Malignant hyperthermia (MH) crises may induce morbidity or death in MH-susceptible (MHS) individuals. The only sensitive method of determining susceptibility is the caffeine-halothane contracture test, requiring muscle biopsy. Early research on MH demonstrated an abnormal response to catecholamines in MHS individuals. The purpose of this study was to determine whether MHS B lymphocytes would demonstrate an increased sensitivity to norepinephrine as indicated by an adrenergic augmentation of intracellular calcium ion (Ca\(^{2+}\)) accumulation, to possibly develop a less invasive laboratory assay for determining MH susceptibility. The fluorescent Ca\(^{2+}\) indicator dye fura-2 acetoxymethyl was used to identify Ca\(^{2+}\) flux within Epstein-Barr virus–immortalized MH-negative (MHN) and MHS B cells exposed to the RyR1 agonist 4-chloro-m-cresol (4-CmC) before and after administration of 1 μM of norepinephrine. In the presence of 4-CmC and norepinephrine, the area under the curve dose responses were significantly elevated in MHS B cells compared with MHN B cells (F[1,10] = 27.37; P < .01). Epstein-Barr virus–immortalized B cells from MHS humans displayed an increased sensitivity to norepinephrine compared with those from MHN individuals. These data suggest that an abnormal response to exogenous norepinephrine could potentially be used to develop a diagnostic laboratory assay to determine MH susceptibility.

**Keywords:** B cells, diagnostic assay, malignant hyperthermia, norepinephrine.
agonist 4-chloro-m-cresol (4-CmC). Because B cells also express both α- and β-adrenergic autonomic receptors on cell membranes, they provide a valid scientific model that can be used to investigate adrenergic sensitivity differences between MHS and MHN individuals. Therefore, we used a B-cell model to determine whether adrenergic stimulation would correlate with increased intracellular Ca2+ accumulation in B cells from MHS individuals. Our hypothesis was as follows: Immortalized human B lymphocytes derived from a known MHS population will display increased sensitivity to catecholamines as evidenced by an increase in myoplasmic RyR1 Ca2+ accumulation compared with MHN controls. Specific aims of the study are listed below and are illustrated in more detail in Figure 2.

1. Compare fura-2 acetoxymethyl (fura-2-AM) Ca2+ dose-response fluorescence ratios in response to the RyR1 agonist 4-CmC in the presence and absence of norepinephrine, 1 μM, and isoproterenol (Isoprot).

2. Compare how these dose-response fluorescence ratios varied in response to 4-CmC with 1 μM of norepinephrine in the presence of the α- and β-adrenergic receptor blockers phenolamine and propranolol.

Methods

- Cell Samples. Institutional review board approval (G161N4) was obtained from the Uniformed Services University of the Health Sciences, Bethesda, Maryland. B-cell samples were obtained from the MH Center at the Uniformed Services University, Bethesda, Maryland. Both MHS and MHN B-cell samples were immortalized at the Tissue Culture Facility at the University of North Carolina, Chapel Hill, using an Epstein-Barr virus (EBV) immortalization protocol (http://unclineberger.org/research/core-facilities/tissueculture/b-lymphocytes-protocol). The immortalization of the cells with the EBV ensured that the same cell lines could be used for repeated experiments.

- The MHS diagnosis was based on a positive result of the CHCT performed according to the North American MH Group diagnostic criteria. Those in the MHN group had a negative CHCT result. Cell lines were cultured and maintained in Roswell Park Memorial Institute buffer (RPMI 1640) and l-glutamine (Quality Biological) constituted with the addition of 5% heat-inactivated fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Gibco Invitrogen).

Using a similar concentration response studied by Sei et al., we determined this investigation should reach 100% power with more than 3 samples per group (DSS Research). However, we decided to use 6 samples to allow for the possibility of varied responses between MHS cell
line with different genetic variants. A blocking technique (randomization of cell lines), was used to address concerns with validity regarding possible seasonal and laboratory environmental variations. Each subject (sample) served as its own control in paired analysis of adrenergic response. Figure 3 depicts the experimental design.

