1. *"Minimum clinically important differences for the primary outcome in the core randomised controlled trials (RCTs), i.e. Computed tomography (CT)-measured lung density, are not established in the literature…" [MSAC CA 1530, p1]*

Lung CT densitometry changes have proven to be the most sensitive marker of disease progression in patients with A1PI deficiency and COPD as compared to pulmonary function tests or quality of life assessments (Dirksen 2009, Chapman 2015). However, in absence of an established minimum clinically important difference (MCID) for lung density decline rates, the results seen in the RAPID and EXACTLE trials may be difficult to interpret. To help address this issue, a group of renowned A1PI researchers in Birmingham, UK are currently working to establish the MCID based on the CT density outcomes from the placebo-controlled trials (Dirksen 1999, Dirksen 2009, Chapman 2015). The researchers recently proposed an MCID of -2.89 g/L (95% CI: -2.59, -3.25) at the American Thoracic Society conference held in May 2018 (Crossley et al 2018).

Based on the annual preservation of lung tissue (0.74 g/L/year) demonstrated in the RAPID trial in favor of A1PI therapy, the proposed MCID would be achieved within 3.9 years as compared to an untreated patient. As the treatment effect was robust and largely consistent between the RAPID and RAPID OLE trials in the Early Start patients who received 4-years of weekly infusions, a patient continuously treated with A1PI 60 mg/kg each week can reasonably expect to maintain a reduced rate of lung density decline well beyond the point at which the proposed MCID has been reached, demonstrating a worthwhile clinical improvement in this rare and often fatal disease.

2. *"No significant differences were observed between A1PI and placebo for the remaining effectiveness outcomes." [MSAC CA 1530, p1]*

Demonstrating clinical efficacy in A1PI deficiency leading to COPD is challenging. It requires quantitative documentation of lung function changes in a chronic and slowly progressive process that may take decades to manifest clinically (Wewers and Crystal 2013). Despite showing a significant effect on lung density, the RAPID study did not show any statistical signal of efficacy in the secondary endpoints.

There are several possible reasons for this: First, and importantly, the study was powered to detect the treatment effect on lung density measures, not changes in pulmonary function tests, diffusion capacity of carbon monoxide (DLco), Incremental Shuttle Walking Test (ISWT), or St. George's Respiratory Questionnaire (SGRQ) scores. The sample size and trial duration reflect those necessary to demonstrate an effect to slow the annual lung density rates, whereas it has been shown that significantly more patients followed for periods longer than 2 years would be required to investigate benefits of A1PI therapy in the secondary endpoints. Furthermore, those estimates are based on the use of placebo which would be considered unethical for the treatment of A1PI deficiency. Secondly, the sensitivity of the clinical endpoints to detect change is much lower compared to CT lung density; EXACTLE, the second largest study in A1PI deficiency, established CT scans and DLco as the most sensitive measures.

3. *"A1PI meets three of the four criteria warranting rule of rescue. It is unclear whether the proposed service provides worthwhile clinical improvement." [MSAC CA 1530, p146]*

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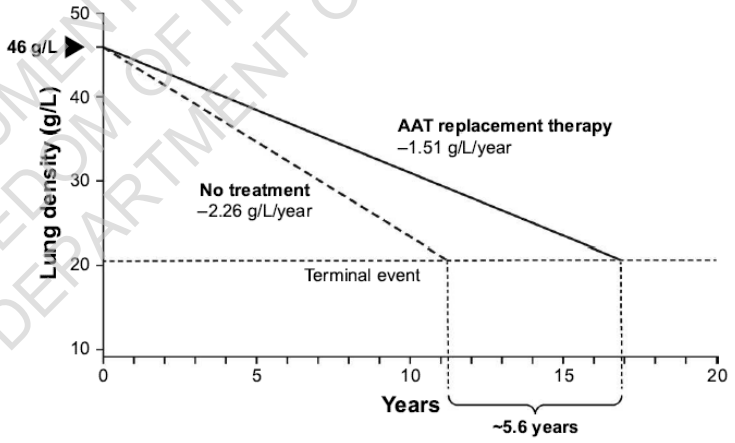
The recent work by Crossley et al to describe the MCID for CT density decline provides further clinical context for the results seen in the RAPID trial, and further demonstrates that A1PI offers worthwhile clinical improvement when evaluated across the appropriate time horizon, noting that A1PI deficiency is a chronic and slowly progressive disease.

Furthermore, evidence from a post hoc analysis of the RAPID programme suggests a mortality benefit following A1PI therapy. During the RAPID programme, the time required for progressive emphysema to develop into respiratory crisis was used to simulate the life-years gained as a result of A1PI therapy. Respiratory crisis was defined as death, lung transplant or a crippling respiratory condition. Seven patients withdrew with an average terminal lung density of 20 g/L. Using the average baseline lung density for all patients (46 g/L) and the rate of decline in lung density in A1PI versus placebo-treated patients, the projected time to terminal lung density was 16.9 years for those receiving A1PI therapy, compared with 11.3 years in the placebo group (Figure 1). This indicates a gain in life-years of 5.6 years with A1PI therapy (McElvaney et al 2017). Although conducted in a small sample size, these data are supported by results from the National Heart, Lung, and Blood Institute observational study showing that patients receiving A1PI therapy had a greater survival than those not receiving treatment (Alpha-1-Antitrypsin Deficiency Registry Study Group, 1998).

Figure 1 Extrapolation of the effect of A1PI replacement therapy on the predicted time to reach terminal respiratory function in RAPID-RCT.



Source: Chapman et al 2018 *International Journal of COPD 18(13): 419-432*

No comments on the economic evaluation or financial implications are provided in this response as Section C, D, E were redacted from the report provided to s47G due to the commercial in confidence nature of the material.

