Research Article

210Pb: A Predictive Biomarker of Retrospective Cigarette Smoke Exposure

Stephen R. Schayer1, Qingshan Qu1, Yanling Wang2, and Beverly S. Cohen1

Abstract

Although cigarette smoking continues to occur worldwide, there are few methods available to assess a person’s retrospective exposure to mainstream smoke. The tobacco of cigarettes contains trace quantities of radioactive 210Pb and 210Po, which are volatilized and inhaled when a cigarette is smoked. It was hypothesized that urinary 210Pb and 210Po activity concentrations could be used as biomarkers of exposure to mainstream tobacco smoke. Human subjects (n = 250) were recruited from Beijing, China, and reported their smoking habits. Each subject provided a 24-hour urine sample, which was assayed for its 210Pb and 210Po activity concentrations. Although the urinary 210Po activity from smoking was very low compared with background levels, the urinary 210Pb activity correlated with the number of cigarettes smoked per day (CPD; r = 0.38, P < 0.001) and the urinary cotinine concentration (r = 0.52, P < 0.001). In a linear regression model, a 1-unit increase in CPD was associated with an increase of 0.13 mBq in urinary 210Pb activity. In a logistic regression model, a 1-unit increase in urinary 210Pb activity was associated with an estimated 25% increase in the odds of being a smoker. These data were modeled using the respiratory, gastrointestinal, and biokinetic models of the International Commission on Radiological Protection. When the final model was applied for a long-term smoker (20 CPD) that suddenly quits, the predicted urinary activity decreased to 50% of the steady-state activity in about 90 days. Based on this half-time estimate and the regression results, urinary 210Pb can be used to assess the probability of having smoked in the past months. Cancer Epidemiol Biomarkers Prev; 19(2); 338–50. ©2010 AACR.

Introduction

Despite an abundance of scientific research over the last several decades that links the smoking of cigarettes with numerous adverse health effects, an alarming number of people continue to smoke. WHO estimates that nearly 1 billion men as well as 250 million women worldwide smoke a total of more than 15 billion cigarettes on a daily basis (1). As such, there are many situations in which an accurate accounting of a person’s current and former smoking intensity is essential. Such information is needed to make accurate predictions of a person’s risk of developing one or more smoking-related diseases, in epidemiologic studies in which smoking may act as a confounder and in clinical trials in which smoking can alter drug metabolism.

Cotinine is the most commonly used biomarker of exposure for cigarette smoking. With an average half-life in plasma of only 16 hours, however, cotinine is indicative of a person’s exposure to nicotine during the recent few days only (2). In addition, single-nucleotide polymorphisms within the gene encoding CYP2A6 have been noted and can affect a person’s ability to metabolize nicotine to cotinine (3, 4).

The lack of a biomarker to date that describes long-term exposure to mainstream cigarette smoke has presented a challenge to the scientific community. One promising new marker in the literature is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which has an elimination half-life from the body of 45 days (5), suggesting that it may give some indication of exposure to tobacco smoke over the recent month. In a study of 61 smokers, Carmella et al. (6) reported that urinary NNAL correlated with urinary cotinine (r = 0.60, P < 0.0001), but not with the number of cigarettes smoked per day (CPD; r = 0.05, P > 0.1). A larger study (n = 374) by some of the same authors indicated, however, that urinary NNAL, when corrected for creatinine, increased with CPD (r = 0.478, P < 0.0001) until it stabilized at 40 cigarettes (7). Others have also been examining the prospect of measuring NNAL or cotinine in hair or toenails, which have relatively long turnover periods. Stepanov et al. (8), in a study of 105 participants, found that toenail NNAL did not correlate with CPD (r = 0.25, P = 0.076) but correlated with toenail cotinine (r = 0.69, P < 0.001); toenail cotinine correlated weakly with CPD (r = 0.26, P = 0.009). Further studies are necessary.

