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Cotinine levels and self-reported smoking status in patients attending a bronchoscopy clinic

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The reliability of self-reported smoking behaviour can vary and may result in bias if errors in misclassification vary with outcome. We examined whether self-report was an accurate measure of current smoking status in patients with malignant or non-malignant respiratory disease. Smoking behaviour was assessed by self-report and by analysis of whole blood for cotinine, a biomarker of exposure to cigarette smoke, in 166 patients attending a bronchoscopy clinic. Cotinine levels ranged from 2.5 to >400 ng ml^{-1} blood and were higher in self-reported current smokers (173 ± 123 ng ml^{-1}) than in never smokers (3.7 ± 8.7 ng ml^{-1}) or ex-smokers (20.5 ± 49.0 ng ml^{-1}). Cotinine levels in self-reported current smokers increased with the numbers of cigarettes smoked ($p = 0.06$), and levels in smokers and ex-smokers decreased with the reported length of time since the last cigarette ($p = 0.001$). Using a cotinine level of 20 ng ml^{-1} and self-report as the gold standard, the sensitivity and specificity for defining current smoking status were 90.2% and 82.4%, respectively. Out of a total of 125 self-reported current non-smokers, 23 (18.4%) had cotinine levels greater than 20 ng ml^{-1}. Smoking prevalence was significantly underestimated by self-report (24.7%) when compared with that defined using blood cotinine levels (36.1%: $p < 0.001$). Misclassification of current smoking status was particularly high in ex-smokers, in patients without malignant respiratory disease, in men, and in those below the median age. Such differential misclassification may result in bias in studies examining associations between current smoking habits and disease risk.

Keywords: smoking, cotinine, misclassification.

Introduction

Cotinine is one of the major metabolites of nicotine (Benowitz 1996). Although it may arise as a result of exposure to non-tobacco products such as certain foodstuffs (Idle 1990, Benowitz 1996), cotinine is now seen as a specific marker of exposure to tobacco smoke, particularly at the levels seen in smokers (Benowitz 1996). Cotinine levels in smokers are typically more than 100 times higher than those in non-smokers, whatever the biological fluid (serum, saliva or urine) analysed (Jarvis *et al.* 1987, Benowitz 1996). As well as distinguishing between smokers and non-smokers, cotinine levels are also higher in individuals passively exposed to tobacco smoke (Emmons *et al.* 1994, Benowitz 1996).

Measurement of cotinine in biological fluids has been used as a more objective measure of current smoking than self-report (Lee and Forey 1995, Perez-Stable *et*

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al. 1995, Wu 1997). Self-report is widely used to measure smoking prevalence in epidemiological studies as it is a cheap and potentially reliable measure. For example, in a case-control study of cervical cancer, cigarette usage was reported accurately 98.5% of the time (Slattery *et al*. 1989). However, a subject may deny current or past smoking habits, particularly in situations where they may have already been given advice to stop smoking or are well informed of the hazards of smoking but still continue to smoke. Hence the reliability of self-report may vary. In a cross-sectional study of dietary intake and hormones, cigarette usage was reported accurately (using serum cotinine as the gold standard) 93.8% of the time, but only 82.8% of the time in a family cohort study of cardiovascular disease and hypertension (Slattery *et al*. 1989).

In a review of 35 studies on the use of cotinine to detect misclassified current smoking, rates of misclassification were reported as varying between 0.5 and 17.4% (Lee and Forey 1995). Even higher misclassification rates have been reported. In a study of pregnant women, 38% of self-reported non-smokers were found to have been exposed to cigarette smoke, but because of the low serum cotinine cut-off level of 6 ng ml^{-1} used this may, in part, reflect passive smoking (Bardy *et al*. 1993). Misclassification rates will vary depending not only on the choice of cut-off value and the methodology used to determine cotinine levels, but also on the study population (Lee and Forey 1995). Denial of current smoking has been reported to be higher in self-reported ex-smokers than in non-smokers (Slattery *et al*. 1989, Wagenknecht *et al.* 1992), and in occasional smokers rather than regular smokers (Lee and Forey 1995). In addition, misclassification rates may vary with ethnicity (Perez-Stable *et al*. 1992, Wells *et al.* 1998) and with educational level (Perez-Stable *et al*. 1992), but not necessarily with gender (Lee and Forey 1995, Wewers *et al*. 1995, Wells *et al.* 1998). In one study the most inaccurate reporting of smoking was by ex-smokers being followed for cardiovascular disease known to be linked to smoking (Slattery *et al*. 1989).