A spectrofluorometer (QuantaMaster, Photon Technology International Inc [PTI], Horiba Scientific) was used to measure the emission fluorescence from the ratiometric Ca\textsuperscript{2+} indicator dye, fura 2-AM (Invitrogen), with cell excitation rendered at 340 nm/380 nm and emission measured at 510 nm. Dye procedures were used as previously described.\textsuperscript{23,32}

- **Reagents.** A sarcoplasmic-endoplasmic reticulum calcium reuptake inhibitor, thapsigargin, 100 nM (Calbiochem, Millipore Sigma), was dissolved in dimethylsulfoxide (DMSO) and maintained at −20°C. Norepinephrine and isoproterenol, 1 μM, were reconstituted in distilled water and free ascorbic acid under darkroom conditions to control for auto-oxidation. Phentolamine, 2 μM, and propranolol, 2 μM, were dissolved in DMSO and diluted with Hank’s balanced salt solution (BSS). The RyR1 agonist 4-chloro-m-cresol (4-CMC), in concentrations of 1.0, 1.5, and 2.0 mM, was dissolved in DMSO. All solutions with the exception of thapsigargin were used within 24 hours. All reagents were analytical grade and purchased from Sigma Aldrich unless otherwise noted.

- **Experimental Protocol.** Epstein-Barr virus–immortalized human B cells were used to calculate differences in fura-2-AM (5 μM final concentration) and ratiometric fluorescence in the absence and presence of norepinephrine, to compare intracellular Ca\textsuperscript{2+} concentrations. B cells were centrifuged at 2,000 revolutions per minute (RPM) for 2 minutes, and then the RPMI growth medium was suctioned from the flask, leaving a pellet of cells. Cells were washed twice with Hanks’ BSS containing 10% FBS and resuspended in 10 mL of fresh Hanks’ BSS with 10% FBS. Fura-2-AM dye in a final concentration of 5 μM was added to the Hanks’ BSS and B-cell solution, and the cells were incubated for a minimum of 40 minutes at 37°C in a dark environment water bath. After 40 minutes the cells in suspension were removed from the water bath and centrifuged for 2 minutes at 2,000 RPM. After the cells were centrifuged, the liquid buffer was carefully suctioned off, and the remaining cells were in the pellet at the bottom of the flask. These cells were then resuspended in 10 mL of Krebs/Ca\textsuperscript{2+} (1.0 mM)–containing buffer solution and placed in a dark environment at room temperature (approximately 22°C) for 40 minutes. It was during this time that adrenergic agonists norepinephrine or isoproterenol as well as the antagonists phentolamine and propranolol were added to the cells to allow for an incubation period. Following the incubation period, 1-mL aliquots of the MHS or MHN B cells were centrifuged at 5,000 RPM for 30 seconds. The buffer solution was removed, and the remaining cell-containing pellet was resuspended in a 3-mL quartz cuvette using 2 mL of Krebs solution with Ca\textsuperscript{2+}. Cuvettes with magnetic stirrer magnets were prewarmed at 37°C. The cuvettes were placed in the QuantaMaster fluorometer and maintained at 37°C. Fluorescence measurements were sampled at the emission wavelength of 510 nM approximately every 0.5 seconds using excitation wavelengths of 340/380 nM. PTI software was used to exponentially smooth the raw fluorescence curves to remove noise.

Before each cell line exposure to experimental conditions or 4-CMC or norepinephrine, thapsigargin (100
nM) was used to compare total releasable \( \text{Ca}^{2+} \) stores between cell lines in the absence of extracellular \( \text{Ca}^{2+} \). This was necessary to demonstrate cell line viability and ensure that the total endoplasmic \( \text{Ca}^{2+} \) in the cell lines was equivalent.

Baseline area was calculated by measuring the first 50 seconds of fluorescence before the addition of 4-CmC to isolate and quantify the effect of norepinephrine on the cells in the absence of the RyR1 agonist. After 50 seconds, 4-CmC was added to the cells in suspension, and the dose response was measured. The dose response was defined as the area under the curve (\( \Delta \text{AUC} \)) value and was calculated beginning with the addition of 4-CmC and ending at 250 seconds. These fluorescence data with and without norepinephrine provided the values for the raw results.

Phentolamine hydrochloride, 2 \( \mu \text{M} \), an \( \alpha \)-adrenergic antagonist, and propranolol, 2 \( \mu \text{M} \), a \( \beta \)-adrenergic receptor antagonist, were used in separate experiments to isolate the \( \alpha \)- and \( \beta \)-adrenergic effects of norepinephrine. Isoproterenol, 1 \( \mu \text{M} \), was used to elicit nonselective \( \beta \)-adrenergic agonist effects. \( \text{Ca}^{2+} \) (1.0 mM) was added to the external media of Krebs solution to elicit detectable 4-CmC–dependent fura-2-AM fluorescence changes after B-cell incubation with norepinephrine.