Because of the lack of a validated biomarker of long-term cigarette smoke exposure, a person’s self-reporting...
of smoking practices is often used. There are several limitations with this method of exposure assessment, including the environment in which the survey is administered and the accuracy of the answers provided. Although several studies (9-11) have found that self-reports of recent smoking activity have correlated to some extent with cotinine levels, others have indicated the prominence of underreporting. One study that used salivary cotinine levels as the “gold standard” estimated self-reporting, as a test, to have a sensitivity of 78.3%. The authors concluded that, although not widespread, underreporting indeed occurs (12). Other studies have found that underreporting of recent smoking habits occurs most often among people who reported being light smokers (13), who are pregnant (14), or who have been previously diagnosed with respiratory disease (15).

Given the limitations of self-reporting, it is clear that additional biomarkers of smoking are needed. Our hypothesis was that lead-210 (210Pb) and polonium-210 (210Po) could serve as biomarkers of exposure to mainstream cigarette smoke. Both 210Pb and 210Po are members of the naturally occurring uranium-238 (238U) series. Briefly, radium-226 (226Ra) decays via α-particle emission to radon-222 (222Rn), a naturally occurring gas that is dispersed throughout the atmosphere. 210Pb, a decay product of the short-lived radon progeny, has a half-life of 22.3 years and undergoes beta decay to bismuth-210 (210Bi). 210Bi has a very short half-life of 5.0 days and decays via beta emission to polonium-210 (210Po). 210Po is an alpha emitter with a half-life of 138.4 days.

It is well known that tobacco contains 210Pb and 210Po. While the roots of tobacco plants absorb these nuclides from the soil, 210Pb in the air surrounding tobacco crops also accumulates on the trichomes of the leaves (17). This results in an ingrowth of 210Po as the 210Pb decays. Our recent compilation of published studies of the 210Po and 210Pb contents of cigarettes yielded a worldwide mean 210Po level of 15 mBq/cigarette (18). Given the long time period that lapses between the cultivation of the tobacco crop and the manufacture and sale of cigarettes, the 210Po is usually in a state of equilibrium with the 210Pb (19). A burning cigarette reaches temperatures between 650°C and 800°C, which are sufficiently high to volatilize a fraction of the 210Pb and 210Po in the tobacco of cigarette (17). Both 210Pb and 210Po are found in the particulate phase of whole cigarette smoke (20). We previously determined that approximately 8% of the 210Pb and 13% of the 210Po present in the tobacco of popular Chinese cigarettes are transferred to the mainstream smoke during simulated smoking on a machine (18). The actual amounts inhaled by a smoker, however, depend on several individual factors that cannot be fully modeled with a standardized smoking pattern on a smoking machine.

Following inhalation, some of the mainstream smoke particles to which 210Pb and 210Po are adsorbed will deposit in the lungs and be absorbed into the bloodstream. After absorption, 210Pb and 210Po each behave differently from a pharmacokinetic perspective. Absorbed lead travels rapidly from the blood plasma to erythrocytes, soft tissues, and bone. More than 99% of the lead in blood is found in the RBC, with the remainder in the plasma (21). In men acutely exposed to lead for less than 124 days, the mean half-life of lead in the total body measured in several studies ranged from 19 to 30 days; in workers chronically exposed to lead for up to 10 years, the initial half-life of lead in the total body after the exposure ended was 20 to 130 days (21). The rate of urinary excretion of lead is proportional to the concentration of lead in the plasma (21).

Leggett et al. have recently developed a pharmacokinetic compartmental model that describes the behavior of absorbed polonium. Although a small fraction (20%) of the polonium rapidly travels from the plasma to the kidneys and is excreted, the remainder of the polonium in the blood travels through a series of compartments that roughly correspond to soft tissue and other organs. The overall biological half-life of 210Po in the soft tissues of the body is estimated to be 20 to 60 days (22).

Several studies have found elevated levels of 210Pb and 210Po in many bodily tissues of smokers relative to nonsmokers. These tissues include the lungs (23-27), blood (28, 29), and bone (23). Two Brazilian studies measured 210Po and 210Pb in the urine of a control population used for comparison with underground miners. The mean activities in smokers were statistically greater than those in nonsmokers (30, 31). Given these previously published results and the fact that 210Pb and 210Po are present in tobacco and inhaled in cigarette smoke, the goal of this research is to determine if the activity concentrations of 210Pb and 210Po in human urine can serve as biomarkers of exposure to mainstream cigarette smoke.

