studied. Although many questions about their use remain unanswered, substantial data exist regarding their validity and utility. This conference reviewed both the general issues surrounding biomarker use and the current state of knowledge regarding the most widely studied and promising biomarkers.

Scope of the Conference

In 2001, the Institute of Medicine issued the report *Clearing the Smoke*, which assessed the science base for tobacco harm reduction (Stratton, Shetty, Wallace, & Bondurant, 2001). This report noted the need to study the potential role of exposure and harm reduction strategies as part of a comprehensive approach to preventing and treating tobacco dependence. A major conclusion of this report was that the impact of harm reduction strategies ultimately must be measured by their effects on health outcomes. Reducing the content of a toxin in a tobacco product cannot be assumed to reduce the exposure of smokers to that toxin, nor can reduced exposure be assumed to result in reduced harm. These outcomes need to be verified experimentally, with consideration for potential changes in individual or population-wide smoking behaviors. Because the most common and important adverse health outcomes associated with tobacco use (cancer, cardiovascular disease, chronic lung disease) are delayed in onset, surrogate measures or biomarkers are needed to provide more timely assessment of the potential for harm reduction. The conference summarized in this paper was intended to review the state-of-the-art with regard to the selection, validation, and potential uses of biomarkers of tobacco exposure or harm (Table 1). Particular attention was paid to the kinds of information needed to inform clinical, regulatory, and public health decisions regarding products, interventions, and policies aimed at exposure or harm reduction.

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Table 1. Potential biomarkers of tobacco exposure or harm

Tobacco/nicotine exposure
Cotinine
Anabasine
Anatabine
Environmental tobacco smoke
Nicotine
Carcinogenesis
1-Hydroxypyrene in urine
Trans, trans-muconic acid in urine
NNAL, NNAL-glucuronide in urine
4-Aminobiphenyl hemoglobin adducts
DNA and hemoglobin adducts of other tobacco smoke carcinogens
Genetic polymorphisms in carcinogen-metabolizing enzymes such as CYP1A1 and GSTM1
Cardiovascular and pulmonary effects
Fibrinogen
Leukocyte count
C-reactive protein
F2 isoprostanes
Postischemic vasodilation
Spirometry
Differential white blood cell count in bronchioalveolar lavage fluid
Pregnancy
Maternal carbon monoxide
Maternal or fetal (umbilical cord, urine, or amniotic fluid) cotinine
Fetal heart rate, movement, breathing movements, vascular resistance (Doppler)

This list is limited to some of the better-studied biomarkers. See text for abbreviations.

Presentations

Overview

Biomarkers and the evaluation of harm reduction

Mitchell Zeller, American Legacy Foundation, Washington, DC

Suitable biomarkers are needed urgently to allow the study of putative harm reduction products and strategies. The train has already left the station, in a manner of speaking, in that the tobacco industry is introducing a wide array of new tobacco products with implied claims of exposure and harm reduction (Anonymous, 2002). The introduction of such products is occurring in advance of scientific study and data with which to assess these claims. It is important for science to inform this debate. Reducing the toxin content of a product does not necessarily reduce toxin exposure. In the case of light cigarettes, reduced tar and nicotine delivery as measured by machines did not result in reduced toxin delivery to smokers because smokers compensated for reduced nicotine delivery by altering their manner of smoking (U.S. Department of Health and Human Services, 2001). In addition, we cannot assume that exposure reduction necessarily results in harm reduction. Harm reduction may fail to result for a variety of reasons including: (a) changes in product formulation that deliver less of one toxin but more of others, (b) increases in the content or delivery of as-yet-unidentified toxins that would not be recognized by current methods, (c) compensatory changes in smoking behavior that mitigate changes in product formulation or toxin delivery, or (d) population changes such as fewer smokers opting to quit because they think that newer products offer less risk. The regulatory process must be brought to bear on the evaluation and approval of new tobacco products and devices. Premarketing evaluation is an essential component of this review process so that the accuracy of harm reduction claims can be assessed prior to the widespread use of these products and so that the public, health care, and public health communities can make informed decisions. An obvious imbalance exists between the regulatory scrutiny applied to pharmaceutical products and that applied tobacco products (''Tobacco Dependence,'' 1998). More scrutiny of tobacco products, and more flexibility regarding pharmaceuticals aimed at smoking cessation, would be appropriate.