We have previously reported on lung cancer susceptibility in relation to metabolic genotypes (Lewis *et al*. 2001, 2002). As the risk of cancer will depend on the degree of exposure to carcinogens in tobacco smoke (as well as the genetic capacity to metabolize them), we examined whether self-report is an accurate measure of current smoking status (as defined biochemically) in patients with malignant and non-malignant disease attending a bronchoscopy clinic.

Materials and methods

Study population

Patients attending bronchoscopy clinics at the North West Lung Centre, Wythenshawe Hospital, Manchester, UK, who were over the age of 18 years and who were well enough to be interviewed were asked to take part in the study. All subjects gave informed consent and were interviewed using a structured questionnaire to obtain information on lifetime history of tobacco use and whether they had been in the presence of people smoking at home or at work. Any patient who reported smoking at least one cigarette per day for as long as 1 year was classified as a smoker; patients who had previously been smokers but who had abstained from smoking for at least 1 week were classified as ex-smokers. All other patients were classified as never smokers. This study population has been described in more detail elsewhere (Lewis *et al*. 2001). Ethical approval for the study was obtained from a local ethical committee. Blood samples ($n = 259$) were collected in ethylene diamine tetra-acetic acid (EDTA) from 178 patients and were frozen immediately and stored at $-80^{\circ}C$ until the assay was performed. Twelve

patients who smoked products other than cigarettes (cigars, pipes, etc.) were excluded from the analyses reported here.

There were 57 patients currently with, or with a history of, tumours of the lung, trachea or bronchus, and 109 other patients who were free of benign and malignant tumours both at the time of, and prior to, the current diagnosis.

Of the patients with lung cancer, 33 had non-small cell cancers (24 squamous cell carcinomas and six adenocarcinomas), 10 had small cell cancers and 14 had tumours that were not defined histologically as the tumours were so far advanced that diagnosis was irrelevant to the palliative treatment given. Of those with non-malignant disease, the most frequent final diagnoses included chronic airflow limitation/bronchitis ($n = 34$), infective disorders/respiratory infection ($n = 25$) and diffuse fibrotic lung disease ($n = 9$). There were no abnormalities found in 38 patients.

Analysis of whole blood for cotinine

Cotinine was measured by gas chromatography-mass spectrometry using the selected ion monitoring mode. Blood samples were thawed at room temperature and 0.2 ml of blood was added to 0.1 ml of H_2O and 0.1 ml of 50 ng ml^{-1} 1-[methyl-d_3]-5-[3-pyridinyl]-2-pyrrolidinone (cotinine-d_3, the internal standard). Then 0.6 ml of 5 M NaOH was added and the samples were centrifuged at 2000 r.p.m. at 4°C for 20 min. Next 0.5 ml of supernatant was removed and 20 µl of antifoam/indicator solution and 100 µl of dichloroethane were added. The samples were then vortex mixed for 60 s and centrifuged twice at 2000 r.p.m. at 20°C for 20 min. The top aqueous layer was removed and the bottom organic layer was dispersed to remove any emulsion at the interface. The clear organic extract was transferred into an injection vial and 1 µl of each extract was injected onto a 30 m × 0.25 mm internal diameter × 0.25 µm film thickness capillary column (J&W DB5MS low bleed column) using a Thermo Unicam 612 autoinjector. Selected ion monitoring was carried out using a Thermo Unicam 610 GC and a Thermo Unicam Automass 2 bench top mass spectrometer. The injection port was set at 275°C; helium was used as the carrier gas and was set at a pressure of 17 p.s.i. The column was held at 70°C for 1 min with the split valve shut. The split valve was then opened and the temperature increased to 265°C at a rate of 25°C per minute. The interface tube was held at a temperature of 250°C and the source at 130°C. Ions with a mass to charge ratio (m/z) of 101 (cotinine-d_3) and 176 (cotinine) were monitored. The ratio of the area under the curve of cotinine to cotinine-d_3 was then measured and compared with a curve generated using standards containing 10, 100 and 1000 ng ml^{-1} cotinine in blood from a non-smoker that were assayed at the same time.

