To the Editor:

The first and major entry point of electrons into the mitochondrial respiratory chain (MRC) occurs through NADH-coenzyme Q reductase (complex I). Decreased complex I activity is associated with a wide range of conditions, including inherited mitochondrial diseases and neurodegenerative conditions such as Parkinson disease (1–2). More than 60 natural and commercial compounds are reported to inhibit complex I activity, among them certain pesticides, agrochemicals, and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin that induces parkinsonism in humans; thus, exposure to these compounds may be harmful (3). We report here that nonylphenol ethoxylate (NP) surfactants leach from disposable laboratory plasticware and inhibit mitochondrial complex I.

During a routine diagnostic workup designed to diagnose mitochondrial diseases from patients’ muscle biopsy samples, we observed a substantial (50%–66%) decrease of mitochondrial enzymatic activities related to complex I compared with historical values determined spectrophotometrically in our laboratory. Furthermore, a 71% decrease in complex I–dependent oxygen consumption was also observed with glutamate + malate as substrates (Oxygraph, Hansatech Instruments) (2). Notably, other MRC activities, as well as citrate synthase, a mitochondrial marker enzyme, were largely unaffected. It was determined that inhibition resulted from a single brief period of contact between assay reagents and blue polypropylene 1-mL pipette tips (“blue tips”) manufactured by Scientific Specialties (Table 1).

A methanol wash of blue tips was subjected to positive ion–mode electrospray mass spectrometry (Waters Mariner BioSpectrometry Workstation oa-TOF); these analyses confirmed the presence of NP compounds, marketed as surfactants from the Tergitol NP series. The prevalent ion at m/z 683.5 was consistent with the use of NP-10 as a dye-solubilizing agent in the plastic manufacturing process, whereas m/z peaks indicative of shorter (NP-9) and longer (NP-11) side-chain surfactants are characteristic of mass spectral data for these compounds (4).

Subsequently we evaluated MRC function in the presence of commercial samples of NP-10 and NP-9 and detected inhibition of complex I enzymatic activity, with IC50 values of 4 μmol/L and 3.7 μmol/L, respectively. Other respiratory chain complexes were unaffected in the presence of these surfactants at 10 μmol/L; however, a marked decrease in complex I–dependent oxygen consumption was evident (Table 1). Oxygen consumption with complex I–independent substrates was not affected (not shown).

The inhibition of oxygen consumption in whole mitochondria by NP-10 and NP-9 indicates that these compounds penetrate the inner mitochondrial membrane. We also studied the impact of leachate from blue tips and of NP surfactants on growing fibroblasts in culture and confirmed that these substances penetrate whole cell membranes. Growth in MRC-dependent, restrictive medium lacking glucose was partially inhibited by NP-10 or NP-9, whereas growth in MRC independent, permissive glucose-containing medium was minimally affected. The use of blue tips to pipette culture medium was deleterious for cell growth in restrictive medium, but affected growth in permissive medium to a lesser extent (Table 1). Other nonionic detergents—dodecyl maltoside and Tween 20—and clear colorless tips from a different manufacturer had no effects under either set of conditions.

Our data clearly show that blue tips leached NP-10 into assay buffers and interfered with MRC complex I while leaving other complexes unaffected. Previously, McDonald et al. reported that disposable laboratory plasticware (pipette tips, microfuge tubes) leached the processing agents DiHEMDA [di(2-hydroxyethyl)methyldecylammonium] and oleamide into buffers, resulting in profound interference with biological assays (5). We did not detect these compounds in blue-tip leachates, neither did they interfere with complex I function (not shown), suggesting that leachates from various disposables might affect a variety of assays in different ways.