Epidemiology of low-yield cigarettes: How did we get it so wrong?

David M. Burns, University of California, San Diego

Given the demonstrated relationship between tar and lung cancer (Wynder, Graham, & Croninger, 1953), the use of low-tar, low-nicotine cigarettes was expected to result eventually in lower lung cancer rates, as long as smokers did not increase the number of cigarettes they smoked or change their smoking behavior in other ways. Examinations of the epidemiology of smoking and lung cancer death rates in the decades following 1970 showed decreasing per-capita consumption of cigarettes and increasing use of low-yield cigarettes, but the expected decrease in lung cancer death rates did not occur. A direct comparison of the lung cancer death rate of smokers in 1959–1965 with that of smokers in 1982–1988 in two American Cancer Society studies indicated that the rates of lung cancer went up rather than down during this period (Thun & Heath, 1997). Two important reasons for the health community's failure to predict this result were: (a) an underestimation of the extent to which tobacco companies would engineer cigarettes and (b) a failure to examine what people actually do rather than accepting the machine-determined yields of cigarettes. Smokers of low-yield cigarettes often covered the ventilation holes designed to decrease tar levels and took deeper, longer puffs. Studies of large samples of smokers also indicated that those who switched to low-yield cigarettes tended to increase the number of cigarettes they smoked per day (Burns, Shanks, Major, Thun, & Samet, 2001). These observations indicate that: (a) the changes in cigarette design and manufacturing over the past several decades have not contributed importantly to reducing the disease burden caused by cigarette use, and (b) measurements of tar and nicotine yields using the Federal Trade Commission method do not provide smokers with meaningful information

about the amounts of tar and nicotine they will receive from a cigarette or about the relative amounts they are likely to receive from smoking different brands (U.S. Department of Health and Human Services, 2001).

Nicotine and tobacco alkaloids

Nicotine and its metabolites

Neal Benowitz, University of California, San Francisco

Nicotine occupies a unique place as a biomarker of tobacco exposure because it is both the primary addictive component of tobacco and a potential toxin. Nicotine intake is regulated by smokers, so that changes in nicotine delivery can result in compensatory changes in smoking behavior (Benowitz et al., 1986). External exposure data (number of cigarettes per day) is an unreliable measure of nicotine delivery. Nicotine itself is not a suitable measure of the internal (absorbed) dose because its short half-life of about 2 hr results in substantial changes in serum nicotine concentrations over the day.

Approximately 80% of nicotine is transformed to cotinine in humans (Benowitz & Jacob, 1994). Because cotinine has an elimination half-life of 16 hr, serum levels vary less than those of nicotine over the day. Serum, saliva, or urine cotinine concentrations have been widely used to estimate nicotine exposure in clinical and epidemiological studies. The validity of this measure depends on how much variability is seen in nicotine and cotinine metabolism between individuals. In fact, nicotine and cotinine clearance may vary up to threefold (Benowitz, Jacob, Jones, & Rosenberg, 1982). Nicotine is converted to cotinine primarily by the hepatic P-450 enzyme CYP2A6 (Tyndale & Sellers, 2001). Nicotine and cotinine also are metabolized by conjugation to glucuronides, and some nicotine is excreted unchanged in urine. Each of these processes is subject to individual variability. Chinese and Hispanic smokers have a lower nicotine clearance than do White or Black smokers, and this lower clearance (resulting in higher serum nicotine concentrations for the same nicotine intake) may be responsible for the lower nicotine intake observed in Chinese and Hispanic smokers (Benowitz, Perez-Stable, Herrera, & Jacob, 2002).

Genetically determined differences in CYP2A6 activity may underlie some of these differences in smoking behavior and health outcomes. Nicotine and cotinine clearance are increased during pregnancy and may account in part for the lower serum cotinine concentrations observed during pregnancy and for underestimation of the nicotine intake of pregnant smokers (Dempsey, Jacob, & Benowitz, 2002). Nicotine clearance also is substantially lower in patients with renal failure (Molander, Hansson, Lunell, Alainentalo, Hoffmann, & Larsson, 2000). In view of this variability, the most straightforward and reliable use of cotinine determinations may be serial measurements in the same subject, a procedure well suited to the assessment of new tobacco products or harm reduction strategies. Cotinine remains the best measure of smoking status, but comparisons across populations or medical conditions should consider the influence of differences in nicotine or cotinine metabolism when estimating nicotine exposure. An important implication of these studies is that the same complexity and individual variability found with nicotine and cotinine pharmacokinetics may apply to other biomarkers and tobacco toxins.