Materials and Methods

Sample Collection

Human subject volunteers were recruited from suburban villages, mostly in Shahe Town, Beijing. After informed consent was obtained, each subject was interviewed and responded to questions about current and past cigarette smoking intensity, occupation, diet, etc. Subjects were also given a physical examination, and those that were deemed healthy provided a 24-h urine sample. A total of 250 samples were collected: 125 in October 2005 and 125 in October 2006. Each subject was asked to record the number of cigarettes smoked on the day before beginning the urine collection as well as the day of the collection. An approximately equal number of subjects were selected in five groups of different smoking intensities. This study was reviewed and approved annually by the New York University School of Medicine (NYUSOM) Institutional Review Board.
After measuring the volume of each sample, a 3-mL aliquot was removed and frozen for later analysis of cotinine and creatinine. The remaining sample was slightly acidified with 10 mL of high-purity nitric acid (HNO₃) and evaporated in a glass beaker on a hot plate until a final volume of approximately 125 mL was attained. Each sample was then shipped to NYUSOM for further analysis. All samples were refrigerated before shipment.

**Assays for Cotinine and Creatinine**

The frozen 3-mL aliquots of each sample were assayed at NYUSOM using the Immunalysis Cotinine Direct ELISA Kit (Immunalysis Corporation) and the Thermo-Trace Creatinine Reagent Kit (Thermo Scientific).

**Assay for 210Po**

On arrival at NYUSOM, urine samples were refrigerated until processing. The method used to measure the 210Po in each sample was a slight modification of HASL-300 Procedure Po-01-RC (32). Briefly, an initial volume of 25 mL of concentrated high-purity HNO₃ (16 mol/L, TraceMetal grade; Fisher Scientific) was added to each sample in a glass beaker covered with a watch glass. A solution of 209Po tracer (NIST standard reference material 4326, 85.42 Bq 209Po g⁻¹ as of March 15, 1994, $T_{1/2} = 102.5$ y, generously provided by Dr. Isabel Fisenne, Environmental Measurements Laboratory, U.S. Department of Energy, New York, NY), to be used as indicator of yield, was added by mass. Each sample was then placed onto a hot plate and heated over medium to high heat. Successive 5-mL portions of HNO₃ (about 200 mL total) were added to digest the organic material present. The end point, at which all of the organic material was destroyed, was indicated when brown gases (NO₂ and nitric oxides) were no longer observed. The remaining sample was slightly acidified with 10 mL of high-purity nitric acid (HNO₃) and evaporated in a glass beaker on a hot plate until the volume was reduced to near dryness, at which point 0.5 mol/L HCl was added. The solution was heated for a short time and then agitated to dissolve any salts that had precipitated. A larger volume (150 mL) of 0.5 mol/L HCl was added to those samples that had precipitated. The solution, at room temperature, was stirred for 4 to 6 h with plastic stir bars attached to overhead motorized stirrers.

After stirring, the nickel disc from each plating was rinsed with deionized water and 70% ethanol and placed side up under a heat lamp to dry.

**Alpha Counting**

The 209Po ($\alpha$-particle energy = 4.9 MeV) and 210Po ($\alpha$-particle energy = 5.3 MeV) activities in each sample were assessed by counting each nickel disc in an alpha spectrometer equipped with a silicon solid surface barrier detector. Background counts of a nickel disc in the regions of interest were assessed at least once weekly. The counting efficiency was 20% and was determined by regularly counting an electroplated source (generously provided by Dr. Isabel Fisenne, Environmental Measurements Laboratory, U.S. Department of Energy, New York, NY) containing the nuclides 235Pu (4.9 MeV), 239Pu (5.1 MeV), 238Pu (5.5 MeV), and 244Cm (5.8 MeV). The plated urine samples were typically counted for 1,500 min. The lower limit of detection calculated for 210Po using the method described in the HASL Manual (32) was 0.6 mBq. Some of the plated urine samples were also counted on alpha spectrometers (15 total) in the counting laboratory at the University of Cincinnati in Ohio.