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Proposal and Validation of a Minimal Clinically Important Difference (MCID) for Annual Pulmonary CT Density Decline

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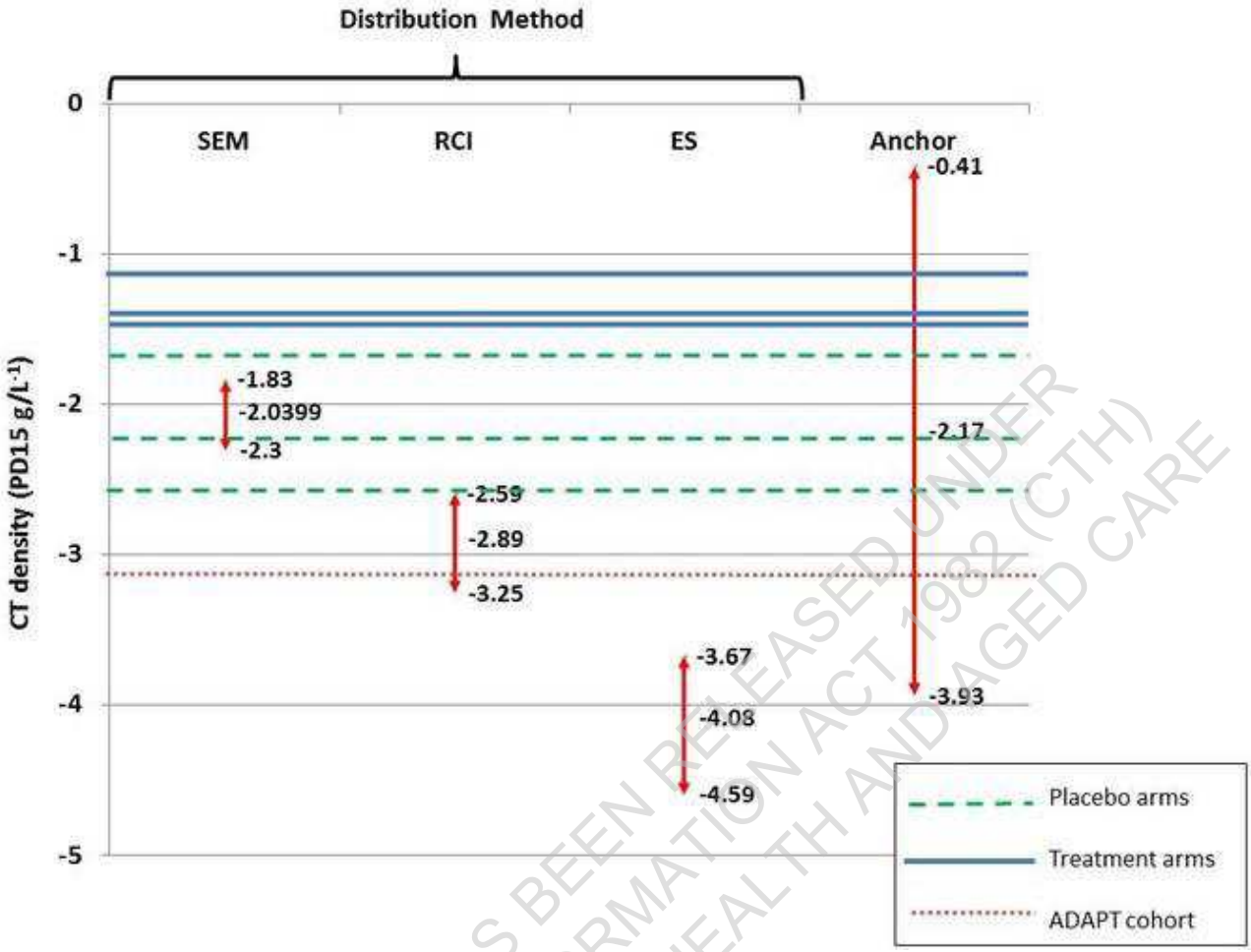
Rationale: Computed Tomography (CT) densitometry has been used as the primary outcome measure in several Randomised placebo Controlled Trials (RCTs) of Alpha one Augmentation Therapy (AAT) to assess amelioration of lung density decline. An MCID for CT density would provide more clarity of density changes that many perceive as having uncertain clinical impact. There are two recognised methods for proposing an MCID; namely the anchor and the distribution method. We aim to determine and validate an MCID for CT density decline in patients with AATD using both recognised methods. This would clarify the effect of AAT, and/or to identify those patients with significant decline who may need intervention. Methods: For the distribution method, studies were sought that reported the mean and standard deviation of CT density (as measured by the 15th ercentile oint; D15 g/L) at baseline and with annual change. These were then used to calculate the MCID using the 3 variations of the distribution method: standard error of measurement (SEM), the reliable change index (RCI), and the effect size (ES). For the anchor method, any papers that reported annual CT density change with the relative change in FEV_1 as measured in mls without exposure to an intervention (the placebo arms) were reviewed. The MCID was then validated using the Birmingham AAT cohort. atients who had received two or more CT scans plus at least three FEV_1 measurements over at least three years were identified. Annual slope FEV_1 (mls) was calculated and compared with the respective annual CT density change and compared by linear regression analysis. Results: Figure one illustrates each MCID and their confidence intervals. The confidence intervals from the anchor method encompassed those of the SEM and RCI. Given that a proposed MCID should originate from a variety of methods, it is reasonable to propose the MCID for CT density as -2.89g/L as this is the middle estimate from the three distribution methods, and still within the confidence intervals of the anchor method. Conclusion: The proposed MCID for CT density in patients with AATD is -2.89g/L. Values in excess of this in patients under surveillance would indicate rapid density decline, which may be one indication for AAT augmentation therapy.



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Biochemical verification of tobacco use and cessation

SRNT Subcommittee on Biochemical Verification*

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Objectives

The charge of our subcommittee was to assess the utility of biomarkers of tobacco use and cessation and make recommendations for their application in clinical trials. The committee addressed five specific questions:

1. Which biochemical markers are most useful for assessing tobacco use, with regards to smoking cessation, smoking cessation given other tobacco use, and concurrent use of nicotine medication to aid cessation?
2. What are optimal cut-off points for biomarker values to distinguish tobacco use vs. no tobacco use, with consideration given to data on specificity and sensitivity at various cut-points and influence of ethnicity?
3. What is an acceptable time window between self-reported last smoking and biochemical verification for different biomarkers?
4. What is the utility (and limitation) of using biochemical markers as indicators of severity of addiction?
5. When is biochemical validation necessary?

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1. Which biochemical markers are most useful for assessing tobacco use, with regards to smoking cessation, smoking cessation given other tobacco use, and concurrent use of nicotine medication to aid cessation?

This section will consider the following issues:

a. Which biomarkers are useful for determining smoking status?
b. Which biomarkers are useful for estimating nicotine intake vs. tobacco smoke exposure?
b. Which biological specimens are useful for various applications?

The pros and cons of various measures will be discussed.