Minor tobacco alkaloids

Peyton Jacob III, University of California, San Francisco

Nicotine exposure can be estimated by measurement of serum, saliva, or urine concentrations of the nicotine metabolite cotinine (Benowitz & Jacob, 1984, 1994). However, these measures cannot be used for studies in which subjects receive nicotine replacement therapy, in which nicotine or cotinine may reflect the therapy rather than tobacco use. Minor tobacco alkaloids are potential alternatives because their only important source is tobacco. Anabasine and anatabine are the best-studied minor tobacco alkaloids. They are structurally related to nicotine and are present at about 0.2%–1% the concentration of nicotine in tobacco (3–15 μg/cigarette) (Schmeltz & Hoffman, 1977). Both alkaloids have biological activity in vitro similar to that of nicotine but likely little in vivo activity owing to their lesser potency and the low dose absorbed. Their elimination half-lives are 16 and 10 hr, respectively, similar to that of cotinine (Jacob, Yu, Shulgin, & Benowitz, 1999). Anabasine and anatabine are readily measured in urine by gas chromatography–mass spectrometry, and levels are about 1%–2% those of cotinine (Jacob, Yu, Liang, Shulgin, & Benowitz, 1993; Jacob et al., 1999). The correlation of anabasine ($r = .7$) and anatabine ($r = .62$) with nicotine intake in a study population is significant but lower than that of cotinine ($r = .8$). Urine anabasine and anatabine levels of greater than 2 ng/ml reliably detect current smokers (100% specificity), but 23%–36% of persons reporting tobacco use while attempting to achieve cessation had lower levels (false negatives). Analytical methods that can measure lower anabasine levels, such as liquid chromatography–tandem mass spectrometry, may improve assay sensitivity. The extent to which the ratios of anabasine or anatabine to nicotine in tobacco vary among products is not clear, although ratios found in several brands of cigarettes were fairly constant (Jacob et al., 1999). Solanesol is a nonalkaloid present in tobacco at relatively high, though variable, concentrations (10–500 μg/cigarette). Typical saliva levels are 50 times higher in smoker than in nonsmokers. The utility of solanesol as a biomarker of tobacco exposure is being studied.

Environmental tobacco smoke

Measurement of environmental tobacco smoke exposure
Katharine Hammond, University of California, Berkeley

Environmental tobacco smoke (ETS) is a complex mixture of chemicals that contains a number of possible markers. The most commonly measured markers are nicotine and particulate matter, but others include solanesol and scopoletin, polonium-210, aromatic hydrocarbons, 3-ethenyl pyridine, myosmine, carbon monoxide, nitrosamines, and tobacco-specific nitrosamines. Nicotine has several advantages as a marker: It is highly correlated with a number of other contaminants in tobacco smoke; it is specific to ETS; it is a major constituent of ETS; and low concentrations can be measured accurately, at a reasonable cost. Passive monitors for measuring nicotine in air have been devised that can monitor an area or be used as a personal monitor to measure the exposure level of a particular person (Hammond & Leaderer, 1987). Studies in real-world settings indicate that concentrations in an environment (e.g., a particular home or office) are often widely variable, even when concentrations are averaged over a long period of time for each measurement. Measurements in homes typically vary between 1 and 5 $\mu g/m^3$. Workplaces that allow smoking often have median concentrations of 8 $\mu g/m^3$, but some are as high as 30 or 40 μ/m^3 (Hammond, 1999). Studies that have measured airborne ETS concentrations have had important policy implications demonstrating, for instance, that flight attendants can receive significant ETS exposure during flights, that high ETS concentrations can occur in nonsmoking sections of restaurants, and that smoking policies in offices are effective in decreasing ETS concentrations (Hammond, 1999).

Cigarette equivalents, or the ratio of the amount of exposure to chemicals from the sidestream smoke from one cigarette to the amount obtained from smoking one cigarette, are often used to make the health effect of ETS appear trivial. It is important to realize, however, that great variability exists in the level of different chemicals in sidestream smoke when compared with mainstream smoke (i.e., the amount inhaled by the smoker). The ratio of sidestream to mainstream smoke of nicotine is 2, whereas that of 4-aminobiphenyl is 31 and that of ammonia is 60. A simple comparison of active smoking to ETS exposure is not possible; one has to compare these exposures chemical by chemical.

