Background: Human papillomavirus (HPV) DNA testing can be crucial for women who have limited access to traditional screening. The current study compared the results obtained through HPV DNA testing with those obtained through cytology-based screening.

Methods: A total of 3068 women aged 18 to 85 years were enrolled in an opportunistic cervical cancer screening program developed by the Barretos Cancer Hospital and performed by a team of health professionals working within a mobile unit from March to December 2012, followed by statistical analyses. For each patient, 2 different cervical samples were collected and preserved in a careHPV assay and SurePath medium, respectively.

Results: High-risk HPV (hr-HPV) DNA was detected in 10.0% of women, with the majority (86.7%) demonstrating no abnormal Papanicolaou test results. The following cytological samples were found to be hr-HPV positive: 8.2% of the normal samples; 39.4% of the samples with atypical squamous/glandular cells of undetermined significance; 38.5% of the samples with low-grade squamous intraepithelial lesions; and 100% of the samples with high-grade squamous intraepithelial lesions. Colposcopy examinations were performed among 33.4% of the women with positive results on at least 1 of the tests (HPV DNA positive and/or cytology with atypical squamous/glandular cells of undetermined significance, cannot exclude high-grade lesion or high-grade squamous intraepithelial lesions), and 59.5% of these women underwent biopsies. Among these samples, 18.2% were confirmed as cervical intraepithelial neoplasia.

Conclusions: The careHPV test was demonstrated to be a feasible alternative to primary screening in low-resource settings accessed through the use of mobile units. Cancer Cytopathol 2016;000:000–000. © 2016 American Cancer Society.

Key Words: cancer screening; cervical cancer; colposcopy; human papillomavirus (HPV) DNA tests; Papanicolaou test.

Introduction

Cervical cancer (CC) is a primary public health problem for women who live in underserved, developing countries. CC is the third most common disease among women worldwide and the second most common disease in Brazil, according to the World Health Organization. Persistent high-risk human papillomavirus (hr-HPV)
infection is associated with high-grade cervical intraepithelial neoplasia of type 2 or higher (CIN2+) and its progression toward invasive CC. Molecular tests for HPV DNA or RNA have been designed to identify hr-HPV in cervical samples; these tests are both reliable and reproducible and can be implemented more easily than cytological analysis. New guidelines for CC screening recommend the combined use of HPV testing and cytology over the use of cytology alone. HPV testing has been shown to increase the sensitivity of the detection of intraepithelial lesions in 45% more cases compared with cytological screening. The detection of hr-HPV cervical infections is important for determining the prevalence of HPV in specific populations, thereby aiding in the planning of appropriate strategies for CC prevention programs. A new HPV DNA test approved by the US Food and Drug Administration (careHPV; Qiagen, Gaithersburg, MD) has been developed for use in low-income regions. The careHPV method is a simplified version of the Hybrid Capture 2 methodology (Digene Corporation, Gaithersburg, MD), and is based on antibody binding to magnetic beads; these beads rapidly capture specific target HPV nucleic acid sequences that are then detected using a chemiluminescent signal.

The careHPV test detects 14 types of hr-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens and exhibits high sensitivity and specificity in the detection of precancerous and cancerous cervical lesions. Populations in many Brazilian regions have difficulty accessing public health assistance because many individuals reside in areas that are a distance from medical care facilities, roads (when they exist) are extremely bad, and public transportation is lacking. These conditions represent some of the principle motivations for the Barretos Cancer Hospital (BCH) to introduce the use of mobile units (MUs) in 1994 to attend to populations living in remote rural areas, including the midwest, southwest, and northern (Amazon) regions of Brazil; women in these regions comprise a significant percentage of the Brazilian population with HPV-induced lesions. The objective of the current study was to evaluate the efficiency of the careHPV test in detecting hr-HPV in women with no precursor lesions and to evaluate its performance as a primary screening method in some of the remote Brazilian regions served by BCH MUs.

