Longitudinal Relation between Smoking and White Blood Cells

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Higher white blood cell counts in smokers compared with nonsmokers have been well documented, but the longitudinal relation between changes in smoking and changes in white blood cells has not been well described. Since 1984, data have been collected semiannually by the Multicenter AIDS Cohort Study (MACS), a four-center prospective cohort study of acquired immunodeficiency syndrome (AIDS) in homosexual men. The study population includes 2,435 participants who were human immunodeficiency virus (HIV) seronegative as of September 1994 and who contributed 20,918 person-visits for this analysis. For individuals who modified their smoking behavior, changes in white blood cell counts occurred primarily during the first 6 months following changes in the amount of cigarettes smoked. Among former smokers who resumed smoking, the extent of the increase in white blood cell count depended on the number of cigarettes smoked. Specifically, increases of 241, 340, and 740 cells/µl were observed for smokers who resumed smoking <1, 1 to <2, and ≥2 packs/day, respectively. Conversely, smokers who quit smoking had a decrease of white blood cell count: −32, −62, and −1,122 cells/µl for men who previously smoked <1, 1 to <2, and ≥2 packs/day, respectively. Long-term ex-smokers, however, still had higher white blood cell counts than did never smokers. There was a high within-individual correlation of white blood cell count in persons who reported a consistent level of smoking (i.e., average correlations between two white blood cell counts 6 years apart were 0.51 for never smokers, 0.48 for ex-smokers, 0.56 for men who smoked <1 pack/day, and 0.43 for men who smoked ≥1 pack/day). These analyses indicate an acute effect of changes in smoking on changes in white blood cell count, a residual effect of having been a smoker, and high long-term tracking for white blood cell count. Am J Epidemiol 1996;144:734–41.

leukocytes; longitudinal studies; smoking

White blood cell count has been associated with coronary heart disease (1–5), respiratory function and symptoms (6, 7), and cancer (2). Mechanisms underlying these associations have been proposed (2). The total leukocyte count and counts of specific cell subtypes are plausible biomarkers of active atherogenesis and other oxidative damage at the tissue level (8–11). White blood cell count may also be a marker of exposure to toxic substances. A relation between smoking and white blood cell count has been long established (12, 13); smokers have higher white blood cell counts than nonsmokers, and the extent of the increase rises with the number of cigarettes smoked. The strength of the association has led to the suggestion that the white blood cell count provides a better indicator of cigarette smoking than the self-reported number of cigarettes (14). However, smoking explains only part of the association between white blood cell count and myocardial infarction (2, 3).

To use white blood cell count as a biomarker of exposure and response, information is needed on its variation over time and on the factors that determine this variation. Short-term variation in white blood cell count has been documented (15), but there is a lack of information on long-term variation. For example, although experimental studies have shown that white blood cell counts returned to the levels of never smokers less than 6 weeks after quitting smoking (16, 17), cross-sectional studies, by contrast, have shown slightly higher white blood cell counts in ex-smokers than in never smokers (18, 19).
Studies on the dynamics of changes in smoking and changes in leukocyte counts require repeat assessment of both variables in the same individual over time. Our objective was to characterize the temporal variation in white blood cell count with changes in smoking among individuals not infected with human immunodeficiency virus (HIV) in the Multicenter AIDS Cohort Study (MACS) (20), a prospective cohort study of acquired immunodeficiency syndrome (AIDS) in homosexual men. We characterize these dynamic relations taking into account the dependency of within-individual measurements of white blood cell count. Furthermore, we compare the intrinsic within-individual correlation (tracking) of the repeated white blood cell count observations according to patterns of smoking.

MATERIALS AND METHODS

Study population and variables

A detailed description of the MACS population and study design has been provided elsewhere (21). Briefly, a cohort of homosexual/bisexual volunteer men from four US sites, AIDS-free and older than age 18 years at entry, was followed semiannually. Seropositivity to HIV was determined at each visit by enzyme-linked immunosorbent assay and confirmed with Western blot. At entry, 2,191 of the participants were seropositive, and of the 3,388 seronegative men, 485 had seroconverted as of September 1994. In this paper, we focus on the participants who remained seronegative during follow-up to September 1994. Median age of this study population in 1994 was 43 years and 91 percent were white.