Carcinogenesis

Urinary markers of carcinogen uptake
Stephen Hecht, University of Minnesota, Minneapolis, MN

This presentation reviewed urinary biomarkers of tobacco carcinogen uptake (Hecht, in press). Urinary biomarkers for some of the principal carcinogens are available. For example, 1-hydroxypyrene is a good biomarker of uptake of carcinogenic polycyclic aromatic hydrocarbons, and *trans*, *trans*-muconic acid is a useful biomarker for uptake of the human carcinogen benzene. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides are excellent biomarkers of uptake of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific lung carcinogen. The NNAL assay has been applied widely in assays of urine from smokers and other tobacco users (Carmella, Akerkar, & Hecht, 1993; Carmella, Akerkar, Richie, & Hecht, 1995; Hecht et al., 1999, 2002). It also has been used to investigate ethnic differences in NNK metabolism and the effects of diet and chemopreventive agents on NNK metabolism in humans (Hecht et al., 1995; Richie, Carmella, Muscat, Scott, Akerkar, & Hecht, 1997; Taioli, Garbers, Bradlow, Carmella, & Hecht, 1997). The NNAL assay has been particularly useful for studies in nonsmokers exposed to ETS. In several studies, levels of NNAL and its glucuronides in the urine of people exposed to ETS were significantly elevated compared with control subjects (Anderson et al., 2001; Hecht et al., 1993, 2001; Parsons, Carmella, Akerkar, Bonilla, & Hecht, 1998).

Hemoglobin adducts

Paul Skipper, Massachusetts Institute of Technology, Cambridge, MA

This discussion reviewed the use of 4-aminobiphenyl hemoglobin adducts as a biomarker of uptake and metabolic activation of the known human bladder carcinogen, 4-aminobiphenyl. This adduct is reproducibly detected at elevated levels, compared with nonsmokers levels, in smokers' hemoglobin (Bryant, Vineis, Skipper, & Tannenbaum, 1988). Levels decrease after cessation of smoking (Maclure, Bryant, Skipper, & Tannenbaum, 1990). A large case–control study of bladder cancer was completed recently in Los Angeles County (Skipper, Yu, Ross, Gan, & Tannenbaum, 2001). This study used 4-aminobiphenyl hemoglobin adducts as well as hemoglobin adducts of other aromatic amines as biomarkers. The results showed a statistically significant relationship between bladder cancer incidence and 4-aminobiphenyl-hemoglobin adducts. Similar data were observed for most of the other aromatic amine adducts. The effect was stronger in women than in men (Castelao et al., 2001).

Genetic polymorphisms: Relationship to carcinogenesis

Peter Shields, Georgetown University Medical Center, Washington, DC

This presentation reviewed the role of environment and genetics in tobacco-induced cancer and the possible use of biomarkers to study the relevant interactions (Shields, 2000; Shields & Harris, 2000). Carcinogens in cigarette smoke are the DNA-damaging

"environment;" and polymorphisms in various genes involved in metabolic activation, detoxification, and DNA repair are the interacting genetic component. Some gene polymorphisms, such as CYP1A1 and GSTM1, contribute to the metabolic activation and detoxification of carcinogens. Ultimately it may be possible with gene array approaches to measure multiple genotypes in an individual and thus to determine risk for cancer on exposure to tobacco smoke carcinogens. Studies to date using ^{32}P-postlabelling for DNA adduct detection and quantitation have provided some information on adduct levels in human lung, and at least one prospective study has found a relationship between DNA adduct levels and lung cancer induction (Tang et al., 2001).

Histologic biomarkers of carcinogen exposure

William Rom, New York University Medical Center, New York, NY

The *p53* tumor suppressor gene and the *K-ras* oncogene frequently are mutated in human lung cancer (Hecht, 1999). Studies have attempted to map the mutations induced in these genes by activated carcinogens (Chen, Zheng, West, & Tang, 1998). These studies have found that activated carcinogens such as benzo[*a*]pyrene-7,8-diol-9,10-epoxide react preferentially at CpG sites (deoxycytidine attached through phosphate to deoxyguanosine) in these genes and at codons that often are mutated in human cancer. These results are consistent with the hypothesis that cigarette smoke carcinogens cause the mutations seen in these tumors. The complex interaction of oncogenes, tumor suppressor genes, growth factors, and other cellular regulators on cell cycle control and cell growth is likely important in the pathogenesis of lung cancer. Abnormalities in these genes, as caused by cigarette smoke carcinogens, are involved in lung tumor induction (Sekido, Fong, & Minna, 1998). The detection of such changes with bronchoscopy and other techniques promises to lead to early detection of lung cancer, which would be important in improving cure rates.