In addition to being used in plastic manufacturing, NP surfactants are also found in household cleaners, laundry detergents, spermicides, and cosmetics. Consequently these compounds are present in sewage waters and soil in micromolar quantities, and their alkylphenol breakdown products are estrogenic and persist in the environment. Furthermore, human exposure and absorption have been confirmed through detection of NP surfactants in human urine (4). Our data show that NP-10 and NP-9 penetrate both the human cell membrane and the mitochondrial inner membrane, and other investigators have confirmed these compounds as substrates for the human P-glycoprotein transporter (4). The possibility thus exists that these and...
related compounds may pose an environmental hazard through inhibition of oxidative phosphorylation via the MRC. Of greater concern to researchers is the addition of these surfactants to the growing list of bioactive compounds present in disposable laboratory plasticware, highlighting the need for individuals to screen plastics as a potential source of interferences in life science experiments.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


Corinne Belaiche2
Andrew Holt1
Ann Saada2

1 Department of Pharmacology
University of Alberta
Edmonton, Canada

2 Metabolic Disease Unit
Hadassah-Hebrew University Medical Center
Jerusalem, Israel

* Address correspondence to this author at:
Metabolic Disease Unit
Hadassah-Hebrew University Medical Center
POB.12000, Jerusalem 91120, Israel
E-mails annsr@hadassah.org.il, saada-ann@macam.ac.il

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False-Negative Results from Point-of-Care Qualitative Human Chorionic Gonadotropin (hCG) Devices Caused by Excess hCGβ Core Fragment Vary with Device Lot Number

To the Editor:

Recently, we reported that increased concentrations of human chorionic gonadotropin β core fragment (hCGβcf)\(^1\) can cause false-negative results on the OSOM\(^\text{®}\) hCG Combo Test (Genzyme Diagnostics) and ICON\(^\text{®}\) 25 hCG (Beckman Coulter) qualitative urine hCG devices (1). We reported that the hCG Combo SP\(^\text{®}\) Brand Rapid Test device (SP\(^\text{®}\) hCG Rapid Test; Cardinal Health), however, was not subject to the same effect. We demonstrate that the false-negative effect is observable with certain lots of the Combo SP devices.

Approximately 9 months after our hospital system switched to the Combo SP Rapid Test device to avoid potential false-negative results in patients with high concentrations of urine hCGβcf, we encountered 2 patients with false-negative results. Patient 1 was 18 years of age with a live intrauterine pregnancy that was seen on ultrasound and estimated at 12 weeks gestation. She presented to the emergency department (ED) with abdominal pain (no bleeding). Her diagnosis at discharge was hyperemesis gravidarum and dehydration. Her urine was cloudy with a pH of 6.5, a specific gravity of 1.026, and a 1+ leukocyte esterase result. A point-of-care urine pregnancy test was performed in the ED (Combo SP Rapid Test device; lot no. 9020102), and the result was negative. A second urine sample was collected, and it also tested negative. The serum hCG concentration was 166 659 IU/L with the Siemens Centaur ThCG assay. The 2 urine samples were also quantified with the Siemens Centaur assay and were 173 949 IU/L and 155 625 IU/L, respectively. Tests of both samples diluted to 1 part in 10 and to 1 part in 100 gave clearly positive results with the Combo SP Rapid Test device. Tests of these samples with the OSOM and ICON devices produced negative results as well, as previously described (1). The urine hCGβcf concentration was 2.13 \(\times\) 10\(^6\) pmol/L (67% of total immunoreactive hCG) in patient 1 and 1.097 \(\times\) 10\(^6\) pmol/L (58% of total immunoreactive hCG) in patient 2. These hCGβcf concentrations and percentages of total immunoreactive hCG were similar to those in samples with demonstrated false-negative results obtained with the OSOM and ICON devices (1), suggesting that hCGβcf might also be the cause of the false-negative effect seen here.

To investigate this possibility, we repeated our original inhibition studies with purified hCGβcf purchased from the National Institute for Biological Standards and Controls [first WHO reference reagent, 2001 (hCGβcf, 99/708)]. hCGβcf was diluted in hCG-negative urine to a concentration of 3 \(\times\) 10\(^6\) pmol/L. A randomly chosen positive urine sample was mixed with purified hCGβcf at concentrations from 6.25 \(\times\) 10\(^5\)

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\(^1\) Nonstandard abbreviations: hCGβcf, human chorionic gonadotropin β core fragment; ED, emergency department.

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Fig. 1. The effect of hCGβcf on 2 lots (8080021 and 9020102) of the hCG Combo SP Brand Rapid Test (SP hCG Rapid Test; Cardinal Health) qualitative hCG device.