Although the first of the semiannual visits in the MACS took place in 1984, we restricted attention to data collected between visits 8 and 20 when a uniform questionnaire on smoking was used. Hence, time of follow-up spanned 6.5 years, from visit 8 in 1987–1988 to the end of visit 20 in 1994. At each visit, blood samples were collected for hematologic studies, including complete blood counts and automated differential, and for determination of percent and number of T-lymphocytes by flow cytometry. A comprehensive quality control program for flow cytometry exists at the four study sites (22). Of the 2,519 HIV-seronegative persons who attended visits 8–20, 2,435 provided at least one measurement of white blood cell count and answered the smoking questionnaire. After excluding white blood cell count values >15,000 cells/µl, which are likely to be associated with occurrence of acute infections, hematologic information was available for 20,918 person-visits over the 6.5-year period of follow-up. Specifically, the median number of individuals seen at each visit was 1,849 (range from 1,892 at visit 9 to 1,121 at visit 20). At each visit, smoking was categorized as never or ever. For men who had smoked, daily cigarette consumption was classified in six categories: not currently smoking (as of one month ago), occasionally smoke (<1 cigarette/day), more than occasionally smoke but <0.5 pack/day, 0.5 to <1 pack/day, 1 to <2 packs/day, and ≥2 packs/day.

Statistical analysis

Due to the small number of observations in the categories of more than occasionally smoke to <1 pack/day, we combined them. Thus, we categorized cigarette smoking as never, smoked in the past but not currently (i.e., ex-smoker), occasional smoker, and current smoker: <1 pack/day, 1 to <2 packs/day, and ≥2 packs/day. Prior to the analysis, we used the longitudinal data on a given individual to adjust for inconsistencies in smoking history. In particular, we made the following three adjustments: 1) a report of never having smoked after a previous report of cigarette consumption was recoded as ex-smoker, 2) missing information on visits prior to a report of never having smoked was recoded as never smoker, and 3) if in two consecutive visits there was a change from never to ex-smoker, then the ex-smoker was recoded as an occasional smoker. Of the 20,918 person-visits, we made adjustment 1 for 2,529 subjects, adjustment 2 for 27 subjects, and adjustment 3 for 151 subjects. The longitudinal information on smoking minimizes misclassification of the smoking status.

First, we assessed the relations between current smoking, white blood cell count, and the composition of white blood cells (i.e., neutrophils, basophils, eosinophils, monocytes, CD4+ T-cells, CD8+ T-cells, and non-T-lymphocytes calculated as the subtraction of CD4+ and CD8+ T-cell counts from the lymphocyte counts) for all person-visits. Next, we determined the net cross-sectional effects of smoking on the total white blood cell count and components by analyzing data from the participants with the same smoking status over the full follow-up period by using linear regression models for within-individual correlation of repeated observations (23).

To assess the longitudinal association between smoking and white blood cell count, we let changes in white blood cell count between consecutive visit pairs be the outcome measures and changes in smoking status be the predictor variables (24). Because the correlation structure of differences is complex and the within-individual correlation of the differences was of no prior interest, we used the generalized estimating equation (25) method, with unstructured correlation
matrix, for analysis. A model was fit for each smoking category at the previous visit, and the model included a covariate indicating the smoking category of the next visit. To determine if changes in white blood cell count persisted beyond the first 6 months after stopping smoking, data from men who successfully quit smoking (i.e., men who always reported ex-smoking after last report of smoking) were selected and their average white blood cell levels were described over time.