Cardiopulmonary effects

Cardiovascular disease mechanisms

Neal Benowitz, University of California, San Francisco

Potential mechanisms for tobacco-induced cardiovascular disease include relative hypoxemia, endothelial dysfunction, lipid abnormalities, hemodynamic stress, coronary vasoconstriction, inflammation, hyperhomocystinemia, insulin resistance, and enhanced arrhythmogenesis. Thrombosis plays a greater role in the risk of acute cardiac events in smokers than in nonsmokers. Chemicals in tobacco smoke suspected to contribute to cardiovascular disease are oxidizing gases, carbon monoxide, and nicotine (Benowitz & Gourlay, 1997).

The evidence for smoking-induced thrombosis includes enhanced platelet aggregation, decreased plasminogen activator release, increased levels of plasminogen activator inhibitor, and increased blood viscosity. Cigarette smoke produces high levels of oxidant stress. This stress is manifest by increased levels of oxidized low-density lipoprotein cholesterol, which is highly atherogenic, as well as other proteins such as fibrinogen. Oxidant stress induces endothelial dysfunction and reduces nitrous oxide release and nitrous oxide-mediated vasodilation. Oxidation of free fatty acids generates isoprostanes, which are associated with platelet activation. Carbon monoxide binds to hemoglobin and reduces oxygen delivery; it also decreases the amount of exercise required to precipitate angina or peripheral claudication and decreases the threshold for ventricular fibrillation. Chronic carbon monoxide exposure leads to polycythemia, which also contributes to hypercoagulability and thrombosis.

Nicotine may contribute to vascular injury by increasing blood pressure and heart rate and, therefore, myocardial oxygen demand. Nicotine also results in coronary vasoconstriction and may contribute to endothelial dysfunction (Nitenberg & Antony, 1999). Treatment of cardiovascular disease with transdermal nicotine may result in higher plasma nicotine levels but less cigarette consumption and less exercise-induced reversible myocardial ischemia (Mahmarian et al., 1997) and does not pose increased risk compared with placebo (Joseph, Norman, Ferry, Prochazka, Westman, & Steele, 1996).

Biomarkers can be used to assess inflammation, oxidative stress, endothelial cell dysfunction, and thrombotic pathways that contribute to cardiovascular risk. Biomarkers of inflammation include fibrinogen levels, leukocyte count, and C-reactive protein. Oxidative stress can be assessed by measuring vitamin C levels, F2 isoprostanes, and other oxidized proteins such as fibrinogen. Endothelial cell function can be assessed with noninvasive measurement of postischemic vasodilation, P-selectin, and ICAM. Hemostasis can be assessed by measuring red blood cell mass and markers of platelet activation. Other potential biomarkers of tobacco-induced cardiovascular disease are homocysteine; total, high-density lipoprotein, and low-density lipoprotein cholesterol; and markers of insulin resistance. However, the relationship between these surrogate endpoints and clinical disease states is variable; therefore, their ability to predict clinical outcomes is limited.

Clinical markers of cardiovascular disease

Ed McFalls, Minneapolis Veterans Administration Medical Center, Minneapolis, MN

Clinical markers of tobacco-induced cardiovascular disease are challenging to measure because large sample sizes are required to capture endpoints such

as death, myocardial infarction, stroke, transient ischemic attacks, and loss of limb (peripheral vascular disease). Cardiac studies such as coronary angiography, coronary Doppler flow velocities (Tanaka, Oka, Tawara, Sada, & Kira, 1998), and coronary venous thermodilution are accurate techniques for diagnosing obstructive coronary artery disease but are too invasive to be practical in studies involving large numbers of participants. Magnetic resonance imaging and ultrafast computed tomography (CT) scanning are evolving technologies that may help quantify atherosclerotic burden, but they may not be feasible because of high cost and low availability.