**Assay for 210Pb**

To determine the 210Pb present in the sample, the supernatant solutions from the digested urine samples following the initial polonium plating were stored for approximately 1 y to allow for the ingrowth of 210Po from 210Pb. 208Pb (United Kingdom National Physical Laboratory, 70.5 Bq 208Pb g⁻¹ as of May 17, 1999, $\alpha$-particle energy = 5.1 MeV, $T_{1/2} = 2.9$ y, purchased from QSA Global) was added as a second tracer of yield because some of the 209Po that was originally added remained in solution. Each sample was heated in a covered glass beaker on a hot plate until the volume was reduced to near dryness, at which point 0.5 mol/L HCl was added. The polonium (residual 208Po and 210Po remaining from the initial plating, 208Pb as an indicator of current yield, and 210Po from the decay of 210Pb during storage) from each solution was then spontaneously deposited at room temperature onto a nickel disc. Discs were again counted with an alpha spectrometer. The lower limit of detection for 210Pb was 0.8 mBq.

Measured activity levels were corrected for the relevant decay interval between plating and counting. The measured 208Pb activity at the first plating was used to calculate the residual fraction of 210Po that would have remained in each solution at the second plating. The calculated value of the residual 210Po—generally quite small—was subtracted from the total measured 210Pb in each solution at this second plating. The net 210Po activity ($N_{210Pb}$) was used to calculate the 210Pb activity ($N_{210Pb}$) present at the initial time of plating.
according to the following equation describing radioactive equilibrium:

\[
\lambda_{\text{Po}} N_{\text{Po}} = \frac{\lambda_{\text{Po}} (\lambda_{\text{Pb}} N_{\text{Pb}})}{\lambda_{\text{Pb}}} \left[ e^{-\lambda_{\text{Pb}} t} - e^{-\lambda_{\text{Po}} t} \right]
\]

in which \(\lambda_{\text{Po}}\) is the decay constant \((5.008 \times 10^{-3} \text{ d}^{-1})\) of \(^{210}\text{Po}\), \(N_{\text{Po}}\) is the number of \(^{210}\text{Po}\) atoms, \(\lambda_{\text{Pb}}\) is the decay constant \((8.516 \times 10^{-5} \text{ d}^{-1})\) of \(^{210}\text{Pb}\), \(N_{\text{Pb}}\) is the number of \(^{210}\text{Pb}\) atoms present initially, and \(t\) is the elapsed time between the first and second plating.

The calculated \(^{210}\text{Pb}\) activity at the initial time of plating was then corrected back to the \(^{210}\text{Pb}\) activity at the time of sample collection. The \(^{210}\text{Po}\) present at the first plating that had been produced by the \(^{210}\text{Pb}\) present at the time of sample collection was then calculated. This \(^{210}\text{Po}\) activity that had been fully produced by the \(^{210}\text{Pb}\) in the sample was subtracted from the total \(^{210}\text{Po}\) activity measured at the first plating. The time interval between the first plating...
and the sample collection was used to calculate the $^{210}$Po activity at the time of sample collection. In all calculations, the yield on each sample was used. It should be noted that $^{208}$Po was not initially available commercially when the assay for $^{210}$Pb was begun; as a result, there were some samples ($n = 65$) that were not spiked with $^{208}$Po. Instead, the recovery was estimated.

**Quality Control**

Quality control was accomplished by regularly running reagent blanks, the average of which was subtracted from the results. Some samples (6%) were also counted both at NYU and at the University of Cincinnati to ensure reproducibility. The activities of counting standards were also verified in alpha scintillation counters at the Department of Environmental Medicine Whole Body Counting Laboratory at NYUSOM. The activities of the $^{209}$Po and $^{208}$Po tracer solutions were verified periodically by depositing a known activity onto a nickel or stainless steel disc and counting. Blanks (nine water blanks and two acid blanks) were included throughout the experiment and there was no detection of any $^{209}$Po or $^{208}$Po.

**Data Analysis**

All data were analyzed using the statistical software package Minitab 15 or PASW 17 (SPSS, Inc.). Spearman

![Figure 2. Scatterplots of total urinary cotinine concentration by CPD (A) and urinary cotinine/creatinine ratio by CPD (B).](image-url)
correlation coefficients ($p$) are presented, unless otherwise noted.