Positive controls, assayed in each batch of samples, consisted of (i) a neat blood sample from a smoker (high positive control), and (ii) a 1:10 dilution of the smoker's blood in the blood of a non-smoker (low positive control). The negative control was filtered water containing cotinine-d_3. The mean ($\pm$SD) cotinine value of the high positive controls was 267.6 ± 17.4 ng ml^{-1} ($n = 12$) and that of the low positive controls was 29.5 ± 7.8 ng ml^{-1} ($n = 12$). The detection limit of the assay was ~ 2.5 ng ml^{-1}.

Statistical analysis

Cotinine levels in self-reported smokers, ex-smokers and never smokers, and variation in levels since the time last smoked were compared using analysis of variance (ANOVA). Smoking prevalence based on self-report and cotinine levels was compared using McNemar's test. Associations between age, sex, disease status and concordance between self-report and cotinine levels were examined using logistic regression.

Results

The study population consisted of 166 people (98 men and 68 women). The mean age of the population was 62.3 ± 13.0 years (range 22–89 years). Of the patients without malignant disease, 24 were smokers, 56 were ex-smokers and 29 were never smokers, whereas only two patients with malignant disease were never smokers, 38 were ex-smokers and 17 were smokers. In current smokers, the mean ($\pm$SD) number of cigarettes smoked per day was 20.5 ± 8.5 in patients with malignant disease and 22.3 ± 10.8 in those without ($p = 0.57$).

Cotinine levels in self-reported current smokers (173 ± 122.6 ng ml^{-1}) were higher than in never smokers (3.7 ± 8.7 ng ml^{-1}) or ex-smokers (20.5 ± 49.0 ng ml^{-1}) ($p < 0.001$) (Table 1). The majority of all self-reported smokers (29 out of

Table 1. Cotinine levels by self-reported smoking status and exposure to passive smoke.

	All			Exposed to passive smoke			Unexposed to passive smoke		
		Cotinine levels (ng ml^{-1})			Cotinine levels (ng ml^{-1})			Cotinine levels (ng ml^{-1})	
Smoking status	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range
Current smokers	41	173.0 ± 122.6	0–441	14	188.0 ± 142.2	0–441	27	165.2 ± 113.3	0–385
Ex-smokers[a]	94	20.5 ± 49.0	0–274	24	30.1 ± 40.5	0–153	69	17.5 ± 51.8	0–274
Never smokers[a]	31	3.7 ± 8.7	0–35	11	3.2 ± 10.6	0–35	19	3.1 ± 6.8	0–24

[a] Information on passive smoke exposure was unavailable for one ex-smoker and one never smoker.

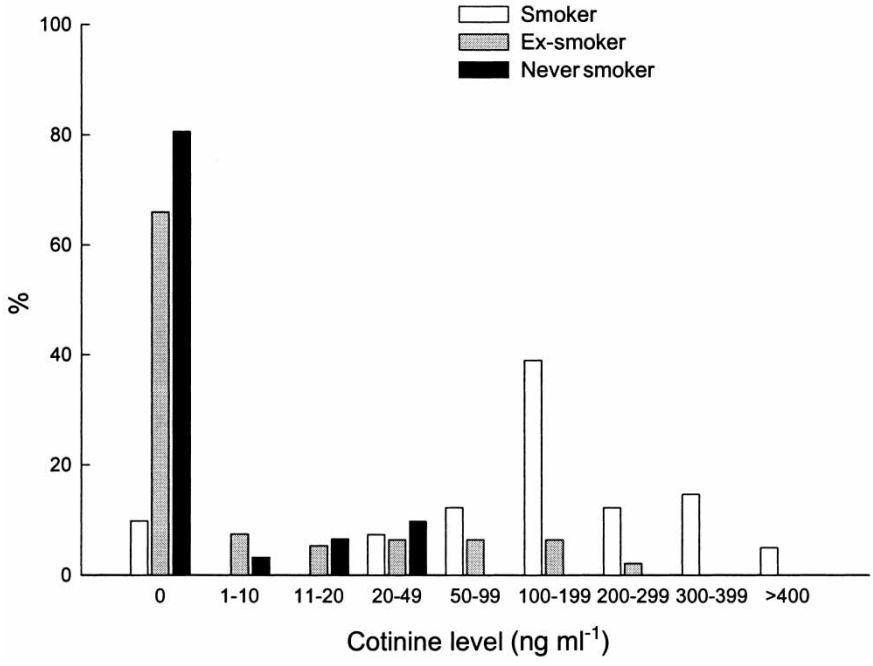

Figure 1. Distribution of cotinine levels in self-reported smokers, ex-smokers and never smokers.