The most practical techniques that are clinically relevant provoke a supply-demand mismatch by increasing oxygen demand (usually by increasing cardiac work) or decreasing oxygen supply (by decreasing coronary perfusion). The extent of myocardial ischemia in response to exercise can be quantified using a combination of the 12-lead electrocardiogram and nuclear medicine tracers of perfusion with single photon emission CT (SPECT) (Mahmarian et al., 1997). Cardiac imaging with positron emission tomography (PET) has generated the most interest in noninvasive testing, because it can quantitate regional myocardial blood flows accurately with serial interventions (Czernin, Sun, Brunken, Bottcher, Phelps, & Schelbert, 1995; Deanfield, Shea, Wilson, Horlock, de Landsheere, & Selwyn, 1986; Kaufmann et al., 2000). Although nuclear medicine studies with PET and SPECT have the advantage of high predictive accuracy, they are not ideal because of high cost and radiation exposure. Two-dimensional echocardiography with Doppler techniques provide an alternate noninvasive means of estimating myocardial ischemia, with measurements of coronary flow velocities in response to pharmacological vasodilators such as adenosine. Two-dimensional echocardiography stress tests also can identify regional wall motion abnormalities in response to an infusion of dobutamine.

The sensitivity of any noninvasive testing for the detection of coronary artery disease is variable and may be an important factor in test selection. For example, exercise electrocardiogram testing is approximately 50%–60% sensitive, whereas imaging with echocardiography and SPECT is approximately 85% sensitive. Noncardiac markers also have gained interest, particularly regarding atherosclerosis in cerebral and peripheral vascular arteries. Doppler ultrasound can be used to measure intimal-medial wall thickness of the carotid arteries serially. By calculating the ankle-brachial index or the severity of claudication measured during a fixed exercise time or distance, one can assess the extent of atherosclerotic burden in lower extremity vessels with serial measurements (Yataco & Gardner, 1999). Subjective assessment of quality of life in patients with cardiovascular disease can be described with the Short Form 36-Item Health Survey, Sickness Impact Profile, or Seattle Angina Questionnaire.

Biomarkers of tobacco-associated pulmonary disease

Steve Rennard, University of Nebraska Medical Center, Omaha, NE

Chronic obstructive pulmonary disease has heterogeneous manifestations that include chronic bronchitis and emphysema, and it can progress in a gradual or stepwise fashion. Biomarkers of pulmonary disease associated with tobacco use can be identified in exhaled air, sputum, bronchoscopy (bronchoalveolar lavage, brushings, biopsy of bronchial epithelium), and lung tissue samples. The lungs can be imaged using high-resolution computed tomography scans that are noninvasive and can quantify changes such as emphysema. The lungs can be tested functionally using spirometry, which is accurate and inexpensive. Pulmonary function declines in nonsmokers after early adulthood but at a more rapid rate in smokers (Piquette, Clarkson, Okamoto, Kim, & Rubin, 2000). Smoking cessation is associated with slight improvement in pulmonary function shortly after cessation, followed by restoration of the slope of the age-related decline in pulmonary function observed in nonsmokers (Anthonisen et al., 1994).

Hydrogen peroxide levels reflect oxidant burden and can be measured in condensate from exhaled air. Levels are increased in smokers but remain elevated in former smokers, suggesting that inflammatory damage may continue even after smoking cessation (Nowak et al., 1996). Hydrogen peroxide levels vary with severity of chronic obstructive pulmonary disease and degree of disease activity. Spontaneous or induced sputum samples can be used to obtain cell counts and differentials as well as epithelial and potentially malignant cells. Differential cell counts in the sputum of smokers show higher proportions of eosinophils and neutrophils than in nonsmokers. Potential cell markers include pigment-laden macrophages and the presence of CD8 lymphocytes. Markers of lower respiratory tract inflammation, such as polymorphonuclear cells, improve after smoking cessation and can be followed up in intervention studies (Skold, Hed, & Eklund, 1992). Other lung sampling techniques, such as bronchoalveolar lavage, can improve the precision of obtaining samples (e.g., avoiding contamination with saliva) (Klech & Pohl, 1989). Bronchoalveolar lavage in smokers yields more cells than in nonsmokers, with a higher proportion of pigment-laden macrophages. These cells can persist for at least 1–2 years after smoking cessation, but the time course for normalization of cell counts is not known. In addition, increased neutrophil counts obtained from bronchoalveolar lavage are better correlated with sputum production than with clinical outcomes, such as air flow. Neutrophil counts, however, are well correlated with exercise tolerance tests.