Nicotine can be measured in various biological specimens including plasma, saliva, and urine (Davis & Curvall, 1999). Its specificity for tobacco use is excellent except for persons using nicotine-containing medications. There are dietary sources of nicotine, but they are insignificant compared to tobacco use (Benowitz, 1988; Davis, Stiles, deBethizy, & Reynolds, 1991). Nicotine concentrations are moderately expensive to measure, and a variety of methods are applicable, including gas chromatography (GC; Jacob & Byrd, 1999), high-performance liquid chromatography (HPLC; Crooks & Byrd, 1999), and immunoassays (Langone, Gjika, & Van Vunakis, 1999). Plasma levels, especially taken in the afternoon of a smoking day, correlate well with nicotine intake and may be used to estimate the extent of tobacco use (Benowitz & Jacob, 1984; Lawson *et al.* 1998a). Urine levels also correlate fairly well with nicotine intake (Jacob, Yu, Shulgin, & Benowitz, 1999; Lawson *et al.*, 1998b). Because of the short half-life of nicotine (about 2 h; Benowitz & Jacob, 1994; Benowitz, Jacob,

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Denaro, & Jenkins, 1991), nicotine levels are not useful in assessing tobacco use that occurred more than 8–12 h previously.

Cotinine, the major proximate metabolite of nicotine, can be measured in various biological specimens including plasma, saliva, and urine (Davis & Curvall, 1999). Its specificity for tobacco use is excellent except for persons using nicotine-containing medications (Benowitz, 1988; Davis *et al.*, 1991). A variety of methods are available for measuring concentrations, including GC (Jacob & Byrd, 1999), HPLC (Crooks & Byrd, 1999; Harihan, VanNoord, & Greden, 1988), and immunoassays (Langone *et al.*, 1999). Some immunoassays overestimate cotinine concentrations because of cross-reactivity with other nicotine metabolites (Anderson, Proctor, & Husager, 1991; Schepers & Walk, 1988; Zuccaro *et al.*, 1997). There is a good correlation between levels of cotinine in biological fluids with nicotine intake from tobacco (Benowitz, Kuyt, Jacob, Jones, & Osman, 1983; Rickert & Robinson, 1981). The relatively long half-life of cotinine allows detection for a few days after cessation of tobacco use (see section 3).

Colorimetric or 'dipstick' methods measure a combination of nicotine and metabolites in urine and may be useful for determining smoking status (Barlow, Stone, Wald, & Puhakainen, 1987; Peach, Ellard, Jenner, & Morris, 1985; Puhakainen, Barlow, & Salonen, 1987). These assays are simple and inexpensive, and are semi-quantitative, allowing a crude estimation of the extent of tobacco use. Certain drugs and dietary substances such as isoniazid, high doses of niacin, and other substances containing a pyridine ring may interfere with the assay and cause false positives (DynaGen, no date; Ubbink, Lagendijk, & Vennaak, 1993).

Carbon monoxide (CO) can be measured in expired air or in blood. The measurement of expired CO is simple and relatively inexpensive. Instrumentation for measurement of expired CO is commercially available (measures the rate of conversion of CO to CO_2 as it passes over a catalytically active electrode), and blood carboxyhemoglobin (COHb) can be measured spectrophotometrically (Sonnenworth & Jarrett, 1980). Expired CO and blood COHb are highly correlated (Jaffe, Kanzler, Friedman, Stunkard, & Vereby, 1981; Rickert & Robinson, 1981). CO is reasonably specific for detecting heavy cigarette smoking but is of marginal utility for detecting light smoking because CO levels from smoking are low, and there are environmental sources of CO of similar magnitude (Sonnenworth & Jarrett, 1980). One downside to using expired air CO is the initial cost ($800–2000) for the CO monitor. Measurement of CO is not applicable to detection of smokeless tobacco use because CO is a combustion product.

Thiocyanate (SCN) can be measured in plasma, saliva, and urine. Relatively simple and inexpensive spectrophotometric assays are available (Giraudi & Grillo, 1981). SCN is reasonably specific for heavy smoking, but specificity is not good for detecting light smoking, possibly because of dietary sources (Foss & Lund-Larsen, 1985; Galanti, 1997; Swan, Parker, Chesney, & Rosenman, 1985). It is not applicable to detection of smokeless tobacco use because it is a metabolite of a combustion product, hydrogen cyanide.

Anabasine and anatabine are two nicotine-related alkaloids present in tobacco. Concentrations in urine can be determined using combined gas chromatography–mass spectrometry (GC-MS; Jacob *et al.*, 1999), which is relatively expensive. Because they are not present in nicotine-containing medications, measuring concentrations of these alkaloids is useful for detecting tobacco use in persons undergoing nicotine replacement therapy (NRT). Because concentrations in urine correlate well with nicotine intake from tobacco, they can be used to estimate the extent of tobacco use (Jacob *et al.*, 1999). At present, only urine levels have been measured, but with more sensitive methodology under development it should be possible to measure concentrations in plasma and saliva. After cessation of smoking, half-lives are 16 h for anabasine and 10 h for anatabine (Jacob *et al.*, 1999).

In summary, nicotine measurement is highly specific for tobacco use (in the absence of NRT), but because of its short half-life and technical difficulty and expense in measurement, it is not recommended for general use. Cotinine is highly specific and sensitive for tobacco use (in the absence of NRT) and has the advantages of a fairly long half-life and moderate cost for analysis. When NRT is not employed, cotinine measurement appears to be the best biomarker for smoking cessation. CO measurement is useful for determining smoking status. Its sensitivity is limited by the rapid elimination of CO, such that after 1 day of not smoking, CO levels are no different than those of non-smokers (see section 3). Specificity is limited by endogenous and environmental sources of CO. For this reason, CO may not distinguish light smokers from non-smokers. SCN is not recommended as a biomarker for tobacco use because of inadequate sensitivity and specificity. Anabasine and anatabine are most useful for determining tobacco use in the presence of treatment with NRT. A potential limitation is the relatively high expense of the assay.

Which biological specimen should be used?

