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White Blood Cells Count and Incidence of Type 2 Diabetes in Young Men

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OBJECTIVE—Association between white blood cell (WBC) count and diabetes risk has been recently suggested. We assessed whether WBC count is an independent risk factor for diabetes incidence among young healthy adults.

RESEARCH DESIGN AND METHODS—WBC count was measured in 24,897 young (mean age 30.8 ± 5.36 years), normoglycemic men with WBC range of 3,000 to 12,000 cells/mm3. Participants were periodically screened for diabetes during a mean follow-up of 7.5 years.

RESULTS—During 185,354 person-years of follow-up, diabetes was diagnosed in 447 subjects. A multivariate model adjusted for age, BMI, family history of diabetes, physical activity, and fasting glucose and triglyceride levels revealed a 7.6% increase in incident diabetes for every increment of 1,000 cells/mm3 (P = 0.046). When grouped in quintiles, a baseline WBC count above 6,900 cells/mm3 had an independent 52% increase in diabetes risk (hazard ratio 1.52 [95% CI 1.06–2.18]) compared with the lowest quintile (WBC <5,400 cells/mm3). Men at the lowest WBC quintile were protected from diabetes incidence even in the presence of overweight, family history of diabetes, or elevated triglyceride levels. After simultaneous control for risk factors, BMI was the primary contributor of the variation in multivariate models (P < 0.001), followed by age and WBC count (P < 0.001), and family history of diabetes and triglyceride levels (P = 0.12).

CONCLUSIONS—WBC count, a commonly used and widely available test, is an independent risk factor for diabetes in young men at values well within the normal range.

Diabetes Care 36:276–282, 2013

Obesity and type 2 diabetes are leading causes of morbidity and mortality, and their prevalence is increasingly rising in the younger population (1). There is solid evidence to support low-grade inflammation as a key component in the pathophysiology of the metabolic syndrome and type 2 diabetes, linking adiposity and insulin resistance (2). Inflammatory cells have been shown to infiltrate the adipose tissue in obese humans, associated with increased production and secretion of inflammatory cytokines that may contribute to whole-body inflammation (3,4). Chronic inflammation has been associated with an increased incidence of diabetes even in the absence of obesity (5,6), such as in patients with rheumatoid arthritis and psoriasis, and treatment with anti-inflammatory medications in these conditions significantly decreased the rates of diabetes (7).

Several epidemiological studies (8–11), but not all (12,13), have shown links between various markers of inflammation and diabetes risk prediction, including interleukin-6 (IL-6) and C-reactive protein (CRP). Total peripheral white blood cells (WBC) count, a nonspecific marker of inflammation, has also been suggested to be associated with diabetes risk in some cohorts (14–16), but observations were not consistent (10,17). A recent meta-analysis of 20 studies including ~90,000 participants demonstrated a positive correlation between increased WBC level and diabetes risk (18). However, most studies in this meta-analysis enrolled middle-aged participants and were based on cross-sectional data, with only partial adjustments for other diabetes risk factors. In addition, whether elevated inflammatory markers can predict the risk for diabetes independent of adiposity is not yet clear. Although a few studies showed significant associations between CRP and incident diabetes after adjustment for obesity indexes (8,9,19–21) others have argued that the association is mediated entirely by increased adiposity (11,13,17).

The aim of our study was to assess whether an increased WBC count within the normal range can predict diabetes incidence in young adults. Using the Metabolic, Life-style and Nutrition Assessment in Young adult (MELANY) cohort, a large prospective, population-based cohort, we report that an elevated WBC count in young, apparently healthy, normoglycemic men, already at levels well within the normal range, is an independent predictor of future diabetes. In addition, men with known risk factors for obesity, but with low-normal WBC count, were relatively protected from type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study population

The MELANY cohort has been conducted at the Israel Defense Forces Staff Periodic Examination Center (SPEC), to which all career service personnel aged older than 25 years are referred every 3 to 5 years for a routine health examination and screening tests, as described previously (22,23). At each visit to the SPEC, participants completed a detailed questionnaire assessing demographic, nutritional, lifestyle, and medical factors. Blood samples were drawn after a 14-h fast and immediately...
analyzed. Height and weight were measured, and a physician at the center performed a complete physical examination. Primary care for all Israel Defense Forces personnel between scheduled visits to the center is obtained at designated military clinics, and all medical information is recorded in the same central database, thereby facilitating ongoing, tight, and uniform follow-up.