Pregnancy

Biomarkers of fetal outcomes
Cheryl Oncken, University of Connecticut Health Center, Farmington, CT

Maternal smoking contributes to a number of poor pregnancy outcomes including spontaneous abortion, placenta previa and abruption, low birth weight, sudden infant death syndrome, childhood learning problems, and probable increased risk of smoking in offspring (Walsh, 1994). These risks are dose related to maternal smoking. The most accessible biomarker of fetal outcome is birth weight, which increases with smoking cessation and smoking reduction (Li, Windsor, Perkins, Goldenberg, & Lowe, 1993). Birth weight is sensitive to tobacco effects, and studies using this continuous outcome require far fewer subjects than do studies that follow infrequent endpoints such as spontaneous abortion or sudden infant death syndrome. In pregnant smokers, the biomarkers cotinine and exhaled carbon monoxide (CO) have the advantages of being easy to obtain and relatively inexpensive; however, cotinine is not useful in trials that include nicotine replacement therapy, and exhaled CO may be less reliable with the low levels of smoking frequently observed during pregnancy. The lower limit of exhaled CO to confirm abstinence from smoking may need to be reduced in pregnancy because of higher minute ventilation. For example, one study found that an exhaled CO level of less than 5 ppm predicted low birth weight (Secker-Walker, Vacek, Flynn, & Mead, 1997). Thiocyanate also has been used to verify smoking status in pregnancy, it is easy to obtain, and it predicts birth weight (Hebel, Fox, & Sexton, 1988). However, this measure is less specific than other measures of tobacco exposure.

It is unclear which components of tobacco smoke, either alone or in combination, cause low birth weight. The most widely studied components have been carbon monoxide and nicotine. Animal research suggests that either CO or nicotine decreases birth weight, but CO has more deleterious effects (Dempsey & Benowitz, 2001). Human studies suggest that nicotine alone does not cause low birth weight.

Other potential assessments include indirect measurements of vascular resistance (obtained by Doppler) and measures of fetal well-being such as fetal breathing movements and fetal heart rate and variability. Physiological measures vary with gestational age and have the disadvantage of being highly variable when studied in the acute setting; they are more reliable in the third trimester of gestation and as serial measurements in longer-term studies. Biomarkers of fetal exposure or effects can be obtained from maternal sources (saliva, exhaled CO, blood, urine), umbilical cord blood, fetal urine, amniotic fluid, and placental pathology. Metabolites of a tobacco-specific carcinogen, NNAL and NNAL-gluc, have been measured in the first voided urine of infants exposed prenatally to tobacco smoke (Lackmann, Salzberger, Tollner, Chen, Carmella, & Hecht, 1999).

New products

Assessment of new nicotine delivery devices
Jack E. Henningfield, Johns Hopkins University School of Medicine, Baltimore, MD, and Pinney Associates, Bethesda, MD

Since the late 1990s, a variety of new nicotine delivery devices have been marketed as tobacco products (Stratton et al., 2001). Although these products utilize tobacco or tobacco-derived materials, they have different operation or physical make-up compared with conventional products. The introduction of these diverse products will challenge present methods of assessing tobacco use and toxin exposure using biomarkers and behavioral measures. This difficulty arises from differences in the chemistry of the products and potential differences in the relationship among various biomarkers as compared with conventional tobacco products such as cigarettes and smokeless tobacco. Scientific challenges raised by these products include identifying appropriate biomarkers and determining the dose–response relationships among them and in relation to behavioral measures (e.g., frequency of use).