• **Statistical Analysis.** Fura-2-AM baseline area and AUC values were calculated by the PTI software. The raw AUC fluorescence data were analyzed using a 2-way analysis of variance (ANOVA) with the independent variable, 4-CmC concentrations (1.0, 1.5, 2.0 mM), as the “within B-cell lines” factor. The MH status (MHS or MHN) was the “between B-cell lines” factor. The B-cell line type (6 MHS vs 6 MHN cell lines) was the random effect used for each concentration. The raw AUC values, which represented the dose response, were considered to be the dependent variable. Post hoc tests of the simple main effects (1-way ANOVA) assessed whether MHS or MHN status had an effect at each concentration with no adjustment for multiple comparisons. The raw AUC results followed a skewed distribution, which violated the normality and equal variance assumptions of ANOVA, so the data were log transformed. The transformed data yielded results similar to an untransformed dataset, so the nontransformed raw AUC data were used for analysis.

The raw AUC values were then normalized to the mean AUC result calculated for the MHN B cells at 1.0 mM, as in the following equation.

\[
\text{Normalized AUC} = \frac{(\text{Raw AUC} - \text{Mean AUC at 1 mM 4-CmC})}{\text{Mean AUC at 1 mM 4-CmC}}
\]

The ratio provides a unitless “fold increase” greater than the AUC detected at the lowest concentration of 4-CmC in MHN B cells. This ratio was calculated for each B-cell trial in the MHN and MHS groups.

The normalized dose-response (“\( \Delta \text{AUC} \)”) results were calculated by subtracting the fold increase values in nontreated norepinephrine B cells from the fold increase values in norepinephrine-treated B cells. The differences in AUC results were corrected by subtracting the baseline area value for each B-cell line (6 MHN and 6 MHS).
difference in AUC fluorescence data were then analyzed using a 2-way analysis of variance with similar parametric assumptions to the raw AUC data. The $\Delta$AUC was considered to be the dependent variable. The difference in AUC results followed a similar distribution pattern to the raw AUC data. We used the statistical program SAS (SAS Institute Inc) for the 2-way ANOVA analyses.

We used the raw AUC value as the dependent variable for the phentolamine, propranolol, and isoproterenol experiments. Independent 2-tailed Student $t$ tests were performed to compare the mean differences for the raw AUC values. All comparisons resulting in $P < .05$ were considered significantly different.

**Results**

- **Malignant Hyperthermia–Susceptible vs Malignant Hyperthermia–Negative Internal Ca$^{2+}$ Stores.** The use of thapsigargin ensured that the variance in dose response was not related to varying amounts of Ca$^{2+}$ available in the endoplasmic reticulum of each cell line. The sarco-/endoplasmic reticulum Ca$^{2+}$ adenosine triphosphatase (SERCA) pump sequesters Ca$^{2+}$ from the intracellular space to refill the “internal” Ca$^{2+}$ stores. Thapsigargin blocks the SERCA pump from refilling the Ca$^{2+}$ internal releasable stores (see Figure 1). The AUC values for the MHN and MHS thaps-

<table>
<thead>
<tr>
<th>MH Group</th>
<th>4-CmC Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 mM</td>
</tr>
<tr>
<td>ΔAUC MHS</td>
<td>0.287 ± 0.066 (12)$^b$</td>
</tr>
<tr>
<td>ΔAUC MHN</td>
<td>0.037 ± 0.066 (12)$^b$</td>
</tr>
</tbody>
</table>

**Table.** Malignant Hyperthermia (MH)-Negative (MHN) vs MH-Susceptible (MHS) Dose Response ($\Delta$AUC) at Different Concentrations of 4-Chloro-m-Cresol (4-CmC)$^b$

$^a$Data are mean dose response ($\Delta$AUC) with standard error of the mean values, followed in parentheses by the number of tests per group. MHN and MHS B cells’ dose responses using fura-2-acetoxymethyl fluorescence (eg, 380 nm/340 nm; emission: 510 nm) were measured to compare the effects of adding norepinephrine (NE) and increasing concentrations of the RyR1 agonist 4-CmC. A total of 69 tests were performed. One test per cell line per NE status was included for each 4-CmC condition: (6 MHN cell lines − NE * 1 test) + (6 MHN cell lines + NE * 1 test) = 12. For the 2.0-mM groups, several cultures were not viable at the time of experimentation, hence, the decrease in number of tests. At each concentration, the $\Delta$AUC MHS significantly differs from the $\Delta$AUC MHN mean values.