C, control region; T, test region; S, sample well.
pmol/L to $1 \times 10^6$ pmol/L and tested with 2 different lots of the Combo SP device (lot nos. 8080021 and 9020102). The results showed a lot-to-lot difference in the effect of hCGβcf on the results for hCG-positive urine (Fig. 1). Lot 8080021 was unaffected by up to $1 \times 10^6$ pmol/L hCGβcf. On the other hand, lot 9020102 was inhibited by in a dose-dependent manner and at an hCGβcf concentration of $1 \times 10^6$ pmol/L produced a result that was nearly invisible (Fig. 1). These data demonstrate the same inhibition by hCGβcf, in a single lot of the Combo SP Rapid Test device, that was previously reported for the OSOM and ICON devices.

Why these devices exhibit such between-lot variation is unclear. The package insert states that the device uses 1 monoclonal antibody and 1 polyclonal antibody. It is possible that one lot used a new batch of polyclonal antibody with a more avid specificity for hCGβcf or that it used polyclonal antibody at a concentration that yielded a lower binding capacity for hCG.

We previously provided data showing that caution is warranted when hCG devices in which hCGβcf causes negative interference are used to test women who are pregnant beyond 5–8 weeks gestation, because false-negative results may occur (1). Our new data suggest that the same caution should be used when interpreting a negative result from any qualitative hCG-testing device. At this time, we know of no device that is free from the inhibition effect by hCGβcf. This observation has been reported to the manufacturer and to the US Food and Drug Administration.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Reference**


**Ann M. Gronowski**

**Michelle Powers**

**Ulf H. Stenman**

**Lori Ashby**

**Mitchell G. Scott**

**Author**

**Letters to the Editor**

**Use of Likelihood Ratios Can Improve the Clinical Usefulness of Enzyme Immunoassays for the Diagnosis of Small-Vessel Vasculitis**

Antineutrophil cytoplasmic antibodies (ANCAs) are associated with small-vessel vasculitis (SVV), such as Wegener granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome (1). Indirect immunofluorescence (IIF) analyses can distinguish 2 major patterns: cytoplasmic and perinuclear. The cytoplasmic ANCA pattern is typically associated with antibodies to proteinase 3 (PR3), whereas the perinuclear ANCA pattern is typically associated with antibodies to myeloperoxidase (MPO). PR3 ANCs are predominantly seen in patients with Wegener granulomatosis, whereas MPO ANCs are predominantly seen in patients with microscopic polyangiitis (1–3).

Studies that have addressed the clinical usefulness of specific enzyme immunoassays (EIAs) for PR3 and MPO for the diagnosis of SVV have used a single cutoff value. In this letter, we illustrate how the likelihood ratio (LR) for SVV depends on the titer of anti-PR3 or anti-MPO antibodies. Our calculations are based on a clinically well-defined group of 37 consecutive patients with newly diagnosed SVV (data collected over a 10-year period) and 285 consecutive control individuals with disease (data collected over 21 months) that a physician suspected to be a vasculitis. Patients suspected of or having
a diagnosis for a gastrointestinal problem (e.g., inflammatory bowel disease) were excluded. The characteristics of the included patients have previously been described (4). One patient with antiphospholipid syndrome who had PR3 and MPO ANCs was excluded because SVV could not be ruled out. Two of the control individuals with disease were excluded because no residual serum sample was available. IIF analysis was performed with ethanol-fixed human neutrophil preparations (INOVA Diagnostics). PR3 ANCs and MPO ANCs were measured in international units per milliliter with the Wieslab capture kit from Euro-Diagnostica, in units with the QUANTA Lite™ method from INOVA Diagnostics, and in units per milliliter with the EliA™ assay platform from Phadia. The recom- mended cutoffs were 5 IU/mL, 20 U, and 7 U/mL, respectively. Patients were considered to have tested positive if either the PR3 ANCA result or the MPO ANCA result was positive.