Modified cigarettes, such as Omni and Advance, use tobacco-derived material that produces significantly different smoked chemistry, compared with conventional cigarettes, through novel process and manufacturing methods, and they contain additives such as palladium-containing filter materials. Cigarette substitutes, such as Eclipse and Accord, use carbon and electrical heating elements and, compared with conventional cigarettes, provide significantly different ratios of carbon monoxide and other constituent intake in relation to markers such as nicotine and cotinine. Novel oral products include oral snuff (e.g., Exalt) and a tobacco-derived lozenge (Ariva), in which different processing methods and perhaps different additives may alter the ratio of potential tobacco markers such as anabasine to nicotine. The extent to which new potential biomarkers are needed is unknown. Similarly, the extent to which new measures of toxicity are needed to evaluate new materials, such as the glass fibers released by Eclipse, the palladium contained in Omni, and the various metals and other materials used to construct the heating and insulating system of Accord, is not clear. The marketing of many of these products for use when smoking is not allowed potentially complicates researchers' ability to use conventional biomarkers developed and validated on the assumption that most tobacco users used a single product and that dose–response relationships were reasonably well understood. A major implication of these developments is that a strong commitment to

research will be required to investigate the new products, their design and chemistry, and their patterns of use and to determine appropriate biomarkers. In some cases the biomarkers might be the same as existing, commonly used biomarkers, but the dose–response relationships among them may vary; in other cases altogether new biomarkers might be required for studies to determine extent of use, dependence, or health effects.

Panel discussion and conclusions

Dietrich Hoffman, American Health Foundation, Valhalla, NY
Neal Benowitz, University of California, San Francisco
Jack Henningfield, Johns Hopkins University School of Medicine, Baltimore, MD
Mitchell Zeller, American Legacy Foundation, Washington, DC

Presently we are faced not only with scientific challenges but also with public health and regulatory challenges. Public health challenges include determining whether the new products reduce overall risk of tobacco disease if they are fully or partially substituted for conventional tobacco products, as well as the total public health impact of these products, including their potential to undermine prevention and cessation efforts. Regulatory challenges include determining whether the advertising claims for the products, which in some case imply reduced risk of cancer, are valid, and whether the products should be regulated as tobacco products, drugs, foods, or drug delivery devices. Because none of these products has been introduced following regulatory review, little information is available about them, other than what their developers offer. The physical nature of the products and how they are being marketed has varied widely and will complicate efforts to develop biomarkers and guidelines for biomarker use (e.g., appropriate thresholds to determine if use has occurred).

The science and public health communities must move rapidly, decisively, and with consensual agreement and comprehensive strategic plans in this area. Independent testing of these products is necessary, and, though limited, biomarkers for measuring tobacco toxins are available. For example, we have at our disposal measures to assess various classes of exposures such as: (a) nicotine, cotinine, and tobacco alkaloids, (b) carcinogens as measured by nitrosamines and polycyclic aromatic hydrocarbons, (c) gaseous toxins as measured by carbon monoxide and oxidant gases, and (d) disease markers. Assessments can be conducted using these indicators of exposure, even with the understanding that some of the biomarkers may or may not be associated with untoward health consequences and that not all tobacco toxins present in these changing tobacco products have been identified.

In assessing tobacco toxin exposure, it is also important to consider factors that contribute to variability of responses to tobacco toxin exposure and disease susceptibility. Factors associated with individual differences include: (a) pattern of smoking resulting from ethnic, sociocultural, genetic, and income sources, (b) metabolism of nicotine based on genetic and ethnic differences, and (c) the interaction between gene mutations associated with cigarette smoking and disease, or vice versa (i.e., the interaction between disease susceptibility and cigarette smoking).

Procedures for medication testing are currently available, and a parallel process should be followed for tobacco- or cigarette-like products. The first step would involve product characterization, that is, examining and identifying the constituents within the product itself. The second step would be *in vitro* and animal testing using tests such as DNA binding or mutations. The challenge would be in examining mixtures of compounds and determining whether a change in balance of these constituents affects toxicity. Completion of this step would then lead to human clinical trials, which would follow the same phases of testing involved in the development of medications. Phase I would include testing the product in a short-term (e.g., 1 week) study using exposure markers (e.g., nicotine) and effect markers (e.g., platelet activation, inflammatory response). If the markers assessed in these short-term trials show no evidence of increased exposure to tobacco toxins compared with conventional cigarettes, then the product could proceed to Phase II testing, in which the humans are exposed to the products for a minimum of 3–4 months. This phase would test exposure and effects using biomarkers associated with longer half-lives or that take longer to show a response (e.g., nitrosamines, DNA adducts, and lipid profile). Trials would proceed to Phase III if the tested products or strategies do not result in higher exposure than existing products. These assessments must include a dose–response curve to represent the extent of possible exposure. Finally, a surveillance system is needed to monitor the extent to which a product or strategy leads to initiation or reuptake of tobacco use or to fewer smokers choosing to quit.

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