Generally, any specimen in which the biomarker can be measured is suitable for determining smoking status. Plasma levels of nicotine are likely to correlate best with the pharmacological effects of tobacco. Urine and saliva for cotinine are non-invasive and do not require venipuncture. The ratio of cotinine in saliva, compared to plasma, serum, or blood, averages about 1.3, with a range of 1.1–1.4 in various studies. Saliva cotinine concentration may also vary according to whether it is stimulated (such as with candy or wax). Saliva cotinine concentrations are lower in stimulated compared to unstimulated saliva. One study showed that cotinine concentrations were 26% lower with stimulation using a sugar cube and 6% lower with stimulation by chewing on paraffin wax,

Table 1. Time after smoking cessation to reach the cut-off concentration used to distinguish smokers from non-smokers (assuming average pre-cessation smoking rate)

	Cut-off	Half-life (h)	Time to cut-off (h)
Cotinine (saliva)			
General population	15ng/ml	16	80
African-American	15ng/ml	20	100
Pregnant women	10ng/ml	9	45
CO (expired air)			
Active	8–10ppm	2	6
Sedentary	8–10ppm	4	12
Sleep	8–10ppm	8	24
SCN (plasma)			
General population	78–89 μM/l	3–14 days	6–28 days

compared to unstimulated saliva levels (Schneider *et al.*, 1997). With some biomarkers, e.g. nicotine, cotinine, anabasine, and anatabine, urine levels are generally higher than levels in plasma or saliva, thus facilitating measurement and increasing the time period during which the biomarker can be measured. For SCN, better specificity/sensitivity has been observed using plasma compared to urine or saliva (Degiampietro, Peheim, Drew, Graf, & Colombo, 1987; Dourdoux, 1995). CO has the advantage of being measurable both in blood and in expired air. In addition, the results of expired CO measurement are available immediately.

2. What are optimal cut-off points for biomarker values to distinguish tobacco use vs. no tobacco use, with consideration given to data on specificity and sensitivity at various cut-points and influence of ethnicity?

A number of markers have been used as biochemical indicators of tobacco consumption, including nicotine, cotinine, SCN, and CO (Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987; Ruth & Neaton, 1991; Saloojee, Vesey, & Russell, 1982). They vary in terms of cost and ease of administration, specificity to tobacco, and half-life. Measuring CO in expired air is the cheapest (once the instrument to measure it has been purchased) and most easily measured, providing feedback within seconds, and its sensitivity and specificity are both around 90%. Plasma or saliva cotinine perform best, with 96–97% sensitivity and 99–100% specificity, respectively. An advantage of cotinine is that optimal cut-points are little affected by the prevalence of smoking in the population sampled (Jarvis *et al.*, 1987). For markers whose concentrations are affected by factors other than tobacco use, such as SCN (diet) and CO (traffic, heating, and cooking emissions), optimal cut-points may vary according to prevalence of smoking, with higher cut-points being more appropriate where expected prevalence is lower (Cummings & Richard, 1988; Jarvis *et al.*, 1987; McNeill, Jarvis, West, Russell, & Bryant, 1987). Finally, the cut-points presented in this section are based on studies in general populations. For some populations, such as African-Americans or pregnant women, nicotine and cotinine metabolism differ from the general population, and optimal cut-points are likely to differ as well (Benowitz *et al.*, 1999; Klebanoff, Levine, Clements, DerSimonian, & Wilkins, 1998).

The following cut-points for the main biomarkers have been widely used and are likely to be appropriate for most circumstances:

- Plasma or saliva cotinine 15 ng/ml;
- Urinary cotinine 50 ng/ml;
- Expired air CO 8–10 ppm;
- Plasma SCN 78–84 μmol/l.

The sensitivity and specificity of cotinine does not vary much across a range of cut-points – from about 10 to 20 ng/ml – and is little influenced by variation in the prevalence of smoking in the underlying population. In many studies, urinary cotinine levels are adjusted for creatinine concentration. However, unadjusted levels may be entirely adequate for determining smoking status. Recommended cut-points for plasma and saliva nicotine are not given. This is because, in the case of salivary nicotine, concentrations are highly influenced by local exposure in the mouth because ambient tobacco smoke, for example, may not be a good indication of systemic levels. Plasma nicotine has not been widely used as a marker of smoking status, for reasons of short half-life and the need for invasive blood sampling. Salivary and urinary SCN lack sufficient sensitivity and specificity to make their use advisable.

3. What is a useful time window between self-reported last smoking and biochemical verification for different biomarkers?

The useful time window for the use of a biomarker to assess tobacco use depends on the specific biochemical marker, the level of exposure, and the selected cut-off point. The characteristic of the biomarker that is most useful in determining how long it will remain in the body

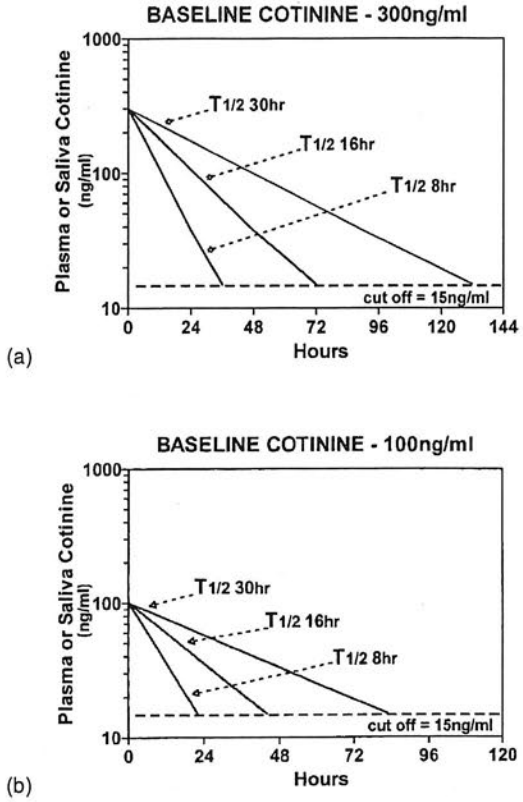

Figure 1. (a) Simulated blood or saliva cotinine concentrations over time, assuming an initial concentration of 300 ng/ml, and different half-lives. The dashed line represents a non-smoking cotinine level cut-off of 15 ng/ml. Cut-off would be reached at 130, 70, and 35 h if cotinine half-life was 30, 16, and 8 h, respectively. (b) Similar simulation to (a), but starting from a baseline cotinine level of 100 ng/ml. Cut-off would be reached at 83, 44, and 22 h if cotinine half-life was 30, 16, and 8 h, respectively.

is the half-life. The half-life of a drug is determined by the rate of clearance from the body and the extent of distribution of the drug in body tissues. Half-life for a particular biomarker can vary considerably from person to person. Therefore, estimates using half-life data need to consider not only the mean or median value but also the range of values.

The half-life of a chemical describes the time it takes for the level of the chemical to decline by 50%. At two half-lives, the level has declined to 25%, at three half-lives to 12.5%, and at four half-lives to 6.25% of the original value. Thus, knowing the initial level of a chemical in the body and the half-life, one can estimate how long it will take to reach a given cut-off point.