41, 70.7%) had cotinine levels of at least 100 ng ml^{-1}, whereas only 8.5% (eight out of 94) of self-reported ex-smokers and 0% (zero out of 31) never smokers had cotinine levels that high (Figure 1). Conversely, 83.9% (26 out of 31) of never smokers and 73.4% (69 out of 94) of ex-smokers had blood cotinine levels of less than 10 ng ml^{-1}. Only four (6.7%) smokers had cotinine levels that low.

There was no difference in cotinine levels between those who reported exposure to passive smoking and those who didn't, especially in those who were self-reported never smokers (Table 1). Fourteen of the 24 ex-smokers reporting passive smoke exposure had detectable cotinine levels, but cotinine was found in only 18 of the 69 patients reporting no such exposure ($p = 0.004$). Only one of the 11 never smokers who reported exposure to passive smoke had a detectable cotinine level, whereas four of the 19 who reported no exposure had detectable levels ($p = 0.40$).

There was a weak association between cotinine levels in self-reported current smokers and the numbers of cigarettes smoked per day (Figure 2) ($p = 0.06$). Two individuals who reported smoking between five and ten cigarettes per day had no detectable blood cotinine levels (Figure 2). Cotinine levels in smokers and ex-smokers decreased with the reported time since the last cigarette (Table 2) ($p = 0.001$). Smokers who reported having smoked a cigarette that day had higher cotinine levels (198 ± 113.7 ng ml^{-1}) than those who reported smoking within the last week (138.8 ± 125.3 ng ml^{-1}); smokers who reported smoking more than a year ago had cotinine levels of 13.2 ± 35.2 ng ml^{-1}. There was no difference between cases and controls in the time since the patient had last smoked (data not shown).

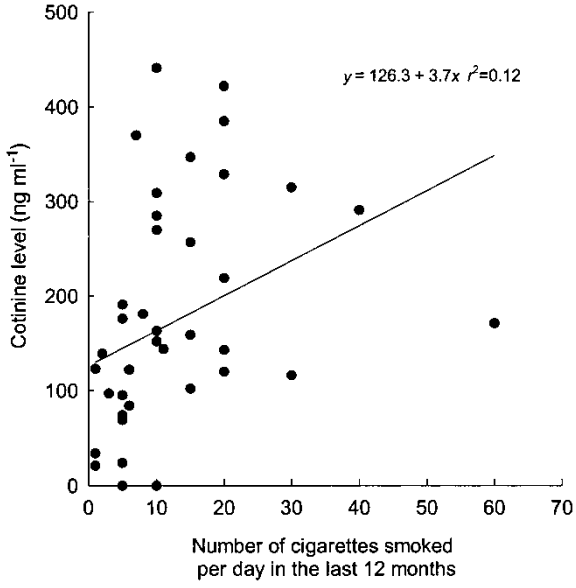

Figure 2. Variation of cotinine level with numbers of cigarettes smoked per day in self-reported current smokers.

Table 2. Time since last smoked a cigarette and cotinine levels in smokers and ex-smokers.

		Cotinine levels (ng ml^{-1})	
Time since last smoked	*n*	Mean ± SD	Range
This morning	24	198.5 ± 113.7	0–422
< 7 days	18[a]	138.8 ± 125.3	0–441
< 4 weeks	6	74.3 ± 114.6	0–274
< 12 months	11	27.6 ± 44.9	0–117
> 1 year	76	13.2 ± 35.2	0–227

[a] Includes one individual who classified himself as an ex-smoker but who had a cotinine level of 171 ng ml^{-1}.