Analysis of the tracking of white blood cell count was carried out in men who did not change their smoking habit in the follow-up period. For the $I$ individuals in a given smoking category, we denote by $Y_{it}$ the white blood cell count of the $i$th individual, $1 \leq i \leq I$, at time $t$, $1 \leq t \leq T$. The within-individual sample mean and variance are given by

$$m_i = \frac{\sum Y_{it}}{T_i} \text{ and } s_i^2 = \frac{\sum (Y_{it} - m_i)^2}{(T_i - 1)}.$$ 

The variance between individuals in a given smoking category is given by

$$s^2 = \frac{\sum (m_i - m)^2}{(I - 1)},$$

with $m$ being the mean of the $m_i$. The tracking, $r_i$, of the within-person measurements of white blood cell count was described as the ratio of the between-individual variation of the corresponding smoking group divided by the sum of the between-individual variance and the within-individual variance, i.e.,

$$r_i = \frac{s^2}{s_i^2 + s^2}.$$ 

In addition, using the regression methods with damped exponential correlation (23), we assessed change in measures lengthened.

**RESULTS**

Table 1 describes smoking and white blood cell counts at all visits. Smoking (i.e., occasionally or more often) was reported in 5,790 of 20,918 (27.7 percent) person-visits. During the study period, the proportion of men who had quit smoking increased, while the proportions of those who had never smoked and who smoked $\geq 1$ pack/day decreased (figure 1).

Cross-sectionally, a dose-response relationship was observed between white blood cell count and smoking (table 1). With respect to the composition of white blood cells, smokers had elevated percentages of neutrophils and eosinophils and lower percentages of CD8$^+$ T-cells and non-T-lymphocytes.

Men who stayed in the same smoking category during the entire follow-up period showed the following mean ± standard error of white blood cell count/µliter: 6,287 ± 42 in never smokers, 6,502 ± 51 in ex-smokers, 6,610 ± 221 in occasional smokers, and 8,606 ± 153, 7,939 ± 124, and 8,107 ± 136 in smokers of <1, 1–2, and ≥2 packs/day, respectively. Long-term ex-smokers had significantly higher white blood cell counts than never smokers (a difference of 215 ± 59 cells/µliter, $p < 0.01$). Because never smokers, ex-smokers, and smokers of 1 to <2 packs/day showed a stable smoking behavior, results for all person-visits were similar to those obtained by selecting only those who stayed in a smoking category. The number of men who persisted in the other smoking categories was lower and the data differed slightly.

Figure 2 depicts the frequency of the within-individual correlation of white blood cell count for individuals who remained in the same smoking category. The median of the within-individual correlation of white blood cell counts was 0.72, 0.70, 0.74, and 0.69 for never smokers, ex-smokers, smokers of 1 to <2 packs/day, and smokers of ≥2 packs/day, respectively. Heavy smokers (≥1 pack/day) had a significantly lower within-individual correlation ($p < 0.05$) compared with never smokers. Average correlations (95

**TABLE 1. Descriptive statistics of number and components of white blood cells (WBC), by smoking status reported at all visits by 2,435 seronegative participants in the Multicenter AIDS Cohort Study, 1987–1994**

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>No.†</th>
<th>No. of person-visits</th>
<th>WBC count (mean ± SE$^s$)</th>
<th>WBC composition (% of total WBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neutrophil</td>
<td>Basophil</td>
</tr>
<tr>
<td>Never smoked</td>
<td>980</td>
<td>7,709</td>
<td>6,265 ± 18</td>
<td>55.8</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1,108</td>
<td>7,419</td>
<td>6,501 ± 19*</td>
<td>56.1</td>
</tr>
<tr>
<td>Occasionally smoke</td>
<td>361</td>
<td>639</td>
<td>6,412 ± 60*</td>
<td>55.1</td>
</tr>
<tr>
<td>&lt;1 pack/day</td>
<td>553</td>
<td>2,080</td>
<td>7,113 ± 40*</td>
<td>56.2</td>
</tr>
<tr>
<td>1–2 packs/day</td>
<td>493</td>
<td>2,185</td>
<td>7,922 ± 42*</td>
<td>58.6*</td>
</tr>
<tr>
<td>≥2 packs/day</td>
<td>210</td>
<td>906</td>
<td>8,279 ± 64*</td>
<td>58.5*</td>
</tr>
</tbody>
</table>

$^* p < 0.05$ (reference category: never).
† Number of individuals (note: the same individual may appear in several smoking categories).
‡ Number of person-visits that provided data on WBC.
percent confidence intervals) between two white blood cell counts 6 years apart, estimated from the regression models, were 0.51 (0.50–0.52) for never smokers, 0.48 (0.46–0.50) for ex-smokers, 0.56 (0.49–0.62) for men who smoked <1 pack/day, and 0.43 (0.40–0.46) for men who smoked ≥1 packs/day. Dampening was moderate, although statistically significant at all the smoking categories (p < 0.05).