The three biomarkers that will be considered in this section are cotinine, CO, and SCN (Table 1). Cotinine levels peak in the body 1–2 h after the last dose of nicotine. African-Americans and Chinese-Americans metabolize cotinine more slowly than do Caucasians, and the half-life of cotinine is longer in these populations (Benowitz *et al.*, 1999). Pregnant women metabolize cotinine more quickly than when they are not pregnant, so their cotinine half-life is shorter (Dempsey, Jacob, & Benowitz, 2002).

For an initial cotinine level of 300 ng/ml, which is a typical level in a daily smoker, it would take five half-lives for this level to decline below the cut-off of 15 ng/ml (Figure 1). Assuming a half-life of 16 h, the duration of abstinence required to reach the non-smoker cut-off level would be 80 h. If a smoker has a lower level of cotinine, it would require four or even three half-lives, which would correspond to 64 or 48 h, respectively, to reach the cut-off concentration. Considering the range of half-lives in the population, for an 8-h half-life the interval from abstinence to reach cut-off would range from 24 to 40 h (three to five half-lives), whereas for a long half-life of 30 h it would take from 90 to 150 h. Considering the longest cotinine half-life (so as to minimize false positive results) and typical initial cotinine levels, 7 days is a reasonable interval to use to assess compliance with non-smoking in most studies. It should be recognized that false negatives may be seen in individuals who have stopped for 2 or more days prior to cotinine measurement, depending on the half-life.

The estimates for CO are more complicated because the half-life for CO depends on the level of physical activity. CO is eliminated in expired air, and the rate of elimination depends on the individual's pulmonary ventilation rate. With sedentary activity, the half-life of CO is 2–3 h, while during sleep the half-life is up to 4–8 h (Coburn, Forster, & Kane, 1965). During exercise, the half-life can be as brief as 1 h. The long half-life during sleep explains why smokers who have not smoked overnight can awaken in the morning with COHb levels as high as 5% (approximately 30 ppm in expired air).

A typical cut-off point for CO in expired air is 8 ppm. Assuming the level of CO during cigarette smoking begins at 40 ppm, it would require about three half-lives to decline to 8 ppm. Assuming a half-life of 2–3 h, this would correspond to 6–9 h to reach cut-off. In an individual who is physically active, the interval would be less. However, during sleep it would decline much more slowly. For an average smoker, considering the sleep/wake cycle, 24 h can be taken as a time point in which the CO level would almost certainly have declined to cut-off. However, it would be possible for someone to smoke a few cigarettes within 24 h and still be below cut-off, depending on the half-life. It should be noted that CO exposure from environmental sources can result in expired CO levels of 2–6 ppm, depending on the extent of exposure to traffic exhaust and other pollution.

SCN is eliminated from the body by excretion in the urine. Its excretion rate depends on kidney function. With normal kidney function, half-lives of 3–14 days have been reported in different studies (Schulz, Bonn, & Kindler, 1979). Even non-smokers have a considerable level of SCN in the body, derived from foods. On average, smokers have SCN levels two to four times higher than those of non-smokers (Vogt, Selvin, & Hulley, 1979). Assuming the level of SCN is four times

higher in smokers than in non-smokers, it would take an average of two half-lives, or 1–4 weeks, for the level to decline from a smoker's level to that typical of a non-smoker.

Studies of persons in the natural environment and the time to reach non-smoker levels or cut-off points were reviewed for empirical evidence to support the half-life data. Because more specific information was available for cotinine as a biomarker than for CO or SCN, cotinine is the focus of this discussion. Nine studies on the topic were identified and summarized. Study samples included subjects who quit smoking in the natural environment, those who were abstinent after completing a 5-day series of nicotine capsules, inpatient and outpatient subjects receiving placebo NRT, and those in 6- and 7-day inpatient studies where cotinine during abstinence was measured (Abrams, Follick, Biener, Carey, & Hitti, 1987; Ahijevych, Kihm, Dhatt, & Weed, 1999; Benowitz *et al.*, 1983; Carey & Abrams, 1988; Dale *et al.*, 1995; Hecht *et al.*, 1999; Jarvis, Russell, Benowitz, & Feyerabend, 1988; Trudgill, Smith, Kershaw, & Riley, 1998; Wewers *et al.*, 2000). Sample sizes were generally small, ranging from five to 31 subjects (mean, 13.7 subjects). Baseline cotinine levels and cigarettes per day (cpd) were not available in two of the nine studies. The mean plasma baseline cotinine levels across four studies ranged from 156 to 302 ng/ml. Saliva baseline cotinine ranged from 93 to 350 ng/ml (two studies). Urine cotinine averaged 1394 ng/ml in one study, and urine cotinine to creatinine ratio averaged 9.7 in another. Mean cpd by study ranged from 12 to 24. The cut-off points for cotinine were 10 or 14 ng/ml in plasma and saliva, 50 ng/ml in urine, 0.4 μg in 24-h urine sample, and a cotinine:creatinine ratio <2. Cut-points were not identified in two reports.

With heterogeneous study designs, small sample sizes, and some gaps in data, a definitive statement on time to reach non-smoker levels is problematic. However, a range of acceptable window estimates can be described. The shortest time to reach non-smoker levels was a mean of 2.8 days (SD, 1.3; range 1–5 days) for the sample with a low average baseline salivary cotinine level of 93 ng/ml and a baseline smoking rate of 12 cpd (Carey & Abrams, 1988). A similar time frame of 2.9 days to reach non-smoker levels was estimated for persons abstinent following administration of nicotine capsules (Jarvis *et al.*, 1988). A mean of 0% cotinine replacement was reported the evening of Day 4 of smoking abstinence in placebo NRT subjects, which would be comparable to non-smoker level (Dale *et al.*, 1995). An average of 4.7 days (range 3–6 days) was evident to reach <14 ng/ml in a small inpatient study (n=6) where smoking abstinence was monitored (Wewers *et al.*, 2000). Sixteen per cent of subjects (5/31) had plasma levels >14 ng/ml at 136 h of smoking abstinence on discharge (5.7 days) in a similar inpatient-monitored protocol (Ahijevych *et al.*, 1999). At 7 or more days of self-reported abstinence in the natural environment, cotinine was not detected in 96% (24/25) of subjects (Abrams *et al.*, 1987). Seven days to reach non-smoker levels was also reported for persons in a study using cotinine in 24-h urine samples as the biomarker (Hecht *et al.*, 1999). Approximately 80% (9/11) of quitters had cotinine to creatinine ratios in the non-smoker range at 7 days (Trudgill *et al.*, 1998).

