Practical Laboratory Medicine 5 (2016) 47–56

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Reference change values in concentrations of urinary and salivary biomarkers of exposure and mouth level exposure in individuals participating in an ambulatory smoking study

Oscar M. Camacho*, Johan Sommarström, Krishna Prasad, Anthony Cunningham

Group Research and Development, British American Tobacco (Investments) Ltd, Southampton, UK

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form
12 May 2016
Accepted 16 May 2016
Available online 17 May 2016

Keywords:
Reference change value
Biomarker of exposure
Smoke toxicant
Cigarette
Tobacco
Marker variability
Smoking
MRTP

ABSTRACT

Background: Modified-risk tobacco products (MRTPs) are being developed that may contribute to tobacco harm reduction. To support reduced exposure or risk claims, a scientific framework needs to be developed to assess the validity of claims and monitor consumers after product launch. We calculated reference change values (RCVs) for biomarker of exposure (BoE): salivary cotinine and hydroxycotinine; and urinary total nicotine equivalents, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and creatinine. Mouth-level exposure (MLE) to nicotine and tar were also recorded in an ambulatory setting to characterise variation among smokers in their everyday environment.

Methods: This non-residential, observational study was conducted over 3.5 years across 10 sites in Germany. Smokers of the same commercial 10 mg ISO tar product were included in the study (N = 1011). Urine samples, questionnaires and cigarette filters were collected every 6 months for a total of seven timepoints.

Results: Greater variability in BoEs was observed compared with confined clinical studies. Gaussian distributed data showed 2-sided values over 100%, which are uninformative for decreases. The proportion of significant changes increased slightly among switchers, probably as a result of additional variability due to the range of products used post-switching. Overall proportions of changes remained small, consistent with literature reporting that when switching to a different tar yield cigarette, smokers partially compensate by changing their smoking behaviour.

Conclusion: Variability estimates and RCVs can be useful for monitoring subjects' BoE and MLE endpoints in longitudinal smoking studies where subjects are followed in their own environment and to aid sample size calculation of studies involving these endpoints.

1. Introduction

Combustible tobacco products have been identified as one of the worldwide leading causes of disease. During the 20th century 100 million deaths were tobacco-related [1]. Despite health awareness campaigns and tobacco control policies, smoking prevalence is $<20\%$ in only seven of the European Union member states (2012) [2].

* Corresponding author.
E-mail address: oscar_m_camacho@bat.com (O.M. Camacho).

http://dx.doi.org/10.1016/j.plabm.2016.05.003

In 2001, the US Institute of Medicine issued a report [3] that suggested the development of potential reduced exposure products (PREPs), later termed modified-risk tobacco products (MRTPs) [4], as a complementary approach to tobacco control policies to promote tobacco harm reduction. In their draft MRTP application guidance published in 2012, the U.S. Food and Drug Administration (FDA) defined these products as "*any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products*"[5].

The potential incentives emerging for reduced exposure or risk claims make it necessary to develop a scientific framework to assess the validity of these claims. In 2012 the FDA Center for Tobacco Products listed 56 research priorities in this area, one of which states: "*What methods and measures best assess biologically relevant changes in harmful and potentially harmful constituents in tobacco products and smoke in both clinical models and humans?*"[6].

Several studies have suggested that differences in levels of biomarkers of exposure (BoEs) to tobacco smoke toxicants can be observed in populations smoking cigarettes containing different levels of toxicants. [7,8] Hatsukami et al. reviewed the usefulness of several established biomarkers used in the assessment of tobacco products and concluded that no existing biomarkers were predictive of tobacco-related disease [9]. However, biomarkers such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) have since been associated with lung cancer, [10] although this has not been validated as a predictor of cancer risk. In the absence of acceptable biomarkers for risk of disease, reductions in BoEs have been suggested as an alternative parameter for the evaluation of MRTP use by consumers, while noting the lack of validation of correlations between exposure reduction and risk reduction [11]. The initial challenge is to determine what constitutes meaningful changes in BoE levels that may lead to reductions in disease risk. Subsequently, risk reduction associated with biomarker reduction could be assessed through prospective epidemiological and surveillance studies [12].

However, BoE levels are variable both within individuals and (more so) between individuals [13]. Such variability is believed to be driven by variations in metabolism [12,14], smoking behaviours, and within-product variation. Furthermore, variations in the yield of smoke constituents can occur when smokers switch between different products with different design specifications [15]. In this complex landscape, Hecht et al. recognised that the ultimate goal should be to aim for the levels of biomarkers observed in non-smokers, who are at lower risk for tobacco-related cancers. However, as this is not likely to be feasible, a realistic but meaningful target level should be set [12].

Reference change values (RCVs) are used in clinical settings to compare two sequential analytical results [16]. RCVs are defined as the minimum critical difference that must be exceeded between two sequential results for their difference to be considered significant, rather than due to random variation [17]. An advantage over classic population reference intervals is that RCVs allow assessment of significant changes within the accepted population intervals, i.e. a subject's analyte levels can lie within the expected ranges, but be classified as a significant change. RCV methodology assumes that analytes fluctuate randomly within individuals around a homeostatic set point following a normal distribution [17]. However, the log-normal distribution has since been found to be more appropriate for many analytes [18]. Hammond et al. have reported high correlation of cigarette consumption over time within smokers [38] which would lead to homeostatic levels of biomarkers of exposure. Therefore, we consider that the RCVs methodology could provide valuable information to monitor these biomarkers between consecutive timepoints.

RCVs have been applied in a wide range of clinical settings and endpoints [19,20]. RCVs for biomarkers of smoke exposure were calculated in a clinical confinement setting to assess metabolic and behavioural variation in subjects who switched between their conventional cigarette and an MRTP [21]. In this study, RCVs for biomarkers were calculated based on the variability observed in an ambulatory setting in which subjects were free to switch between cigarette products. It was expected that the biomarkers of tobacco smoke exposure and mouth-level exposure assessed in this non-invasive unrestricted setting would more closely reflect real variation among smokers [22]. The urinary biomarkers measured in this study were chosen for their specificity to tobacco smoke exposure and smoking behaviour. For example, NNAL has been shown to be sensitive to changes in exposure to tobacco smoke [23] and has been linked to lung cancer [10], whilst cotinine is frequently used in smoking studies to assess compliance. [24] Mouth-level exposure (MLE) endpoints have proved useful in assessing smoking intensity, showing correlations with urinary and salivary nicotine metabolites [25,26].

Here, we report RCVs calculated in conditions representative of those expected during monitoring potential MRTPs. The sensitivity of these references was evaluated by quantifying significant changes between successive observations when subjects switched to products of a lower ISO tar and nicotine yield.

2. Methods

2.1. Study design

A non-residential, observational, ambulatory study was conducted over 3.5 years across 10 sites in Germany (Berlin, Cologne, Dresden, Gelsenkirchen, Hamburg, Leipzig, Munich, Nuremberg, Rostock and Stuttgart). The study was conducted in accordance with the principles documented in the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice (trial registration ID: ISRCTN95019245). All subjects provided written informed consent and the study protocol and informed consent forms were approved by the Ethics Committee of Bayerischen Landesärztekammer.

Full details of the study protocol have been described previously [27]. Briefly, all subjects were daily smokers of