Changes in white blood cell count among individuals who stop smoking are depicted in figure 3. White blood cell counts in these men decreased after quitting smoking, reaching white blood cell counts similar to the levels in those who entered as and remained ex-smokers. The steepest decline in the white blood cell count after quitting smoking occurred in the first 6 months for those who smoked ≥1 pack/day. A stable level of white blood cell count was attained at approximately 1.5 years after quitting.

Finally, we assessed changes in white blood cell count at the individual level. A total of 16,982 pairs of person-visits provided information on changes in white blood cell counts between two visits. Of these, 36.9 percent and 33.1 percent were in never and ex-smokers, respectively, who remained in the same smoking category. Table 2 shows changes in white blood cell count for those who smoked. Participants who did not change their smoking status showed small variations in white blood cell count, similar to those observed in never smokers (mean ± standard error, 19 ± 7 cells/μliter) and in persistent ex-smokers (8 ± 9). Changes from nonsmoking to smoking were mainly in persons who previously had stopped smoking. Among men who restarted smoking (n = 394 pairs), the higher the number of cigarettes first reported after they restarted smoking, the greater the white blood cell count increase. Conversely, smokers who quit smoking (n = 571 pairs) had a monotonic decrease of white blood cell count according to the amount of cigarettes previously smoked. Changes between smoking categories (n = 547 and 554 pairs who increased and decreased, respectively) were also consistent with the previous findings, i.e., the increase (decrease) of cigarettes was followed by an increase (decrease) in the white blood cell count, except for changes between smoking 1 to <2 packs/day and ≥2 packs/day.
DISCUSSION

Our analysis confirms the previously described dose-response relationship between smoking and white blood cell count, and provides new findings on changes in smoking and changes in white blood cell count. Longitudinal data have the advantage that they allow characterization of change at the individual level in contrast to comparison of groups of individuals in cross-sectional analyses. The longitudinal information on smoking also facilitated quality control of the data, because inconsistencies over time could be examined and misclassification of smoking status minimized.

Leukocytosis in smokers has been well established based on cross-sectional (12) and longitudinal studies in the general population (2). In contrast, information on changes in cell subtypes, particularly T-cell subtypes, has been limited to small laboratory studies (16, 17) and cross-sectional studies (26-28). We corroborated a moderate increase of neutrophils and eosinophils and decrease of lymphocytes, as percent of total white blood cells (12, 29, 30). Discrepant findings have been reported on the effect of smoking on monocytes. In our study, monocytes were not increased, as has been previously reported (29). We observed a moderate decrease of CD8+ T-cells and non-T-lymphocyte cells and no changes in CD4+ T-cells in percent over total leukocytes in smokers of $\geq 1$ pack/day. These findings agree with previous observations of an increase of CD4+ T-cells and no changes in CD8+ T-cells in smokers of $\geq 1$ pack/day when lymphocyte subtypes were analyzed as percent over total lymphocytes (20, 26, 27).