Clearly, baseline cotinine levels are critical in determining a useful time window to biochemically verify non-smoking status. In field studies where baseline cotinine data are not readily available for every participant to estimate half-life and when non-smoker levels would be reached, a 7-day window of self-reported smoking abstinence would most likely capture and accurately classify almost all persons as non-smokers using cotinine as the biomarker.

4. What is the utility (and limitation) of using biochemical markers as indicators of severity of addiction?

Defining addiction

In the field of addictive disorders, the definition of addiction is made almost exclusively on behavioral or symptomatic indices and, for the most part, ignores drug (and by inference biomarkers of) intake. This has evolved over the years, as it became evident that intake was not a good assessment tool for whether a person was dependent upon that drug. For example, on a given day, an alcoholic might be at a very low level of intake and, because the half-life is short, have a low blood alcohol level. On the same day, an occasional non-problem drinker might be at a higher level of intake and have higher blood alcohol level. At one point, the National Council on Alcoholism used blood alcohol concentrations to define tolerance and, if the levels were high enough, to define dependence (Criteria Committee, NCoA, 1972). An individual with a blood alcohol concentration of 1500 μg/ml of alcohol without showing signs of intoxication was considered to be exhibiting tolerance. An individual who had a blood alcohol concentration of 3000 μg/ml at any time was considered to be alcoholic because in order to drink enough to reach this blood level would require tolerance. Blood alcohol concentrations have not been a part of the DSM classification of alcoholic dependence (APA, 1994).

Tobacco appears to be an exception, because levels of intake tend to be stable in most smokers, and biomarkers such as cotinine are related to the level of nicotine intake. There is considerable inter-individual variability in the relationship between levels of cotinine and the daily intake of nicotine from tobacco, but cotinine levels predict nicotine intake better than cpd (APA, 1994). The reasonably long half-life of cotinine means that a particular level reflects nicotine intake over the past 2–3 days.

Nicotine dependence and its severity may be defined both in behavioral terms and in terms of a biomarker(s). Most researchers would agree that higher levels of

consumption of tobacco products are related to the severity of dependence, i.e., the more consumed, the more likely a higher level of dependence. When smokers are categorized according to light, moderate, and heavy, then biomarkers such as nicotine and cotinine are correlated with the level of smoking. In some studies where there has not been a relationship between smoking rate and cotinine, the smokers may not have been stratified well enough to have a clear demarcation in the smoking rate (Lawson *et al.*, 1998b). The Fagerström test for nicotine dependence (FTND) includes, as one of its more important questions, quantity of smoking (Fagerström, 1978). A relationship between the FTND score and plasma cotinine levels has been observed (Pomerleau, Pomerleau, Majchrzak, Kloska, & Malakuti, 1990). In that study, shorter latency to the first cigarette in the morning was also related to the cotinine levels, as was the question, 'Do you smoke if you are so ill that you are in bed most of day?' While the FTND has not been consistent in predicting successful treatment outcome for nicotine dependence across all studies, it has been reported to be predictive in many studies (Fagerström & Schneider, 1989). Furthermore, using the 4-mg dose of nicotine gum in people with higher FTND scores has been shown to be more effective than the 2-mg dose (Sachs, 1995).

Biomarkers and behavioral predictors of outcome

Cotinine has also been shown in some (but not all) studies to be a predictor of treatment outcome, with those with higher levels of cotinine having a poorer outcome during nicotine dependence treatment, whether it be behavioral treatment or nicotine replacement (Hall, Herning, Jones, Benowitz, & Jacob, 1984). For NRT, these outcome data may simply be related to inadequacy of nicotine replacement, which might be improved were the doses to be increased. CO levels and the FTND scores have also been shown to be related, reflecting most likely the number of cigarettes smoked per day (Fagerström & Schneider, 1989).

Biomarkers and addiction

Because tobacco intake and severity of addiction appear to be related, we believe that biomarkers can be valid indices of dependence level, medication need, or both. As discussed previously, the biomarkers to be considered are nicotine, cotinine, SCN, and CO levels of expired air. Each has its own strengths and weaknesses. In brief review, SCN is not specific for tobacco use and may have too long a half-life to make it clinically useful in defining the severity of dependence. On the other end of the spectrum, CO and serum nicotine levels may have too short a half-life such that they are highly dependent on the recency of last smoking. Cotinine appears to be the best marker to gauge the severity of dependence, although CO, SCN, and cotinine in combination could be used in a complementary way to assess an individual's nicotine intake over time. Clinically, cotinine levels reflect nicotine intake over time in a way similar to the use of glycosylated hemoglobin in assessing the adequacy of control of an insulin-dependent diabetic. In this analogy, plasma glucose is used much in the same way as serum nicotine, for example, and a more immediate marker.

Thus, from an assessment and treatment standpoint, plasma cotinine levels could be used to assess severity of nicotine dependence and assess the level of medication needed, especially with regards to NRT.

5. When is biochemical verification necessary?

In considering statistical issues in smoking treatment research, we limited ourselves to randomized trials and focused our discussion on two variants of these trials frequently reported in the literature.

The first is the 'clinic-based randomized trial,' which has a sample size usually under 500, typically 150–250. Participants are volunteers. These volunteers participate in a good deal of face-to-face contact with researchers. There are multiple assessments – frequently at baseline, post-treatment, and 6–12 months. The primary outcome variables are point-prevalence abstinence and 12-month continuous abstinence, biochemically verified. By the end of 1 year, about 10–20% of the sample has dropped out. 'Missingness' does not differ between conditions, but it is related to gender and number of cigarettes smoked at baseline.

The second is the population-based trial that is characterized by a much larger sample size – usually 1000+ – often recruited through healthcare settings or worksites or by random-digit dialing from a defined geographic region. The goal of a population-based study is to produce a sample that is representative of a defined population. Biochemical validation could produce a selection bias unrelated to smoking status. The primary outcome variables are the same as in the clinic-based trial, but biochemical verification is not generally used. Follow-up periods tend to be longer, often stretching to 24 months. Missing data rates tend to be somewhat higher – perhaps more like 30% than 20% at the end of follow-up – and may well differ between groups, particularly if one group received more active and time-consuming interventions. The higher missing data rates may also reflect the somewhat longer follow-up rates. They may also be related to baseline variables such as number of cigarettes smoked, education or gender.