**MATERIALS AND METHODS**

The current study was performed from March to December 2012 using 4 MUs (Fig. 1) from the Cancer Prevention Department of the BCH in São Paulo, Brazil. MUs 2, 3, 4, and 5 were used. Each MU consists of an adapted truck, with 3 rooms for patient examinations (Papanicolaou [Pap] testing and prostate and surgical centers), a reception area, a document cabinet, and a supply room. The cervical specimens used in this study (careHPV and cytology tests) were collected in the Pap test room. The careHPV test was performed in the neighboring room. A stand was added in this room so that it would be possible install the careHPV system and perform the HPV test. HPV DNA analysis was performed in 2 settings: 1) in the MU while visiting the first 10 cities by one of the authors (A.T.L.) who was responsible for the research and...
molecular analyses (approximately 15% of the samples were analyzed in the MUs); and 2) at the BCH Molecular Oncology Center in coordination with the same individual. The analyses were performed at 2 locations because space in the MU is limited, and an additional nurse was required to support patient services and maintain the sample collection schedule in the cities. The disease prevalence results were similar between the 2 laboratory settings.

Study Subjects
A total of 3068 volunteers (all women) were enrolled in an opportunistic screening program that was regularly performed in the BCH MUs. The women ranged in age from 18 to 85 years (median, 47 years; standard deviation, 12.4 years). During this period, the MUs visited >50 cities in 4 states, including São Paulo, Minas Gerais, Mato Grosso, and Goiás.

Ethics and Procedural Overview
All participants who agreed to participate were informed about the objectives and methods of the study, provided written informed consent, and answered a structured questionnaire. The study was previously approved by the BCH Research Ethics Committee (no. 404/2010). The women’s privacy was guaranteed.

Preparation and Interpretation of the Samples
Two samples were collected from each participant, including 1 for HPV testing (careHPV) and a second for Pap testing (SurePath; Becton, Dickinson and Company, Franklin Lakes, NJ). The samples were properly labeled and stored at 4 to 8°C or at −20°C when the test was not performed within 7 days.

careHPV Test
The careHPV rapid HPV-DNA detection method involves specific target HPV nucleic acid sequences that are rapidly bound by antibodies attached to magnetic beads and then detected through chemiluminescence. The ratio between relative light units and a cutoff is calculated by the careHPV system, and samples are considered positive for hr-HPV when this ratio is >1.0. This method can detect 14 types of hr-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and >90 specimens can be processed within 2.5 hours.12,13,19

Pap Test
Specimens were collected for liquid-based cytology using SurePath medium by the same well-trained professional who collected the careHPV samples. The samples were obtained using a Rovers Cervex-Brush equipped with a removable brush head (Rovers Medical Devices, Oss, the Netherlands). Ectocervical and endocervical canal samples were immediately placed in SurePath medium. Specimens were subsequently forwarded to the Department of Pathology at BCH for preparation, analysis, and classification using the BD FocalPoint Slide Profiler (TriPath Imaging, Burlington, NC) for the first triage step. The specimens were first screened by cytotechnicians using an automated microscope, and all abnormal cases were rescreened by a senior cytopathologist.

Follow-Up
The study proceeded according to the results of HPV and cytology testing. If the cytological test classified the sample as normal or atypical squamous/glandular cells of undetermined significance (ASC/AGC-US) and the hr-HPV test was negative, the participants received a letter including their laboratory results without any follow-up recommendations, and were advised to follow only periodic screening adherence. If the hr-HPV test was positive and/or the abnormal cytology was classified as atypical squamous/glandular cells of undetermined significance, cannot exclude high-grade lesion (ASC/AGC-H) or high-grade squamous intraepithelial lesion (HSIL), the participants were referred to the hospital for colposcopy and/or biopsy, if necessary.

Statistical Analysis
Statistical analysis was performed using SPSS statistical software (version 20.1; IBM Corporation, Armonk, NY) and MedCalc statistical software (version 11.1; MedCalc, Ostend, Belgium). Chi-square tests were used to compare frequencies among groups and the Fisher exact test was used when the chi-square test was insufficient. The Student t test was performed to compare means between groups. P values <.05 were considered statistically significant. A screening criterion was statistically analyzed considering only those women with a positive hr-HPV test.