This study assessed 37,418 men who had documented WBC counts within the normal range (3,000–12,000 cells/mm³) at their first visit to the SPEC between the years 1995 and 2010. The analysis excluded 1) men with pre-existing type 1 or type 2 diabetes, men with fasting plasma glucose (FPG) level ≥100 mg/dL at the first visit (n = 3,638), or men with newly diagnosed diabetes (FPG ≥126 mg/dL, n = 602); 2) men with a follow-up shorter than 2 years (n = 8,001); or 3) men missing fasting glucose levels or WBC count (n = 280). The institutional review board of the Israel Defense Forces Medical Corps approved this study on the basis of strict maintenance of participants’ anonymity during database analyses.

Follow-up and outcome
Participants aged 25 to 45 years were followed up prospectively from their first visit to the SPEC until retirement from military service. Follow-up ended at the time of diabetes diagnosis, death, retirement from military service, or 8 March 2011, whichever came first. Mean total follow-up was 7.71 ± 3.83 years, corresponding to ~85 and 67% completing at least 3 and 5 years of follow-up, respectively. All participants were censored at the end of their follow-up period (based on the above criteria for end of follow-up); thus, none of the study participants were lost to follow-up. Of note, follow-up duration for subjects who did and did not develop diabetes was similar (7.72 ± 2.84 and 7.44 ± 3.84 years, respectively, P = 0.13), and baseline WBC levels did not affect the number of visits to the SPEC.

Screening for diabetes was performed at each visit to the SPEC using FPG. The diagnosis of 447 incident cases of diabetes was based on the American Diabetes Association criteria by documenting two FPG levels of ≥126 mg/dL (7.0 mmol/L) or a glucose level ≥126 mg/dL 2 h after ingestion of 75 g of glucose. The diagnosis was made after the abnormal screening result at each visit to the SPEC in 198 subjects (4.3%) or between visits by the participants’ Israeli Defense Force primary care physician, followed by confirmation by a military physicians’ committee, in 249 (55.7%). All laboratory studies were performed on fresh samples in an International Organization for Standardization (ISO)-9002 quality-assured core facility laboratory.

Statistical analysis
The cohort was divided into WBC quintiles, and their baseline characteristics are presented in Table 1. (Baseline characteristics of patients who developed diabetes vs. those who did not are detailed in Supplementary Table 1.) The medians of the quintiles were fit as continuous variables to estimate the trend of variables across quintiles in a linear regression model (adjusted R² = 0.99, B = 900 cells/mm³ per quintile, P = 0.001). Cox proportional hazard models were used to estimate the hazard ratios (HR) and 95% CIs for developing diabetes. We gradually added to the age-adjusted model (model 1) additional parameters known as classical risk factors for diabetes (Table 2). In model 2, BMI was added as a continuous variable. In model 3, the following categorical variables were added to model 2: smoking status (current smoker, former smoker, never smoked), family history of diabetes (yes or no), and physical activity (not active, <150 min/week, ≥150 min/week). In model 4, serum triglyceride levels (quintiles) and fasting glucose level (as continuous variables) were added to the model. An additional multivariate model was conducted analyzing WBC count as a continuous variable. Of note, the presence or absence of a family history of diabetes was recorded for each participant from the last available visit to the examination center.

Log-minus-log plots for each variable were inspected to verify the assumption of proportionality of the hazards. All variables used in the model were tested for collinearity using the Pearson correlation. The maximal R recorded was 0.357 (triglyceride and BMI). Omnibus tests of model coefficients were used to assess the relative contribution of the various variables to the model. To evaluate the power of the models to discriminate events from non-events we calculated the area under the receiver operating characteristic (ROC) curve for each of the variables as well as in a multivariate model (C statistic). To study potential threshold in WBC count for diabetes prediction, a restricted cubic spline (using R software) and a decision tree procedure with CHAID (χ² Automatic Interaction Detection) method were used. Values are reported as mean ± standard deviation (SD), unless mentioned otherwise. Statistical analyses were performed with SPSS 19.0 software.

RESULTS
Characteristics of study participants
Data of 24,897 apparently healthy young normoglycemic men who were followed up as part of the MELANY cohort met the inclusion criteria. Participants were categorized by quintiles according to their WBC count at enrollment. Baseline characteristics are presented in Table 1. Mean WBC count was 6,620 ± 1,480 cells/mm³ (range 3,000–12,000) with an average increment of 900 cells/mm³ between consecutive quintiles. WBC level was directly correlated with BMI, systolic and diastolic blood pressure, triglyceride level, LDL-cholesterol, and rates of current smokers. Physical activity and HDL-cholesterol were inversely correlated with WBC level (P < 0.0001 for trend).