Clinic-based studies

Biochemical verification has been generally recommended for clinic-based randomized trials. However, empirical data have been lacking to support this recommendation (e.g., Glasgow *et al.*, 1993; Patrick *et al.*, 1994; Velicer, Prochaska, Rossi, & Snow, 1992).

A review was conducted to address the following questions:

a. Does biochemical verification affect outcome in clinic-based trials?
b. Which populations are more likely to have biochemical verification affect outcome?
c. What other study conditions/characteristics are related to such effect?

A literature search using the *MEDLINE* database was conducted for studies published between 1990 and 1999, with 'smoking cessation' as subject, and 'controlled clinical trials, or randomized clinical trials' as publication type. From a total of 471 studies that met the search criteria, we identified 163 non-duplicate studies that met the following criteria: (1) some participants received smoking cessation treatment; (2) there was more than one treatment condition; (3) abstinence rates were reported; and (4) the total sample size was less than 500. Out of the 163 studies, 101 (61.9%) of the studies indicated using biochemical verification for smoking abstinence reported. Eighteen (11.0%) studies provided sufficient data for treatment outcome to be re-analyzed based on self-report abstinence rates alone vs. rates verified by biochemical verification. Three additional studies from Hall and colleagues (Hall, Munoz, & Reus, 1994; Hall *et al.*, 1996, 1998), for which we had the raw data for both self-reported and biochemically verified rates, were included in the analyses with the 18 studies. Thus, 21 studies provided data for this review. Eighteen of these had sufficient data for analysis in the published reports; three, those from Hall's laboratory, were based on data not available in the published articles.

For the purpose of this review, outcome data containing abstinence rates that were self-reported or biochemically verified were abstracted from these 21 studies at two time points: end of treatment, and 12-month follow-up or the longest follow-up assessment available. When end-of-treatment assessment data were not available, data were abstracted from the first and the last assessments reported. For each study, data were re-analyzed using Pearson χ^2 tests based on self-reported abstinence rates alone and biochemically verified rates (self-report plus biochemical verification).

Treatment outcome was concluded to be affected by biochemical verification when the result from the analysis based on self-reported abstinence alone was different from that based on biochemical verification using $p<0.05$. The data were analyzed using two approaches: (1) complete-case analysis: excluding participants with missing data (self-reported smoking, biochemical verification, or both) and (2) intent-to-treat: including all participants who were randomized into treatment conditions and treating missing data as smoking or relapse. The complete-case analysis would yield the 'highest' abstinence rates; and the intent-to-treat approach would yield the 'lowest' abstinence rates. These two approaches were, therefore, chosen to evaluate the impact of biochemical validation on outcome analyses.

Data from a total of 34 comparisons derived from 21 studies were included in the analyses. Twelve studies provided data from only one assessment. Seven studies provided data from two assessment points: end-of-treatment, and 12-month follow-up or the longest assessment available. These seven studies thus accounted for 14 comparisons. Two studies (Hall *et al.*, 1996, 1998) used a 2×2 factorial design and provided data from two assessments; in these two studies, treatment outcome was compared separately for each factor, resulting in four comparisons from each of these two studies. Thus, these two studies account for eight comparisons.

Does biochemical verification affect outcome in clinic-based trials with sample size less than 500?

Using the complete-case approach, four out of 31 comparisons (12.9%) derived from four out of 19 studies (21.0%) showed that treatment outcome was affected by biochemical verification (three comparisons derived from two studies were excluded from the complete-case analysis because attrition information was not available). Under the intent-to-treat approach, five out of the 34 (14.7%) comparisons derived from five out of the 21 studies (23.8%) showed the impact of biochemical verification on treatment outcome. Most of the differences occurred where a significant treatment effect was found based on self-reported abstinence, but not when based on biochemical verification. Two comparisons derived from two studies, however, indicated that analyses based on biochemical verification yielded a significant treatment effect when self-report did not. Both of these were studies of pregnant women.

Which populations are more likely to have biochemical verification affect outcome?

The data included in the current review were abstracted from various study populations. Out of the 34 comparisons, 15 (44.1%) were from special populations, which were derived from 12 studies (57.1%). The special populations studied in the current review included pregnant women, primary care patients, VA patients, hospitalized patients, cancer patients, drug dependence patients, and individuals with a history of depression and alcohol dependence. Using complete-case analysis, among the four studies where outcome evaluation was affected by biochemical verification, three of them were from a special population. Similarly, using the intent-to-treat approach, three out of five studies that showed an impact of biochemical verification on treatment outcome used special populations. The special populations, on which the data suggested an impact of biochemical verification on treatment outcome, were pregnant women, patients with alcohol and depression history, and VA-hospitalized patients after surgery. Among the four studies of pregnant women, two indicated an impact on

outcome from biochemical verification. Both of these studies showed that the outcome analyses based on biochemical verification yielded a significant treatment effect under either or both of the analytic approaches.

What other study conditions/characteristics are related to the effects of biochemical verification on outcome?

Other study characteristics included: what portion of the sample was asked to provide biochemical verification: all subjects vs. subjects reporting abstinence only, percentage of participants providing biochemical verification, or assessment time points? The percentage of participants providing biochemical verification was the only characteristic that appeared to determine whether biochemical verification affected outcome. Using the complete-case approach, three out of four studies in which outcome was affected by biochemical verification had more than 10% of biochemical verification data missing. Similarly, under the intent-to-treat approach, four out of five studies that were affected by biochemical verification had more than 10% of these data missing.

This review did not yield definitive findings with respect to the necessity of biochemical verification in clinical trials. Indeed, the most striking finding was that only 18% of the studies identified reported enough self-report and biochemical data to make comparisons possible. Reporting of both outcomes in a format that allows ready comparisons would contribute to our understanding of when biochemical verification is useful.

Also, given the usual requirements around disclosure to human participants, it is a reasonable assumption that most participants in the studies reviewed knew that the veracity of their self-report would be evaluated by biochemical verification. Results may be different when this is not the case.

Even so, some findings warrant further study. First, it appears that special populations may indeed be more likely to provide self-report data that is discordant with biochemically verified data. Second, a high rate of missing biochemical data and discrepancies between biochemical data and self-report appear to be related.