We calculated the likelihoods and the LRs (likelihood of the patients with disease divided by likelihood of the control individuals) for different test-result intervals for each of the EIAs and for IIF. Table 1 summarizes the results. For the Wieslab capture kit, the LRs were 0.17, 5.1, 20, and 123 for 0–5 IU/mL, 5–7 IU/mL, 7–100 IU/mL, and >100 IU/mL, respectively. For the INOVA assay, the LRs were 0.14, 7.7, 58, and 131 for 0–14 U, 14–20 U, 20–100 U, and >100 U/mL, respectively. For the Phadia assay, the LRs were 0.14, 5.1, 100, and 131 for 0–7 U/mL, 7–20 U/mL, 20–100 U/mL, and >100 U/mL, respectively. For IIF, the LRs were 0.13, 0.8, 8, and 10 for negative, 1:40, 1:80–1:320, and >1:320, respectively. Our data demonstrate that the LR for SVV increases with antibody concentration and that LRs for a positive test result are much higher for EIAs than for IIF. The lower LR observed with IIF is due to the lower specificity of this assay. For the INOVA EIA, a test result between 14 U and 20 U (which is below the cutoff) had an LR of 7.7, which indicates a moderate increase in pretest-to-posttest probability. Use of the manufacturer’s proposed cutoff produces more false-negative results with the Inova assay. The LR for a negative test result was between 0.1 and 0.2 for both the EIAs and the IIF assay, indicating a substantial, but modest, difference in pretest-to-posttest probability. The use of LRs for different test-result intervals allows test results with a very high difference in pretest-to-posttest probability (an LR >10 or <0.1) to be distinguished from test results with a moderate difference (an LR between 5 and 10 or between 0.1 and 0.2) in pretest-to-posttest probability.

In conclusion, we have illustrated how the use of LRs for different test-result intervals can improve the clinical usefulness of EIA testing for SVV. Clinical laboratories might consider providing likelihood ratios for test-result intervals to improve clinical interpretation.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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- **Stock Ownership:** None declared.

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**Table 1. Overview of the LRs as a function of antibody concentration for 3 different EIAs (Wieslab capture kit from Euro-Diagnostica, QUANTA Lite™ from INOVA Diagnostics, and EliA™ from Phadia) and one IIF assay (INOVA).**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Cutoff proposed by manufacturer</th>
<th>Test-result interval</th>
<th>LR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euro-Diagnostica (EIA)</td>
<td>5 IU/mL</td>
<td>0–5 IU/mL</td>
<td>0.17 (0.08–0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–7 IU/mL</td>
<td>5.1 (0.89–29.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7–100 IU/mL</td>
<td>20 (7.6–53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 IU/mL</td>
<td>123 (16.8–903)</td>
</tr>
<tr>
<td>Inova (EIA)</td>
<td>20 U</td>
<td>0–14 U</td>
<td>0.14 (0.06–0.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14–20 U</td>
<td>7.7 (1.1–53.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–100 U</td>
<td>58 (13.8–243)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 U</td>
<td>116 (14.7–850)</td>
</tr>
<tr>
<td>Phadia (EIA)</td>
<td>7 U/mL</td>
<td>0–7 U/mL</td>
<td>0.14 (0.06–0.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7–20 U/mL</td>
<td>5.1 (0.89–29.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–100 U/mL</td>
<td>100 (13.5–744)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 U/mL</td>
<td>131 (17.9–956)</td>
</tr>
<tr>
<td>Inova (IIF)</td>
<td>1:40</td>
<td>Negative</td>
<td>0.13 (0.05–0.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:40</td>
<td>0.8 (0.19–3.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:80–1:320</td>
<td>8 (4.2–16.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;1:320</td>
<td>10 (5.3–19.0)</td>
</tr>
</tbody>
</table>


**Letters to the Editor**

**Increased Plasma Lipoprotein(a) Found in Large-Artery Atherosclerotic, but Not Small-Artery Occlusive, Stroke**

To the Editor:

We previously reported that plasma lipoprotein(a) [Lp(a)] is an independent risk factor for coronary artery disease, abdominal aortic aneurysm, peripheral arterial disease, and ischemic stroke (1). Although the exact pathophysiologic role of Lp(a) has not been definitively established, both atherogenic and thrombogenic mechanisms have been proposed. In this study, we measured plasma Lp(a) in ischemic stroke patients who had been subdivided into etiologic subtypes with the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification to determine if stroke subtype is a confounder of the Lp(a) association.