WBC count and risk for developing diabetes
During 185,354 person-years of follow-up, 447 new cases of diabetes were diagnosed among young normoglycemic men. The incidence of type 2 diabetes increased linearly across quintiles of WBC, with 62 new cases diagnosed in the lowest quintile (Q1; WBC of 3,000–5,400 cells/mm³) and 124 new cases diagnosed in Q5 (WBC >7,810). In model 1, adjusted for age, the risk for type 2 diabetes was 2-fold higher in Q4 (WBC 6,910–7,800 cells/mm³) and 2.44-fold higher in Q5 compared with Q1 (HR 2.44 [95% CI 1.79–3.31]). Further adjustment for BMI (model 2, Table 2) significantly attenuated the risk for diabetes associated with WBC levels. Nevertheless, even in the multivariate model adjusted for age, BMI, family history of diabetes, physical activity, triglyceride level, and fasting glucose, WBC count remained an independent risk factor of incident diabetes at levels >6,910 cells/mm³ (model 4, Table 2), with a HR in Q4 versus Q1 of 1.48 (1.03–2.14; P = 0.011 for trend). When WBC count was modeled as a continuous variable, an increment of 1,000 cells/mm³ in WBC count was associated with a 7.6% increase in the risk for developing diabetes independently of age, BMI, family history of diabetes, physical activity, triglyceride level, and fasting glucose level (HR 1.076 [1.001–1.157], P = 0.046).
WBC and diabetes incidence in young men

Table 1—Baseline characteristics of population cohort

<table>
<thead>
<tr>
<th>Quintile of WBC count</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 5,269</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24,897</td>
</tr>
<tr>
<td>WBC count (cells/mm³)</td>
<td>3,000–5,400</td>
<td>5,401–6,180</td>
<td>6,181–6,900</td>
<td>6,901–7,800</td>
<td>7,801–12,000</td>
<td>6,630 ± 1,480</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>30.5 ± 5.27</td>
<td>30.7 ± 5.28</td>
<td>30.7 ± 5.30</td>
<td>31.0 ± 5.43</td>
<td>31.4 ± 5.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BM1 (kg/m² ± SD)</td>
<td>24.09 ± 3.32</td>
<td>24.84 ± 3.47</td>
<td>25.39 ± 3.74</td>
<td>25.87 ± 3.97</td>
<td>26.64 ± 4.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25 kg/m²</td>
<td>65</td>
<td>56</td>
<td>50</td>
<td>45</td>
<td>38</td>
<td>52</td>
</tr>
<tr>
<td>25 to &lt;30 kg/m²</td>
<td>30</td>
<td>36</td>
<td>39</td>
<td>40</td>
<td>42</td>
<td>37</td>
</tr>
<tr>
<td>≥30 kg/m²</td>
<td>5</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>BP (mmHg ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>115.1 ± 11.8</td>
<td>116.1 ± 11.9</td>
<td>116.7 ± 12.2</td>
<td>117.7 ± 12.5</td>
<td>118.7 ± 13.0</td>
<td>116.8 ± 12.3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73.2 ± 9.1</td>
<td>73.7 ± 9.3</td>
<td>74.3 ± 9.3</td>
<td>74.9 ± 9.6</td>
<td>75.5 ± 9.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≤140/90 mmHg (%)</td>
<td>15.0</td>
<td>17.3</td>
<td>21.2</td>
<td>20.8</td>
<td>25.7</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/dL ± SD)</td>
<td>87.3 ± 6.6</td>
<td>87.6 ± 6.7</td>
<td>87.7 ± 6.7</td>
<td>87.8 ± 6.7</td>
<td>88.1 ± 7.0</td>
<td>87.7 ± 6.7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/dL ± SD)</td>
<td>48.4 ± 11.2</td>
<td>47.3 ± 10.7</td>
<td>46.2 ± 10.5</td>
<td>45.6 ± 10.4</td>
<td>44.1 ± 10.2</td>
<td>46.4 ± 10.7</td>
</tr>
<tr>
<td>HDL</td>
<td>111.6 ± 31.4</td>
<td>115.0 ± 31.6</td>
<td>116.2 ± 32.1</td>
<td>118.6 ± 33</td>
<td>121.0 ± 34.6</td>
<td>116.3 ± 32.6</td>
</tr>
<tr>
<td>LDL</td>
<td>97.1 [61, 116]</td>
<td>107.6 [67, 130]</td>
<td>119.2 [72, 145]</td>
<td>130.4 [78, 158]</td>
<td>145.7 [86, 178]</td>
<td>119.3 [88, 185]</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[25th, 75th]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>8.1</td>
<td>8.3</td>
<td>8.4</td>
<td>7.4</td>
<td>6.1</td>
<td>7.7</td>
</tr>
<tr>
<td>&gt;150 min/week (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of</td>
<td>12.0</td>
<td>13.6</td>
<td>12.8</td>
<td>12.7</td>
<td>14.7</td>
<td>13.1</td>
</tr>
<tr>
<td>diabetes (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Never</td>
<td>67.6</td>
<td>64.8</td>
<td>60.3</td>
<td>55.4</td>
<td>41.4</td>
<td>58.2</td>
</tr>
<tr>
<td>Former smoker</td>
<td>12.8</td>
<td>13.7</td>
<td>13.5</td>
<td>12.7</td>
<td>10.5</td>
<td>12.7</td>
</tr>
<tr>
<td>Current smoker</td>
<td>19.6</td>
<td>21.5</td>
<td>26.2</td>
<td>31.9</td>
<td>48.1</td>
<td>29.1</td>
</tr>
</tbody>
</table>