$^b$P < .05.

$^c$P < .01.

...
Dose-Response Comparison at Varying Doses of 4-Chloro-m-Cresol. The most notable result from these experiments was the significant differences in dose response between the MHS and MHN B-cell population in terms of their fura-2-AM ratiometric response to the RyR1 agonist 4-CmC in the presence of norepinephrine. The 2-way ANOVA detected that intracellular Ca\(^{2+}\) increases in the MHS B cells were greater than in the MHN B cells (\(F(1,10) = 27.37, P < .01\)). This association remained significant for each of the 4-CmC concentrations (\(F(2, 17) = 0.57, P > 0.5\)). The ANOVA tests confirmed significant differences between the MHN and MHS groups in separate tests for the 1.0, 1.5, and 2.0 mM 4-CmC concentrations (\(P = .015, .001, \text{and} .025\)), respectively (Table, Figure 5).

Effects of Phentolamine and Propranolol. The MHS plus norepinephrine B cells exhibited a decrease in the AUC fluorescence following incubation with phentolamine compared with MHS plus norepinephrine B cells not treated with phentolamine (MHS + norepinephrine + phentolamine: 381.77 ± 59.11 vs MHS + norepinephrine – phentolamine: 450.92 ± 60.72; \(P < .05\); \(n = 4\); Figures 6 and 7). No differences between AUC values were detected in MHS plus norepinephrine B cells following propranolol incubation (MHS + norepinephrine + propranolol = 482.29 ± 62.93, MHS + norepinephrine – propranolol = 553.05 ± 66.48, \(P > .05\); \(n = 4\); Figure 8).

Isoproterenol. Following the experiments with norepinephrine, we used the pure adrenergic \(\beta\)-agonist isoproterenol to elicit any pure \(\beta\)-response from representative MHS cell lines. There was no significant increase in intracellular Ca\(^{2+}\) accumulation in dose response to 1.5 mM of 4-CmC in the presence or absence of isoproterenol (MHS 4-CmC + isoproterenol \(M = 362 \pm 55.0\) vs MHS 4-CmC – isoproterenol \(M = 365.03 \pm 42.83\); \(P > .05\); \(df = 3\); Figure 9).

Discussion

Our findings support our hypothesis that immortalized human B lymphocytes derived from a known MHS population would display an increased sensitivity to the catecholamine norepinephrine as evidenced by an increase in myoplasmic Ca\(^{2+}\) accumulation compared with MHN controls. Our results demonstrate that in this model norepinephrine stimulation correlates with increased intracellular Ca\(^{2+}\) levels in MHS B-cell lines in response to an RyR1 agonist. Significant findings from our experiment suggest that norepinephrine stimulation correlates with increased intracellular Ca\(^{2+}\) concentration changes in MHS B-cell lines in our model. Significant findings are described here.

(1) Immortalized human B cells from MHS subjects displayed increased fura-2-AM fluorescence ratios in response to the RyR1 agonist 4-CmC at each dose (1 \(\mu\)M, 1.5 \(\mu\)M, and 2.0 \(\mu\)M). Results are not shown, because this was consistent with previous findings and was an
expected outcome.22,23 Furthermore, intracellular stores of Ca²⁺ were equivalent in all MHS and MHN cell lines, as demonstrated by no significant differences in response to thapsigargin (see Figure 4).

(2) B cells that were MHS yielded a greater fura-2-AM fluorescence signal compared with the MHN B cells after incubation with norepinephrine and 4-CmC, suggesting a higher intracellular Ca²⁺ concentration response from the norepinephrine.

(3) The pure adrenergic β-receptor agonist isoproterenol elicited no significant increase in MHS cell lines in response to the RyR1 agonist 4-CmC. This indicates that in our B-cell model the increase in Ca²⁺ was not a pure β-receptor response.