Ischemic stroke patients (n = 245) and arterial disease–free and stroke-free controls (n = 435) were recruited from the Otago region of New Zealand as previously reported (1). Stroke patients were placed into TOAST classification subtypes by means of a computer algorithm developed by Goldstein and colleagues (2). The TOAST subgroups were large-artery atherosclerosis (LAA), cardioembolism, small-artery occlusion (SAO), undetermined due to 2 or more causes (multiple etiologies) (ME), or undetermined etiology due to negative or incomplete evaluation. Inclusion criteria for controls were an age >52 years and no history of ischemic heart disease or stroke, including transient ischemic attack [Questionnaire for Verifying Stroke-Free Status (QVSFS) score = 0]. Plasma lipoprotein and demographic risk factors were assessed as previously described (1). The study was approved by the local human ethics committee, and all study participants gave written informed consent before being recruited into this study. The χ² test and ANOVA were used to assess nominal and normally distributed variables, respectively. We used the Mann–Whitney U-test to assess Lp(a) because of the nongaussian distribution of this variable. Logistic regression models were used to stratify Lp(a) by control population quartiles. Odds ratios were calculated for the fourth quartile (>50 nmol/L) vs the first quartile (<4.5 nmol/L). Multiple logistic regression was used to identify statistically significant interactive effects of variables on susceptibility to ischemic stroke.

Demographic factors associated with ischemic stroke are shown in Table 1; statistically significant confounders were included in subsequent multivariable logistic regression models. Plasma Lp(a) concentrations were significantly higher in the total-stroke group than in vascular disease–free controls (P < 0.03, Table 1). Lp(a) was differentially associated with TOAST stroke subtypes. Most notably, SAO strokes were not associated with Lp(a), and the association with the all-strokes group was largely attributable to increased concentrations in the “atherothrombotic” stroke subtypes (LAA and ME). Overall, 86% of ME patients had LAA as one of their identified pathologies. The ME cases with LAA were grouped with the LAA TOAST subgroup to form a patient population designated as having an “atherothrombotic” stroke. Compared with stroke-free controls, atherothrombotic cases had an odds ratio of 3.47 (95% CI, 1.26–9.60; P < 0.02) and an ad-

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**References**


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**Pieter Vermeersch**

**Daniel Blockmans**

**Xavier Bossuyt**

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*Laboratory Medicine, Immunology and General Internal Medicine University Hospitals Leuven Leuven, Belgium*

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*Address correspondence to this author at: Laboratory Medicine, Immunology University Hospitals Leuven Herestraat 49 B-3000 Leuven, Belgium Fax +00-32-13-347042 E-mail xavier.bossuyt@uz.kuleuven.ac.be*

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*Nonstandard abbreviations: Lp(a), lipoprotein(a); TOAST, Trial of ORG 10172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; SAO, small-artery occlusion; ME, undetermined due to 2 more causes (multiple etiologies); QVSFS, Questionnaire for Verifying Stroke-Free Status.*