**Results**

**Demographic Data of the Subjects**

The subjects recruited for this study ($n = 250$) ranged in age from 20 to 82 years. There were 196 men (172 smokers and 24 nonsmokers) and 54 women (12 smokers and 42 nonsmokers). The 184 smokers each reported that they currently, at the time of sample collection, smoked cigarettes. The 66 nonsmokers each reported that they did not currently smoke cigarettes. The nonsmokers included 55 subjects who reported having never smoked cigarettes and 11 who reported having completely quit smoking at least 30 days before the urine sample collection.

**Cotinine Results**

Of the 250 urine samples collected for analysis, 2 were initially excluded for quality assurance purposes. Urinary cotinine was first examined in the analyzed data set ($n = 248$) to verify the self-reported recent smoking status of each subject. As expected, the median cotinine level in the urine of self-reported smokers ($6,668 \mu g/d$, mean = $8,638 \mu g/d$, $n = 184$) was orders of magnitude higher ($P < 0.001$, Kruskal-Wallis test) than that in the urine of nonsmokers ($30 \mu g/d$, mean = $139 \mu g/d$, $n = 64$). There was no significant gender effect, as the median cotinine level among nonsmoking females ($29 \mu g/d$, mean = $129 \mu g/d$, $n = 41$) was not statistically different ($P = 0.8$, Kruskal-Wallis) from that among nonsmoking males ($31 \mu g/d$, mean = $158 \mu g/d$, $n = 23$); similarly, the median cotinine level among smoking females ($6,663 \mu g/d$, mean = $6,735 \mu g/d$, $n = 12$) was not

**Figure 3.** Simple linear regression models for CPD by total urinary cotinine concentration (A) and cotinine/creatinine ratio (B).
statistically different \((P = 0.5, \text{Kruskal-Wallis})\) from that among smoking males \((6,668 \, \mu g/d, \text{mean} = 8,771 \, \mu g/d, \, n = 172)\). On average, females smoked fewer cigarettes per day \((\text{mean} = 16 \, \text{CPD})\) than did males \((\text{mean} = 24 \, \text{CPD})\).

A cumulative lognormal probability plot of the urinary cotinine levels displays the differences by smoking status (Fig. 1A). Samples with unusual observations are quite apparent. There are five self-reported nonsmokers whose urinary cotinine levels indicate recent smoking, and thus they were not nonsmokers. Interestingly, three of these five had indicated in the questionnaire that they were former smokers who had completely quit smoking more than 30 days before the sample collection. Conversely, there are six smokers whose urinary cotinine levels were too low to have actively smoked recently. Of those six, five reported that they were current smokers who, for some reason, did not smoke any cigarettes during the urine collection period; this result reaffirms the short biological half-life of cotinine. These five smokers who did not smoke during the collection were placed into a new category of “nonrecent smoker,” whereas the remaining six samples with conflicts between their cotinine and self-reported smoking status were recategorized as “unknown.” With these categories, the separation between smokers and nonsmokers becomes much clearer on the cumulative lognormal plot (Fig. 1B).
cotinine value of 100 μg/d is a useful active smoking breakpoint for this population.

To examine the relationship between the measured urinary cotinine and the self-reported smoking intensity, the six samples with an unknown smoking status were removed from the analysis. With the remaining subjects, urinary cotinine is well correlated with CPD (ρ = 0.70, P < 0.001); a scatterplot fit with a nonparametric Lowess curve (Fig. 2A) indicates a linear increase of cotinine with CPD until about 20 CPD, after which the cotinine concentration stabilizes. Interestingly, when the urinary cotinine concentration is divided by the urinary creatinine concentration and plotted against CPD (Fig. 2B), the correlation observed (ρ = 0.70, P < 0.001) is the same. In simple regression models in which total urinary cotinine (Fig. 3A) or the cotinine/creatinine ratio (Fig. 3B) is used to predict CPD, the R^2 value is 49% in both models, indicating a modest regression fit. The equations of the regression lines in both models are also almost identical.