(4) The α-adrenergic inhibitor phentolamine significantly attenuated the norepinephrine-related increase in intracellular Ca²⁺ (as measured by fura-2-AM fluorescence) response to the RyR1 agonist 4-CmC in MHS B-cell lines. This result supports the importance of the α-adrenergic receptor as the mechanism of action for the increase in intracellular Ca²⁺ exhibited in MHS cell lines.

(5) The β-adrenergic inhibitor propranolol had no significant effect on attenuation of the norepinephrine-related increase in intracellular Ca²⁺ levels in response to the RyR1 agonist 4-CmC supporting the lack of β-agonist effect from the norepinephrine in eliciting increased Ca²⁺ levels in the MHS B cells in our model.

Our findings are consistent with existing evidence regarding B-cell physiology. The presence of the RyR1 calcium release channel and adrenergic receptors on the B cell has been well documented.18,19,27-30 Given that B cells have been used to phenotype and genotype putative MH patients, our experiments attempted to evaluate the contribution of norepinephrine to the altered Ca²⁺ flux associated with MHS B cells.23 In this study, we found the increase in dose response as measured by the AUC value in the 4-CmC–treated MHS B cells were consistently elevated compared with the MHN cells (see the Table and Figure 5). The consistently greater fluorescence values in the MHS B-cell lines in basal and stimulated (norepinephrine and 4-CmC) conditions suggest that greater intracellular Ca²⁺ concentrations may be a prominent characteristic of MHS B cells.

To evaluate the contribution of only intracellular Ca²⁺ pools, we originally planned to perform all measurements using Ca²⁺ free medium, restricting the 4-CmC dose-dependent detection of Ca²⁺ to the endoplasmic reticulum.24 However, in the absence of external Ca²⁺ we found no significant differences in the response to norepinephrine in MHS vs MHN cell lines (data not shown). Adding Ca²⁺ to the physiologic buffer produced strong and reproducible effects on 4-CmC–induced Ca²⁺-dependent fura-2-AM fluorescence after norepinephrine stimulation. Intracellular Ca²⁺ significantly increased in norepinephrine-treated MHS B cells.

Our finding that intracellular Ca²⁺ homeostasis differs...
in MHS B cells compared with MHN B cells is also consistent with prior work. In a 2015 study, Bina et al.33 also found a greater sensitivity of intracellular Ca²⁺ flux in MHS B cells. The prevailing hypothesis suggests that mutations in the \(\text{RYR1}\) gene increase the probability of this Ca²⁺ release channel to open. These “leaky” RyR1 channels (and any other transmembrane channels, such as transient receptor potential channels) release Ca²⁺ into the intracellular space, increasing intracellular Ca²⁺ concentration and generating the greater fura-2-AM difference in dose-response AUC signals that we observed.

The data importantly indicate that external ligand binding to the \(\alpha\)-adrenergic receptor transduces signaling mechanisms to elevate the Ca²⁺ in MHS B cells, thereby supporting previous research findings that suggested that neurotransmitters released by the adrenergic nervous system may exacerbate MH.13-16 We hypothesize that the \(\alpha\)-adrenergic antagonist phentolamine blocked the effects of norepinephrine (see Figure 6) on Ca²⁺ influx in MHS B cells, further substantiating the postulated role of \(\alpha\)-adrenergic receptors in the MH pathophysiology. It is highly likely the Gs/adenylyl cyclase/cAMP/protein kinase A pathway is also being activated, since norepinephrine does bind to \(\beta\)-adrenergic receptors. Past research described increased levels of adenyl cyclase activity and cAMP content in muscle cells from MHS individuals compared with MHN individuals.8 However, our data implicate \(G_{\alpha}/\text{PLC}/(\text{Ins1,4,5})\)P3 pathway that alters the Ca²⁺ levels because the \(\beta\)-adrenergic agonist isoproterenol did not induce significant fura-2-AM ratio-metric changes. Our results support the hypothesis that the MHS population has an increased sensitivity to norepinephrine and possibly other stress hormones. Lister et al.13 previously reported a similar result using high doses of phentolamine in MHS swine.