RESULTS
At baseline, a total of 3068 women with a median age of 47 years (range, 18-85 years) participated in the current
study. Among the participants, 2476 women (80.7%) were aged >35 years. The mean ages of the HPV-negative and HPV-positive women were 46.9 years and 41.0 years, respectively ($P < .0001$). hr-HPV DNA was detected in 10.0% of the samples (307 of 3068 samples). Among the participants, 95.4% (2927 of 3068 participants) had at least 1 previous Pap test result, and 58.6% reported having had only 1 sexual partner in their lifetime. The demographic and histocytopathological characteristics of the participants are shown in Table 1. The results achieved based on the cytology, colposcopy, and biopsy findings for the women who were tested for HPV DNA are presented in Table 2.

Approximately 91.8% of the women (2696 women) who were classified as exhibiting normal cytology (negative for intraepithelial lesion or malignancy) were HPV negative. Similarly, 39.4% of the women (26 of 66 women) diagnosed with ASC/AGC-US, 38.5% of the women (5 of 13 women) diagnosed with ASC/AGC-H, and 55.3% of the women (21 of 38 women) diagnosed with low-grade intraepithelial lesion (LSIL) were also found to be HPV positive. Women aged <34 years exhibited elevated hr-HPV prevalence; this prevalence was found to be lower in women aged 35 to 44 years and was higher in women aged >45 years. Women who were positive for hr-HPV with cytological abnormalities (ASC/AGC-H, LSIL, or HSIL) were referred to BCH for colposcopy and biopsy. The careHPV test was positive for all 15 women who were diagnosed with HSIL on cytology. However, of these 15 cases, 8 women underwent to colposcopy and biopsy, of which, 3 women were diagnosed with invasive carcinoma, 1 woman with CIN3, 2 women with CIN2 and 2 women with CIN1. If the participants were unable to travel to BCH, they were advised to present for examination at the hospital closest to where they lived.

**Molecular Test Performance**

According to the previously described criteria, 10.8% of the women (332 of 3068 women) were referred for follow-up and 33.4% of these women (111 women) underwent colposcopy examinations (1 woman was excluded from this analysis due to death from another cause). Among the women who underwent coloscopies, 92.8% (103 of 111 women) were infected with hr-HPV, 43.2% (48 of 111 women) were diagnosed with CIN2+, and 32.4% (36 of 111 women) were classified as having unsatisfactory findings because the transformation zone or the endocervical borders of the lesion were not identified during the examination.25

Based on these results, the HPV DNA test demonstrated accuracy, with a sensitivity of 100% (95% confidence interval, 75.3%-100%) and a specificity of 10.8% (95% confidence interval, 5.1%-19.6%), considering the cervical biopsy as the gold standard. However, if biopsy was not performed, colposcopy was considered the gold standard (59.4% of the women referred for follow-up [66 of 111 women] did not undergo the procedure). Consequently, women with normal findings or findings that were suggestive of low-grade intraepithelial lesions during colposcopy were categorized as disease free. When the colposcopy examination suggested a high-grade intraepithelial lesion (even without biopsy), the women were classified as being positive for HPV-induced disease.

**DISCUSSION**

All the severe cervical lesions confirmed by biopsy were found to be positive for hr-HPV. Moreover, the careHPV test was easy to perform, and we confirmed previous reports indicating that careHPV is easily used in screening settings.12–14 The careHPV method was primarily designed for use in remote areas in which human resources and laboratory facilities are scarce,13,14 which spurred us to evaluate the implementation of an “easy-to-perform” molecular HPV test for CC screening in underserved areas, because high sensitivity is critical for identifying women with cervical lesions. Carefully designed strategies and efficient screening programs are needed to identify those women at risk of developing cancer because access to hospitals and ambulatory clinics is poor due to the long distances involved and the precariousness of Brazilian roads. The MUs from the BCH are able to visit remote areas in the midwest, southwest, and northern (Amazon) regions of Brazil, accommodating approximately 24,000 visits in 157 cities in 2013, thus serving the needs of a significant percentage of women with HPV-induced tumors. The ability to identify women with cervical lesions and provide an appropriate medical referral is critical for saving lives and reducing mortality due to CC.

Based on the results of the current study, the prevalence of hr-HPV was 10.0% (307 of 3068 women) among women with a median age of 47 years. The results of the current study are consistent with those of other studies regarding HPV detection in Brazilian women. de Aguiar
et al,25 Lorenzi et al,19 Rosa et al,26 and Nonnenmacher et al27 reported HPV prevalence rates of 10.5%, 12.3%, 12.3%, and 15%, respectively. The majority of these women lived in rural or remote areas of Brazil and experienced difficulties in accessing medical care or the health assistance infrastructure. These women lived in resource-poor settings, and the majority had poor literacy, thereby hindering their access to information regarding the role of HPV infection in the development of CC.