All smokers (except the four in whom no cotinine was detected) had cotinine values of at least 20 ng ml^{-1}. Using this as a cut-off value gave a sensitivity of 90.2% and a specificity of 82.4%. A cut-off value of 35 ng ml^{-1} (no self-reported never smoker had a level above this) would have increased the specificity to 87.2%, but the sensitivity for self-reported smoking with this cut-off value was only 82.9%. A cut-off value of 20 ng ml^{-1} consistent with previous results was used for the subsequent analysis. Out of 125 self-reported current non-smokers, 23 (18.4%) had cotinine levels greater than 20 ng ml^{-1}. Of the self-reported current smokers ($n = 41$), four had cotinine levels less than 20 ng ml^{-1} and two had abstained from smoking for 4–5 days. The misclassification rate was higher in the ex-smokers (20 out of 94; 21.2%) than in the never smokers (three out of 31; 9.7%).

Overall, in the study population, the smoking prevalence was 24.7% by self-report and 36.1% when defined biochemically using a cotinine level > 20 ng ml^{-1} ($p < 0.001$) (Table 3). The smoking prevalence defined using blood cotinine levels

Table 3. Smoking prevalence defined by self-report and cotinine levels >20 ng ml^{-1}.

	All			Lung cancer patients			Patients without lung cancer		
		Smoking prevalence			Smoking prevalence			Smoking prevalence	
Variable	*n*	Cotinine level	Self-report	*n*	Cotinine level	Self-report	*n*	Cotinine level	Self-report
All	166	60/106 (36.1%)	41/125 (24.7%)[b]	57	21/36 (36.8%)	17/40 (29.8%)	109	39/70 (35.8%)	24/85 (22.0%)[b]
Male	98	36/62 (36.7%)	22/76 (22.4%)[c]	39	15/24 (38.5%)	12/27 (30.8%)	59	21/37 (35.6%)	10/49 (16.9%)[d]
Female	69	24/44 (35.3%)	19/49 (27.9%)	18	6/12 (33.3%)	5/13 (27.8%)	50	18/32 (36.0%)	14/36 (28.0%)
Age[a]									
$<$ Median	83	37/46 (44.6%)	24/59 (28.9%)[e]	29	13/16 (44.8%)	11/18 (37.9%)	55	21/34 (38.2%)	12/43 (21.8%)[f]
$>$ Median	83	23/60 (27.7%)	17/66 (20.5%)	28	8/20 (28.6%)	6/22 (21.4%)	54	18/36 (33.3%)	12/42 (22.2%)

[a] Median age: all patients, 63.8 years; lung cancer patients, 69.3 years; controls, 60.9 years.
Significant differences in smoking prevalence as defined by serum cotinine levels or self-report: [b] $p = 0.001$; [c] $p = 0.003$; [d] $p = 0.007$; [e] $p = 0.002$; [f] $p = 0.012$.

was higher than when defined by self-report particularly in patients without malignant disease (35.8% versus 22.0%, $p < 0.001$), in men (36.7% versus 22.4%, $p = 0.003$) and in those patients younger than the median age (44.6% versus 28.9%, $p = 0.002$) (Table 3).

Amongst the patients without malignant disease, significant discrepancies between cotinine levels and self-reported smoking status occurred in men: when defined by self-report the smoking prevalence in men was 16.9% but it was 35.6% when defined biochemically ($p = 0.007$) (Table 3). The corresponding figures for women were 36.0% and 28.0% ($p = 0.12$). The smoking prevalence in these patients was significantly greater when defined biochemically than by self-report, particularly in those below the median age (38.2% versus 21.8%, $p = 0.01$). Women were less likely than men to have discordant results (odds ratio 0.30; 95% confidence interval 0.09–1.0, $p = 0.05$), but age (above/below median) was not associated with discordant results (odds ratio 0.56, 95% confidence interval 0.25–2.12). In contrast, amongst those patients with lung cancer, no significant differences between self-reported and biochemically defined smoking status were seen in men, women, or patients greater or less than the median age.