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1888 Clinical Chemistry 55:10 (2009)
Table 1. Demographic profiles of the ischemic stroke populations.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 439)</th>
<th>All strokes (n = 245)</th>
<th>TOAST stroke classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LAA (n = 20)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>52.4</td>
<td>60.2\textsuperscript{c}</td>
<td>55.0</td>
</tr>
<tr>
<td>Age, years</td>
<td>68.8 (6.6)</td>
<td>71.4 (10.5)\textsuperscript{a}</td>
<td>70.3 (8.3)</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>25.5</td>
<td>70.2\textsuperscript{a}</td>
<td>70.0\textsuperscript{a}</td>
</tr>
<tr>
<td>History of dyslipidemia, %</td>
<td>22.9</td>
<td>58.9\textsuperscript{a}</td>
<td>75.0\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>5.1</td>
<td>25.8\textsuperscript{a}</td>
<td>25.0\textsuperscript{a}</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>0 (0–15)</td>
<td>9.5 (0–30.0)\textsuperscript{a}</td>
<td>25.0 (0.5–45.8)\textsuperscript{a}</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>25.9 (3.7)</td>
<td>27.5 (5.2)\textsuperscript{a}</td>
<td>28.7 (5.2)\textsuperscript{a}</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 (0.4)</td>
<td>1.2 (0.4)\textsuperscript{d}</td>
<td>1.1 (0.3)\textsuperscript{a}</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6 (0.5–9.0)</td>
<td>1.6 (0.5–5.3)\textsuperscript{a}</td>
<td>1.8 (0.8–3.6)\textsuperscript{a}</td>
</tr>
<tr>
<td>Lp(a), nmol/L</td>
<td>16.0 (4.6–50.9)</td>
<td>24.6 (7.5–83.5)\textsuperscript{a}</td>
<td>29.2 (7.7–97.6)\textsuperscript{a}</td>
</tr>
<tr>
<td>Lp(a), % &gt;50th percentile</td>
<td>50</td>
<td>58.8\textsuperscript{a}</td>
<td>63.2</td>
</tr>
<tr>
<td>Lp(a), % &gt;75th percentile</td>
<td>25</td>
<td>19.8</td>
<td>10.5</td>
</tr>
<tr>
<td>Fourth quartile Lp(a) odds ratio</td>
<td>—</td>
<td>1.84 (1.21–2.80; \textsuperscript{a} P &lt; 0.005)</td>
<td>3.89 (0.81–18.75; \textsuperscript{a} P &lt; 0.09)</td>
</tr>
<tr>
<td>Adjusted fourth quartile Lp(a) odds ratio</td>
<td>—</td>
<td>1.85 (1.01–3.41; \textsuperscript{a} P &lt; 0.05)</td>
<td>3.47 (0.62–19.51; \textsuperscript{a} P = 0.16)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Results are expressed as the mean (SD) or as the median (interquartile range). Odds ratios include the 95% CI and the P value. The adjusted model includes sex, age, hypertension, dyslipidemia, smoking history, diabetes, and history of coronary heart disease. Note the differential risk association between Lp(a) and TOAST stroke subtypes.

\textsuperscript{b} CE, cardioembolism; UE, undetermined due to negative or incomplete evaluation; BMI, body mass index; HDL-C, HDL cholesterol.

\textsuperscript{c} P < 0.05, vs controls.

\textsuperscript{d} P < 0.01, vs controls.

\textsuperscript{e} P < 0.001, vs controls.

\textsuperscript{f} P < 0.002, vs controls.

\textsuperscript{g} P < 0.05, vs ME.

\textsuperscript{h} P < 0.05, vs SAO.
justed (sex, age, hypertension, dyslipidemia, smoking history, diabetes, and history of coronary heart disease) odds ratio of 3.36 (95% CI, 1.04–10.89; P < 0.05). Lp(a) concentration was also significantly higher in this group than in the SAO stroke subgroup (P < 0.02).

This study therefore suggests that our previously reported association of Lp(a) with ischemic stroke was principally due to the presence of the atherothrombotic (LAA and ME) stroke subgroups. In contrast, the plasma Lp(a) concentration in the SAO stroke group was indistinguishable from that in stroke-free elderly control individuals. This observation is consistent with other studies that have suggested that plasma Lp(a) concentrations in SAO patients are approximately half those of LAA patients (3); however, the present report is the first to simultaneously examine all stroke subtypes and to measure Lp(a) with an apolipoprotein A isoform–insensitive method. We therefore concluded that plasma Lp(a) is associated with atherothrombotic cardiovascular disease phenotypes and appears to show no association with nonatherosclerotic pathologies such as SAO, which is typically associated with lipohyalinosis. Because a large proportion (86%) of ME cases had LAA as one of their associated pathologies, we considered it reasonable to combine these ME cases with LAA cases in the LAA group to further increase the power of the study. Of note, however, is that Lp(a) has been implicated as being both atherogenic and thrombogenic; therefore, high concentrations of this plasma lipoprotein may be associated with a compounded risk of ME stroke. We recommend that this possibility be investigated in future, larger-size studies of stroke populations. Such differential risk associations have also been reported for other stroke-related biomarkers (e.g., brain natriuretic peptide and d-dimer) as independent predictors of cardioembolic stroke, but not of other stroke subtypes (4).

In conclusion, this study suggests that plasma Lp(a) is differentially associated with ischemic stroke subtypes and highlights the importance of etiologic subclassification in assessing stroke-susceptibility biomarkers.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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