Risk prediction of diabetes in young adults using WBC count

To better assess the interrelation between obesity and WBC, we next studied the joint effect of BMI and WBC levels in predicting the risk for developing type 2 diabetes (Fig. 1A). In a multivariate analysis controlled for age, family history of diabetes, physical activity, triglyceride level, and FPG, obese men (BMI ≥30 kg/m²) with elevated WBC count within the normal range (7,810–12,000 cells/mm³) had a more than sixfold increase in the risk for type 2 diabetes (HR 6.1 [95% CI 3.81–9.78]) compared with the reference group. Of interest, a strong attenuation in the risk for type 2 diabetes attributed to overweight and obesity was observed among individuals with low-normal WBC (<5,400 cells/mm³), reaching an HR of 2.63 (1.19–5.88) for obese men and a nonsignificant HR of 1.40 (0.8–2.44) for overweight men. Taken together, these observations suggest that, in the presence of a low-normal WBC count, overweight and obese men are relatively protected from the development of type 2 diabetes compared with overweight or obese individuals with higher WBC count.

Figure 1 demonstrates the joint effects of triglycerides level (Fig. 1B) or family history of diabetes (Fig. 1C) with WBC count. Similar to the observation with BMI, a relatively low WBC level (<5,400 cells/mm³) was associated with complete attenuation of diabetes risk attributed to an elevated triglyceride level or to a positive family history of diabetes.

We next assessed for a potential threshold within the normal range of the WBC count, above which diabetes risk increases significantly. Although a decision tree procedure (with CHAID) suggested a WBC value of 7,230 cells/mm³ as a potential cut point, no evidence for a nonlinear component of WBC was observed using restricted cubic splines (data not shown). We therefore performed the Cox regression multivariate models using a moving cutoff value for WBC in increments as low as 100 cells/mm³ (Supplementary Table 4). A clear threshold could not be delineated; however, the highest HR (1.438) with the lowest P value (0.002) for diabetes incidence was observed when the value of 7,100 cells/mm³ was used. Of note, WBC >7,100 cells/mm³ corresponds exactly to levels observed among the upper one-third of the study population.

Residual contribution of WBC to diabetes prediction

To assess the differential contribution of the various risk factors to the prediction of diabetes, we next calculated the ROC curves for WBC count and for other, well-validated diabetes risk factors. Surprisingly, the AUC of the WBC count ROC curve was not statistically different than that of fasting glucose (0.59 [95% CI 0.55–0.61] vs. 0.58 [0.55–0.61], respectively). BMI was the single most powerful predictor for diabetes (AUC 0.670 [0.643–0.696]), followed by triglyceride level (0.63 [0.60–0.66]), WBC count, and fasting glucose. The addition of all
parameters included in model 4 resulted in an AUC of 0.674 (0.643–0.705) without WBC and 0.676 (0.645–0.707) with WBC. The change in −2 log likelihood with addition of each of the variables to the Cox regression model (omnibus test of model coefficients) revealed that BMI and age had the highest contribution to the model, followed by WBC count, family history of diabetes, and triglyceride level (Supplementary Table 2). As detailed above, the upper one-third of the WBC distribution (corresponding to WBC levels >7,100 cells/mm$^3$) was suggested as a potential threshold for increased diabetes risk. Indeed, a significant contribution to the full multivariate model was observed when tertiles of WBC (<5,900, 5,910–7,100, or ≥7,110 cells/mm$^3$) were used (P=0.004 omnibus test).