The greatest change in white blood cell count after a change in smoking occurred within the first 6 months. Changes in shorter time intervals could not be assessed in our study. Changes after this time period were smaller, and a significant residual smoking effect on white blood cell count after quitting smoking was observed in our follow-up of 6.5 years. Thus, participants who entered the study as ex-smokers and who remained in that category had white blood cell counts an average of approximately 200 white blood cells/μliter higher than those who entered as never smokers. Similarly, participants who quit smoking during the
study period did not decrease to the lower count of
never smokers, but did reach that of subjects who
entered as ex-smokers and remained in that category.
The findings are consistent with previous observations
of an intermediate level for ex-smokers between those
of never and current smokers (1, 2, 18, 19). In addi-
tion, we found that this plateau is reached in less than
2 years, in contrast to previous studies that found
longer times to reach a plateau (18, 19). Biologic
mechanisms for a persistent effect of smoking in white
blood cell counts have been proposed, such as a re-
sidual chronic inflammation of the bronchial tree due
to smoking (18). Although we corrected inconsist-
encies on repeated measurements of smoking status, a
higher misclassification in ex-smokers could not be
excluded, because we were not able to biologically
confirm the smoking status, as a previous study did
(2).

The white blood cell count showed high tracking
whatever the smoking status, although less so in heavy
smokers (in particular, among subjects who smoked
≥2 packs/day, who likely were more heterogeneous in
the amount of cigarettes smoked). This high level of
tracking was seen even without taking into account
factors that acutely modify the white blood cell count,
such as acute infections or hour of the day (15, 31), or
host factors that change over time and that have been
related with white blood cell count, such as age or
body mass index (18, 32). If white blood cell count is
a marker of the oxidant load, the high tracking in white
blood cell count after removal of the effect of smoking
suggests an individual predisposition to the oxidative
damage, invariant over time. On the other hand, if
white blood cell count is a marker of exposure to toxic
substances, the long-term high tracking suggests the
persistence of environmental exposures, possibly re-
lated to white blood cell count, other than smoking
(e.g., diet).

Race has been related to white blood cell count (27).
In this study, because 91 percent of the subjects were
white, we did not have high power to detect differ-
ces according to race; however, race did not appear
to modify the relation between smoking and white
blood cell count (data not shown). Although we stud-
TABLE 2. Estimated average change ± standard error for differences in white blood cell count (cells/µl) for participants who smoked at some time during the Multicenter AIDS Cohort Study, 1987–1994

<table>
<thead>
<tr>
<th>Type of change in smoking status</th>
<th>Smoking (packs/day)</th>
<th>0</th>
<th>1–2</th>
<th>2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change</td>
<td>27 ± 27</td>
<td>7 ± 28</td>
<td>-6 ± 49</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From ex-smoker</td>
<td>241 ± 131</td>
<td>340 ± 200†</td>
<td>740 ± 524</td>
<td></td>
</tr>
<tr>
<td>From occasional smoker</td>
<td>135 ± 126</td>
<td>1,866 ± 721*</td>
<td>-‡</td>
<td></td>
</tr>
<tr>
<td>From smoking &lt;1 pack/day</td>
<td>202 ± 94*</td>
<td>382 ± 357</td>
<td>-123 ± 144</td>
<td></td>
</tr>
<tr>
<td>From 1–2 packs/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To quit</td>
<td>-32 ± 119</td>
<td>-629 ± 168*</td>
<td>-1,122 ± 412</td>
<td></td>
</tr>
<tr>
<td>To occasional smoker</td>
<td>-196 ± 122</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>To &lt;1 pack/day</td>
<td>-172 ± 94†</td>
<td>-265 ± 265</td>
<td>90 ± 161</td>
<td></td>
</tr>
<tr>
<td>To 1–2 packs/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‡ p < 0.05.
† 0.05 < p < 0.10.
Dash given when no. of pairs <5.

ied only men, the present findings contribute to the general understanding of the dynamic relation between smoking and white blood cell count and provide new information about the high level of memory of white blood cell count at the individual level. This may be relevant for understanding the underlying mechanisms of the relation between smoking and disease, and evidence, if smoking behavior is constant, of the utility of a single measurement of white blood cell count as a biomarker in epidemiologic studies.

ACKNOWLEDGMENTS

Supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (cooperative agreements nos. U01-AI-35042, 35043, 35039, 35040, and 35041). The work of Dr. Sunyer was partially funded by the Fondo Investigaciones Sanitarias of Spain, BAE/FIS 95/5259.


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