210Pb and 210Po Activity Results

The 210Pb and 210Po activity levels in the urine samples were next examined to determine whether and how they are related to cigarette smoking. Of the 242 samples for analysis, 10 were excluded because of known laboratory errors. In the remaining sample set (n = 232), there were 177 smokers and 55 nonsmokers; the nonsmokers included 48 never smokers and 7 former smokers who reported having completely quit smoking 1 or more years before the urine sample collection. The median urinary 210Pb activity in the never smokers (7.8 mBq, mean = 8.4 mBq) was not statistically different (P = 0.7, Kruskal-Wallis) from that in the never smokers (8.4 mBq, mean = 9.9 mBq). This confirmed their classification as nonsmokers.

The median total urinary 210Pb activity among smokers (12.4 mBq, mean = 13.6 mBq, n = 177) was statistically greater (P < 0.001, Kruskal-Wallis) than that among nonsmokers (8.2 mBq, mean = 9.7 mBq, n = 55). There were no significant differences by gender, as the median in nonsmoking females (7.8 mBq, mean = 8.9 mBq, n = 37) was not statistically different (P = 0.4, Kruskal-Wallis) from that in nonsmoking males (9.8 mBq, mean = 11.3 mBq, n = 18); the median in smoking females (11.3 mBq, mean = 11.9 mBq, n = 12) was also not statistically different (P = 0.2, Kruskal-Wallis) from that in smoking males (12.6 mBq, mean = 13.7 mBq, n = 165).

The situation for urinary 210Po is more complex. Because of shipping delays from China to the United States, as well as the large number of samples to be processed on arrival, there was a long period, ranging from 101 to 382 days, that lapsed between the date of sample collection and the date of initial plating. As a result, the 210Po present at the time of sample collection had almost fully decayed in 68 of the samples before the chemical analysis. After the exclusion of these samples, the remaining data set (n = 164) was composed of 122 smokers and 42 nonsmokers (5 former smokers and 37 never smokers). Again, the activities in the former smokers and never smokers were not statistically different.

A correlation analysis was next performed for total urinary 210Pb activity, 210Po activity, cotinine, CPD, and years of smoking. The 210Pb activity among smokers (18.6 mBq, mean = 23.0 mBq, n = 122) was not statistically different (P = 0.1, Kruskal-Wallis) from that among nonsmokers (14.5 mBq, mean = 19.1 mBq, n = 42). There were also no significant differences by gender. When subjects were placed into smoking groups by CPD, however, increasing trends were observed for both the 210Pb activity (Fig. 4A) and the 210Po activity (Fig. 4B).

A correlation analysis was next performed for total urinary 210Pb activity, 210Po activity, cotinine, CPD, and years of smoking. The 210Pb activity is correlated with the urinary cotinine concentration (ρ = 0.52, P < 0.001), CPD (ρ = 0.38, P < 0.001), and years of smoking.
The $^{210}$Po activity is correlated with the $^{210}$Pb activity ($\rho = 0.26, P = 0.001$), which is logical because at least some of the $^{210}$Po in the body should be supported by $^{210}$Pb, and weakly with the urinary cotinine concentration ($\rho = 0.16, P = 0.04$). It is also worth mentioning that neither the urinary $^{210}$Pb nor the $^{210}$Po activity in nonsmokers correlated well with each subject’s self-reported total daily exposure to secondary cigarette smoke.

In a simple linear regression model, when urinary $^{210}$Pb activity was tested as a predictor of CPD, a large outlier emerged. This point refers to a nonsmoking subject whose urinary $^{210}$Pb activity (47 mBq) was nearly five times the mean (10 mBq) in nonsmokers. This subject is a male accountant who was 34 years old at the time of sample collection and reported not having any medical conditions and never having smoked. It therefore seems likely that he was somehow exposed to $^{210}$Pb levels well in excess of all of the other subjects, both smokers and nonsmokers, combined. Because this point had a standardized residual greater than 2.5, it was removed.

In the final model (Fig. 5, $n = 231$), the regression is significant ($F = 30, P < 0.001$), with a 1-unit increase in urinary $^{210}$Pb activity (mBq/d) associated with an additive increase of 0.9 CPD. Although only 12% of the variability in CPD is accounted for by the regression, the SE of the estimate, $S$, indicates that CPD can be predicted to within ±30 cigarettes 95% of the time. When the axes are reversed (not shown), such that urinary $^{210}$Pb activity is predicted by CPD, a 1-unit increase in CPD is associated with a $^{210}$Pb activity increase of 0.13 mBq; the equation is $^{210}$Pb (mBq/d) = 10.2 + 0.13 CPD.