Population-based studies

There is an extensive body of evidence relating to the utility of and necessity for biochemical validation in large population-based studies. Four major papers have either reviewed the literature or evaluated an extensive sample:

a. Velicer *et al.* (1992) provide an extensive literature review, including 21 studies involving cotinine and 29 studies involving CO. They conclude that misreporting rates are generally very low, typically near zero and seldom exceeding 5% except in high-risk medical settings, such as involving patients with heart disease or pregnant women, where the misreporting rate averaged 13%.
b. Glasgow *et al.* (1993) concluded that biochemical validation was sometimes not feasible and did not alter the conclusions of low-intensity intervention trials. These authors also dismissed the implications that participants refusing to provide biological fluids for analysis were cessation failures.
c. Patrick *et al.* (1994) performed a meta-analysis on 51 comparisons and concluded that self-reports are accurate in most studies. They also suggested that biochemical validation could improve accuracy in student samples and intervention studies.
d. The COMMIT Research Group performed an ancillary study to investigate the validity of self-report. The full report of that study is not available at this time, but a summary has been presented (COMMIT Research Group, 1995). Preliminary analysis found no difference in misrepresentation between the intervention groups and the comparison group in either the heavy smoker or the light-to-moderate smoker cohorts. The direction of the differences should be noted. Misreporting rates were lower in the intervention condition (5.1% and 6.8%) than in the comparison condition (7.7% and 8.8%).

The results of these four studies are consistent and suggest that biochemical validation is not always necessary in smoking cessation studies. The levels of misrepresentation are generally low. Alternative methods of validation such as employing multiple items to verify smoking status are likely to produce accurate estimates, and there is little reason to expect differential misrepresentation rates in most smoking cessation studies.

The decision to employ biochemical validation

Three broad issues that impact the decision about whether to employ biochemical validation in a specific study will be considered:

a. *Demand characteristics* – That is, aspects of the intervention that increase demand on smokers to quit. Different types of clinical trials present different levels of demand characteristics. Population-based intervention trials typically have very low demand characteristics, while clinic-based studies may involve very high demand characteristics. Special populations are to some extent defined by a contextual demand characteristic. The adolescent is smoking illegally, and parents and teachers often will express strong disapproval. Pregnant women and medical patients face strong demand characteristics from medical service providers and society in general. In large population-based trials with low-demand situations, biochemical validation appears not to be necessary. There are inadequate data for trials in high-demanding settings.
b. *Type of study.* Clinic-based trials are widely employed in the initial stages of evaluating the efficacy of an intervention. They often rely on reactively recruited

volunteer subjects, involve extensive experimental controls, and are of relatively brief duration. Population-based interventions are employed to demonstrate the generalizability of established interventions. Proactive recruitment is employed to recruit a representative sample, the degree of participation is at least partially under the control of the participants, and an extended follow-up is employed.

c. *Type of population.* That is, general population vs. special subgroup. Special subgroups include adolescents, pregnant smokers, intensive group interventions, and medical patients.

Three issues should be considered in making the decision to employ biochemical validation in a high-demand study. Underlying each of these three issues is a need to clearly establish the purpose of knowing a person's smoking status. Each of these issues is important not only with respect to the decision to use biochemical validation but also to such basic issues as accuracy of conclusions and statistical power.

a. What is the likely rate of refusal of biochemical testing, and how will those subjects who refuse be classified? Traditionally, subjects who refuse have been assumed to be smokers. Refusal rates for clinic studies are extremely low (i.e., under 15%). Refusal rates for population-based interventions have been as high as 70%. Refusal-rate problems can result in an overestimate of smoking rates if all refusers are classified as smokers. In some studies, patients receive intervention as part of routine clinical care and are 'informed' there is a study as part of follow-up assessment. Such participants may understandably refuse to provide biochemical data.
b. What alternative explanations exist for false positive testing results? The assumption that all inconsistencies between self-report and biochemical testing are because of inaccurate self-report may not be justified. SCN and CO are particularly vulnerable to environmental influences, especially in light smokers who have relatively low levels of these biomarkers from their tobacco use. Cotinine testing must carefully assess the presence of other forms of nicotine, including the use of nicotine replacement products and extensive exposure to secondhand smoke. The former problem can be addressed by measurement of minor tobacco alkaloids, although such measurements are not currently widely available.
c. What is the likely impact of inaccurate self-report on the evaluation of the intervention? A goal of an intervention study is typically to determine the relative difference in smoking rates between two groups. For biochemical validation to modify the estimates, it would be necessary to demonstrate that a differential misreporting rate exists between the intervention and the control groups and apply a correction to the estimate of the relative difference. Most studies do not have adequate power to detect such a difference because it would involve multiplying the proportion of participants who quit smoking by the proportion of self-reported quitters who fail biochemical validation. It would require considerably larger sample sizes to perform the correction than is feasible in most smoking cessation studies.

These three issues, in combination, suggest that in large-population, low-intensity intervention trials, biochemical verification is neither feasible nor necessary. While it is likely that self-report inflates quit rates, the magnitude of such inflation is small, and there rarely is differential across intervention conditions.

On the other hand, for small-population, high-demand clinical trials of new interventions, where accurate estimation of quit rates is necessary for regulatory approval and for determining benefits vs. risks of a treatment, biochemical verification is feasible and is strongly encouraged. Likewise, biochemical testing is recommended for special populations, where there is an incentive to deceive, such as in adolescents, pregnant women, and medical patients with smoking-related diseases. Finally, biochemical information is mandatory for studies evaluating novel nicotine-delivery products (such as devices that heat rather than burn tobacco, which are currently being test-marketed in the USA) or for harm reduction studies, where the level of exposure to nicotine and other tobacco smoke toxins is an essential end-point.

Recommendations

Considering all of the above, we recommend the following. In most settings, biochemical verification provides additional assurance that the participant's self-reports are accurate. Because currently available methods of verification are relatively inexpensive and not invasive, it is our recommendation that they be used in most new product and all harm-reduction studies. We also recommend that biochemical verification be used in most or all studies of smoking cessation in special populations, such as adolescents, pregnant women, and medical patients with smoking-related diseases. There are circumstances under which the added precision gained by biochemical verification is offset in such a way that its use is not required and may not be desirable. Examples include large-scale population-based studies with limited face-to-face contact and studies where the optimal data collection methods are through the mail, telephone, or Internet. We also recommend that researchers who collect both self-report and biochemical verification data report both separately in published articles, indicating discrepancies between the two in various intervention conditions, so that future research may better address the question of the utility of biochemical verification across a range of studies, treatment modalities, and populations.

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