Discussion

In this study, cotinine levels were measured in whole blood samples to detect potential errors in smoking status as defined by self-report. If self-reported smoking rates are lower than the true smoking prevalence, this could underestimate the effects of smoking on disease occurrence or progression. Using a cotinine cut-off level of 20 ng ml^{-1}, the misclassification rate in this study (self-reported non-smokers identified as smokers biochemically) was 18.4%. This misclassification rate is higher than many other published studies: in 35 studies the rate ranged from 0.5–17.4% (Lee and Forey 1995). More recently it has been reported that typically 3% of non-smokers have cotinine/nicotine levels inconsistent with levels in true non-smokers (Wu 1999). The high misclassification rate in this study is unlikely to be due to the blood cotinine cut-off value used (20 ng ml^{-1}) as values in the range 14–25 ng ml^{-1} are most often used to detect true smokers (Benowitz 1996). Even with a conservative cut-off value of 35 ng ml^{-1}, self-reported smoking (24.6%) underestimates the true (biochemically determined) rate (30.1%). The high misclassification rate more likely results from the specific population examined. All the patients involved in this study were under investigation for pulmonary disease and hence may have been aware of smoking as a risk factor in pulmonary diseases and/or may have been given advice to stop smoking, which is known to increase the rate of false statements (Lee 1988). Similarly, high misclassification rates have been detected in other populations where smoking is a risk factor for disease, for example they were 17.4% in a study of cardiac risks in young people (Wagenknecht *et al*. 1992), or may have adverse consequences, for example a misclassification rate of 38% was reported in pregnant women (Bardy *et al*. 1993).

The relatively small sample size limits the power of our study, particularly in the subgroup analyses. There was evidence to suggest that the misclassification rate in this study was higher in patients without lung tumours than in those with lung

tumours and higher in men than in women. Differential misclassification of smoking status may result in bias in studies of such populations and may obscure the relationship, if any, between smoking habits and an adverse effect. In the present study there was little difference in lung cancer risk when comparing ever versus never smokers when smoking status was based on either self-report or cotinine levels (data not shown). However, misclassification may be more apparent for other outcomes: for example, maternal cotinine concentrations have been shown to be better associated with decreases in gestational age at birth and the size of the baby than self-reported smoking status (Bardy *et al.* 1993, Klebanoff *et al*. 1998).

Consistent with previous studies, cotinine levels were found to be (i) usually greater than 100 ng ml^{-1} in self-reported current smokers and undetected in never smokers (Benowitz 1996); (ii) increased with increased numbers of self-reported cigarettes smoked per day (Law *et al*. 1997, Klebanoff *et al*. 1998, Etter *et al*. 2000); and (iii) decreased with increased self-reported time since the last cigarette. The enzyme cytochrome P450 2A6 (CYP2A6) has been implicated in the metabolism of nicotine to cotinine (Nakajima *et al*. 2000). The considerable inter-individual variation in expression of this enzyme (Camus *et al*. 1993), as well as errors in reporting cigarette consumption, may help to explain the relatively poor association between cotinine levels and cigarette consumption observed in many studies. In the present study, four self-reported smokers had cotinine levels below 20 ng ml^{-1}. While these individuals may not have been smokers or may have smoked their last cigarette days before the blood sample was taken, it is also possible that they had low CYP2A6 expression.

Cotinine has been described as the best available biomarker of environmental tobacco smoke (Benowitz 1996). In the present study, ex-smokers, but not never smokers, who reported exposure to passive smoke were more likely to have detectable cotinine levels than those who were not exposed. This may reflect the relatively crude measure of exposure used (yes versus no), which did not take into account a number of factors, including the number of cigarettes smoked in the presence of a patient or the proximity of the patient to the smoker (Kemmeren *et al.* 1994). Previously it has been reported that current smokers are detected by high cotinine levels more frequently amongst self-reported ex-smokers than never smokers (Lee and Forey 1995). This may be due to denial on the part of professed ex-smokers, where a desire to stop smoking or a substantial reduction in smoking intensity may lead to under-reporting of current smoking habits. Such differential misclassification probably explains why cotinine levels were higher in those ex-smokers who self-reported passive exposure, and why levels in ex-smokers were found to be intermediate between smokers and never smokers even though the half-life of cotinine in blood is of the order of hours rather than days (Benowitz 1996).

In summary, in patients attending bronchoscopy clinics, self-reported smoking status resulted in a substantial misclassification of smoking status, particularly among men and among patients without malignant lung disease. This differential misclassification may result in bias in studies examining associations between current smoking habits and disease outcome or progression.

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