In addition to predicting CPD, given the apparent biological half-life of lead in blood of 20 to 130 days in people who are repeatedly exposed, urinary $^{210}$Pb activity can be used to predict the probability that a person had smoked in the past months. A scatterplot of the $^{210}$Pb activity versus cotinine concentration (Fig. 6) shows that some smokers had a very low urinary cotinine concentration but a very high urinary $^{210}$Pb activity. This indicates that the $^{210}$Pb activity can be useful in detecting some smokers that may not have been readily detected by the cotinine concentration.

In a logistic regression model (Fig. 7) in which urinary $^{210}$Pb activity is used to predict a subject’s smoking status, a 1-unit increase in the $^{210}$Pb activity (mBq/d) is associated with an estimated 25% increase in the odds of being a smoker. The $^{210}$Pb activity is a significant predictor (Wald statistic of 4.9, $P < 0.001$), the regression is significant ($G = 35, P < 0.001$), and the logit function is a good fit to the data ($Hosmer-Lemeshow \chi^2$ of 3.2, $P = 0.9$). The model correctly identifies 75% of the subjects as smokers or nonsmokers and incorrectly identifies 25%. A plot of the predicted probabilities of being a smoker versus the urinary $^{210}$Pb activity is more quantitative; these predicted probabilities, however, may not be reliable to predict that a person from a random population is a smoker because smokers in this study were oversampled relative to nonsmokers. Nonetheless, the sensitivity and the specificity vary depending on the probability cutoff that is used. If a person with a predicted probability of >0.5 is called a smoker, the sensitivity is 95% whereas the specificity is only 11%. On the contrary, if a probability of 0.7 is used, the sensitivity drops moderately to 65% whereas the specificity increases dramatically to 86%, very much reducing the risk of making a type I error. This model, then, indicates that urinary $^{210}$Pb activity can be used to predict a person’s smoking status over the past months.

Figure 6. Scatterplot of urinary $^{210}$Pb activity by cotinine concentration.
To further estimate the half-life of $^{210}$Pb in the body, the data from the simple regression model, in which a 1-unit increase in CPD was associated with an increase in the total urinary $^{210}$Pb activity of 0.13 mBq, were modeled. The models that were combined included the International Commission on Radiological Protection Publication 66 Human Respiratory Tract Model (33), the Publication 30 Gastrointestinal Tract Model (34), and the Publication 67 biokinetic model for lead (35). After defining the intakes, deposition, and pulmonary clearance parameters, a series of coupled first-order differential equations describing the movement of $^{210}$Pb between compartments was written for each combined model. The equations were solved in the Berkeley Madonna software using a 4th order Runge-Kutta integration function with a time step of 0.01 hour (36). The incremental 24-hour urine output, defined as the total activity expected in a 24-hour urine sample ending at the time point indicated, was calculated. Because nearly all of the smokers in this study had been smoking regularly for at least 2 years, and assuming that they did not substantially vary the number of cigarettes that they smoked each day, the $^{210}$Pb activity inhaled from smoking should reach a steady state in the body. In the combined model, a term for the total inhaled $^{210}$Pb activity per day was included, with an additional intake of the same activity occurring every 24 hours.

Because the deposition of mainstream cigarette smoke in the lungs remains quite uncertain, the deposition parameters were varied in the combined model until

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|}
\hline
Variable & Value & Count & Predictor & Coef & SE Coef & Z & P & Odds Ratio & 95% Conf Interval \\
\hline
Current Smoker & 1 & 177 (Event) & Constant & -1.25 & 0.48 & -2.61 & 0.009 & 1.25 & 1.14 & 1.37 \\
0 & 54 & & Pb-210 (mBq/d) & 0.223 & 0.04 & 4.85 & 0.000 & & & \\
Total & 231 & & & & & & & & & \\
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Test that all slopes are zero: $G = 35.004$, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