**Ethnic variations in WBC count**

Of the 24,897 subjects included in this study, 7,939 were of African origin, exhibiting a lower mean of WBC count (5,510 ± 1,440 cells/mm$^3$, P < 0.001) than participants from non-African descent. When WBC count was modeled as a continuous variable, a significant 11.8% increase in diabetes risk was observed for every increment of 1,000 cells/mm$^3$ in this population (HR 1.118 [95%CI 1.01–1.25], P = 0.05). These data indicate that WBC count may be considered as a risk factor even among subjects with a genetic tendency for a lower range of WBC count (further detailed in Supplementary Table 3).

## CONCLUSIONS

In this prospective, large-scale study, we demonstrate that a single measurement of WBC in healthy, normoglycemic young men may predict diabetes incidence independently from other traditional risk factors for diabetes such as BMI, fasting glucose and triglyceride levels, and family history. We report that for every increase in 1,000 cells/mm$^3$ within the normal range of the WBC count, the risk for diabetes increases by 7.6%, and levels of WBC already as low as 6,900 cells/mm$^3$ and above are associated with an obesity-independent ~50% increase in the risk for diabetes. Indeed, many studies have demonstrated a positive association between increased levels of inflammatory markers, such as WBC count, CRP, and inflammatory cytokines, and diabetes incidence, although only a few have tested this prospectively in large, population-based studies (for a systematic review and meta-analysis, please see reference 18).

Previous studies have been inconsistent in whether WBC or other inflammatory markers contribute to diabetes prediction models independent of obesity (8,9,19–21) or whether these markers simply reflect adipose tissue mass (11,13,17,19). The current study, to the best of our knowledge, is the largest
Figure 1—Risk factor for diabetes and WBC count in young normoglycemic patients. Q1 to Q5 indicate increment WBC quintiles as they appear in Table 1. The dark bars indicate a significant change compared with the reference group. A: Joint effect of BMI and WBC count and the risk for incident diabetes. Data are adjusted for age, family history of diabetes, activity status, smoking status, blood pressure, triglycerides level, and fasting glucose. The reference group is in the lower WBC quintile at the lower (lean) BMI tertile. B: Joint effect of plasma triglycerides (TG) level and WBC count and the risk for diabetes. Data are adjusted for age, BMI, family history of diabetes, activity status, smoking status, and fasting glucose. Reference group is the lower WBC quintile at a normal (<150 mg/dL) TG level. C: Joint effect of family history of diabetes, WBC count, and the risk for diabetes. Data are adjusted for age, BMI of diabetes, activity status, smoking status, triglycerides level, and fasting glucose. Reference group is the lower WBC quintile without a family history of diabetes.
screening setting, as a risk factor for type 2 diabetes in addition to the other known risk factors may elaborate the interpretation of this commonly used laboratory test by primary care providers, increasing their attention to increased diabetes risk among young adults, who are usually not frequently screened for this condition. Identifying individuals at higher risk for diabetes may facilitate the consideration of preventive interventions. Indeed, lifestyle and pharmacological approaches have both been reported to be efficient in delaying the onset of diabetes in selected groups at high risk for the disease (37–40). Of specific interest is the observation that specific anti-inflammatory treatments for rheumatoid arthritis patients also resulted in a decreased incidence of diabetes (6).

Targeting the obesity-related chronic inflammatory response to prevent the metabolic derangements associated with increased fat deposition may become an attractive approach for diabetes prevention once additional, more specific anti-inflammatory treatments with more tolerated side effects become available.

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No potential conflicts of interest relevant to this article were reported.

G.T. contributed to the study concept and design, acquisition and interpretation of the data, research methods and statistical analyses, and drafting of the manuscript. A.A. contributed to study concept and design, interpretation of the data, and critical revision of the manuscript for important intellectual content. A.S. contributed to interpretation of the data, research methods and statistical analyses, design, acquisition and interpretation of the data, and critical revision of the manuscript. A.A. contributed to critical revision of the manuscript for important intellectual content. E.D. contributed to acquisition of the data and to critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, research methods and statistical analyses, design, acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. A.S. contributed to interpretation of the data, and critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. E.D. contributed to acquisition of the data and to critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. E.D. contributed to acquisition of the data and to critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. E.D. contributed to acquisition of the data and to critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. E.D. contributed to acquisition of the data and to critical revision of the manuscript. A.T. and G.T. contributed to acquisition and interpretation of the data, and critical revision of the manuscript for important intellectual content. A.S. contributed to interpretation of the data, and critical revision of the manuscript. A.A. contributed to critical revision of the manuscript for important intellectual content. A.T. contributed to study concept and design, research methods and statistical analyses, interpretation of the data, and drafting of the manuscript. G.T. and A.T. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References