These findings reveal that an \(\alpha\)-adrenergic agent significantly contributed to higher intracellular Ca²⁺ in MHS B cells independent of RyR1 activation by 4-CmC. In our model, 4-CmC enhanced intracellular Ca²⁺ differences between the MHS and MHN B cells. However, it is noted that concentrations of 4-CmC at or above 1 mM of 4-CmC partially inhibit the SERCA pump. Inhibition of the SERCA pump blocks the Ca²⁺ refilling of the endoplasmic reticulum, thereby decreasing the available Ca²⁺ for intracellular release. It is possible the inhibition may differ between MHS and MHN B cells. However, our experiments report the overall intracellular Ca²⁺ changes, which included any SERCA pump inhibition.

In summary, we have demonstrated that norepinephrine increases the baseline Ca²⁺ and the 4-CmC–dependent intracellular Ca²⁺ concentrations in immortalized human MHS B-cell lines compared with MHN controls, and that this increase was successfully blocked by phentolamine. Phentolamine is an \(\alpha\)-adrenergic receptor antagonist, blocking the \(\alpha\)-adrenergic receptors from the agonism of norepinephrine. Once the \(\alpha\)-receptor is blocked, the norepinephrine cannot induce the G protein–coupled signaling cascade, thereby limiting the downstream second messenger effects associated with norepinephrine coupling to the receptor. Without phentolamine, we hypothesize an increased Ca²⁺ intracellular concentration in response to the norepinephrine and 4-CmC. Further investigations into the exact relationship of this response would be required. In Figure 10, we provide a possible mechanism that explains how our data may inform the pathophysiologic mechanisms of MH.

At this time, the only definitive diagnostic test for MH

---

**Figure 10. Proposed Pathway for Altered Ca²⁺ Signaling in MHS B cells Following Norepinephrine (NE) Activation of \(\alpha\)-Adrenergic Response in B-Cell Model**

**Abbreviations:** MHN, malignant hyperthermia negative; MHS, malignant hyperthermia susceptible.
is CHCT, with a sensitivity of 97% and a specificity of 78%. Genetic studies have demonstrated that the RyR1 gene, located on chromosome 19q13.1, is the primary locus responsible for MH susceptibility. However with more than 300 different missense RyR1 mutations found in patients with positive CHCT results or a presumptive clinical diagnosis of MH, genetic testing lacks the sensitivity or specificity to be used as a screening tool.

Our research demonstrates that immortalized B cells from MHS individuals have a distinctive sensitivity to norepinephrine at a concentration of 1.0 μM. This critical finding could possibly be the first step in the development of a less invasive and therefore more accessible diagnostic serologic assay using fresh B cells to determine the sensitivity to norepinephrine for the easy identification of MHS individuals.

The study has a limitation. The B-cell model may not provide an exact model to evaluate the intracellular Ca²⁺ flux for 2 reasons. First, the α₁ subunit of the dihydropyridine receptor protein, the CACNA1S gene, is not expressed in B cells; therefore, the B-cell functional assays would not be able to evaluate mutations in this gene. Second, RyR1 receptors in B cells have a lower sensitivity compared with the RyR1 proteins located in skeletal muscle. However, primarily Sei et al provided the functional validation studies to use B cells for studying the ryanodine receptor and the intracellular Ca²⁺ associated with the protein's activation.

REFERENCES


35. Bina S, Capacchione J, Munkhbu B, Muldoon S, Burger R. Is lympho-


AUTHORS

Susan M. Perry, PhD, CRNA, ARNP, FAAN, Col(ret), USAF, NC, is the vice dean for Faculty and Community Affairs at the University of South Florida College of Nursing in Tampa, Florida. Email: susanmperry@health.usf.edu.

Sheila Muldoon, MD, is professor emeritus at the F. Edward Hebert School of Medicine in the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, Maryland.

Luke P. Michaelson, PhD, RN, is a research assistant professor, Department of Anesthesiology, School of Medicine, at the Uniformed Services University. Email: luke.michaelson@usuhs.edu.

Rolf Bunger, MD, PhD, is an emeritus member of the American Physiological Society. He is professor of physiology, molecular biology and anesthesiology at USUHS.

Christine E. Kasper, RN, PhD, FAAN, is dean, University of New Mexico College of Nursing, Albuquerque, New Mexico. Email: ckasper@salud.unm.edu.

DISCLOSURES

The authors have declared no financial relationships with any commercial entity related to the content of this article. The authors did not discuss off-label use within the article.

ACKNOWLEDGMENT

The authors would like to thank Cara Olsen, MS, DrPH, from the Biostatistics Consulting Center, Uniformed Services University, for helping the research team perform the data analyses.