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Figure 7. Logistic regression model for smoking status by $^{210}$Pb activity.
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the predicted urinary $^{210}$Pb activity at steady state matched the results of the simple regression model built from the measured data. As previously described in detail (37), the combined model was then used to predict the length of time it would take for the urinary $^{210}$Pb activity to decrease back to 0 mBq, when disregarding ambient and dietary intakes, if a smoker of several years suddenly were to quit smoking. For a typical person who has smoked 20 CPD for 4 years before quitting, the model predicts that the urinary $^{210}$Pb activity (Fig. 8) would decrease to about 50% of the approximately steady-state activity within 90 days and to about 25% of the activity within 260 days. These predictions further indicate the usefulness of urinary $^{210}$Pb activity to predict smoking over the past months.

Discussion

From the analysis of 250 24-hour urine samples, it was determined that a total urinary cotinine breakpoint of 100 $\mu$g/d can be used to distinguish recent active smokers from nonsmokers in a contemporary Chinese smoking population. In the regression models in which the total urinary cotinine concentration or the cotinine/creatinine ratio was used to predict CPD, the cotinine/creatinine ratio was found to predict CPD just as well as did the total cotinine concentration. The ratio is therefore reliable for use in normalizing the excreted urinary cotinine concentration in urine spot samples, when 24-hour samples are not available or not practical. There has been much debate in the scientific community over whether the ratio is useful (38), and this affirmative finding helps to settle that debate. In addition, the results indicate that cotinine is best used to distinguish recent smokers from nonsmokers. It is not possible to distinguish nonrecent smokers—those that reported being current smokers that did not smoke on the day of urine collection—from nonsmokers. Given the leveling off of the cotinine at higher smoking intensities, it is also difficult to use urinary cotinine to assess a person’s smoking intensity at higher levels (greater CPD) of smoking.

In the radiochemical analysis of the urine samples, the median $^{210}$Pb activity concentration in the 24-hour urine samples of smokers (12 mBq) was statistically greater than that in nonsmokers (8 mBq). The median urinary $^{210}$Po activity concentration in smokers (19 mBq) was also close to being statistically greater than that in nonsmokers (15 mBq). Before the start of this study, no one had examined whether urinary activity concentrations of $^{210}$Pb and $^{210}$Po correlate with cigarette smoking. Although urinary $^{210}$Po was significantly correlated with urinary $^{210}$Pb, and despite an increasing trend between urinary $^{210}$Po and smoking group, there were no significant correlations observed between $^{210}$Po and the smoking indices. The lack of any stronger correlations between $^{210}$Po and the smoking indices is surprising and suggests that urinary $^{210}$Po may be strongly influenced by dietary intakes [studies have indicated that 50% of ingested $^{210}$Po is absorbed (39), whereas only 15–20% of ingested $^{210}$Pb is absorbed (40)] to a larger extent than it is influenced by inhalation intakes or internal body stores. Most recently, a group in central Italy published their study of the $^{210}$Po activity concentration in the 24-hour urine samples of 132 residents and also failed to find a statistically significant difference in the urine of smokers versus nonsmokers (41).

Significant correlations were observed, however, between the total urinary $^{210}$Pb activity and the smoking indices (cotinine, CPD, years). Regression models indicated

![Figure 8. Urinary $^{210}$Pb activity model for smoking 20 CPD and then quitting.](image)
that CPD is statistically predicted by total urinary $^{210}\text{Pb}$ activity, although with less variability being accounted for than in the model using total urinary cotinine as a predictor. $^{210}\text{Pb}$, however, with its longer apparent half-life, indicates past smoking (months), whereas cotinine, with its short half-life, indicates recent smoking (days). A logistic regression model indicated that a 1-unit increase in total urinary $^{210}\text{Pb}$ activity is associated with an estimated 25% increase in the odds of being a smoker, indicating that $^{210}\text{Pb}$ activity can be used to predict the probability of having smoked within the past months. Following validation, practical applications might include both epidemiologic studies and smoking cessation studies. For the latter, repeated intrasubject measurements could be made over time to monitor a person’s progress in quitting smoking. In closing, the results of this study indicate a promising new use of $^{210}\text{Pb}$ as a biomarker in predicting a person’s retrospective smoking status.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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