The prevalence of HPV in women with normal cytology ranges from 10.0% to 24.5%.3,28,29 In the current study, we reported a slightly lower prevalence (8.2%) (Table 2). However, in women aged <34 years with normal cytology, the prevalence of hr-HPV was found to be higher. The majority of women with high-grade lesions (CIN3+) were aged 25 to 44 years; similar results were reported in other studies.3,27,29–32 The prevalence of hr-HPV was found to be lower in women aged 35 to 44 years, except for those women with specimens classified as negative for intraepithelial lesion or malignancy and ASC-US, among whom the prevalence decreased and remained stable or continuously decreased with age, a finding that is consistent with other reports.3,29,30 HPV tests based on the detection of HPV DNA or RNA are considered more efficient than cytological analysis for the screening of underserved populations, due to the simplicity of the automated methodology and the reproducibility of the results, which are advantages that are not obtained using operator-dependent cytology.13,14,33,34 A recent study comparing careHPV and the Pap test35 described these tests as having equal performance in the detection of CIN2 and CIN3 and these findings were not dependent on the age of the women. In addition, other studies have described the careHPV test as having acceptable cost-effectiveness when compared with Hybrid Capture 2, the OncoE6 cervical test (Arbor Vita Corporation, Fremont, Calif), and Liquid-Based Cytology (LBC), thereby recommending it

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the Study Population</th>
<th>Characteristica</th>
<th>Frequency, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous Pap test</td>
<td>Yes</td>
<td>2927 (95.7)</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>No</td>
<td>2638 (86.1)</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>No</td>
<td>2515 (82.2)</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>252 (8.2)</td>
</tr>
<tr>
<td>No. of sexual partners</td>
<td>1</td>
<td>1779 (58.6)</td>
</tr>
<tr>
<td>Cytology</td>
<td>No atypia</td>
<td>2936 (95.7)</td>
</tr>
<tr>
<td>ASC/AGC-US</td>
<td>66 (2.2)</td>
<td></td>
</tr>
<tr>
<td>ASC/AGC-H</td>
<td>13 (0.4)</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>38 (1.2)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>15 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Colposcopy</td>
<td>Normal</td>
<td>27 (24.3)</td>
</tr>
<tr>
<td>suggestive of low-grade intraepithelial lesion</td>
<td>40 (36.0)</td>
<td></td>
</tr>
<tr>
<td>suggestive of high-grade intraepithelial lesion</td>
<td>8 (7.2)</td>
<td></td>
</tr>
<tr>
<td>Unsatisfactory</td>
<td>36 (32.4)</td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>Normal/Chronic cervicitis</td>
<td>27 (40.9)</td>
</tr>
<tr>
<td>CIN1</td>
<td>27 (40.9)</td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>5 (7.6)</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>4 (6.1)</td>
<td></td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>3 (4.5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ASC/AGC-H, atypical squamous/glandular cells of undetermined significance, cannot exclude high-grade lesion; ASC/AGC-US, atypical squamous/glandular cells of undetermined significance; CIN, cervical intraepithelial neoplasia (types 1, 2, and 3); HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou.

a Data were excluded from the analysis when not informative.

<table>
<thead>
<tr>
<th>TABLE 2. Frequency Comparison Between Cytological Results, Colposcopy, and Biopsy According to HPV Testinga</th>
<th>Negative HPV DNA, No. (%)</th>
<th>Positive HPV DNA, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>No atypia</td>
<td>2696 (91.8)</td>
</tr>
<tr>
<td>ASC/AGC-US</td>
<td>40 (60.6)</td>
<td></td>
</tr>
<tr>
<td>ASC/AGC-H</td>
<td>8 (61.5)</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>17 (44.7)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Colposcopy</td>
<td>Normal</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>suggestive of low-grade intraepithelial lesion</td>
<td>4 (10.0)</td>
<td></td>
</tr>
<tr>
<td>suggestive of high-grade intraepithelial lesion</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Unsatisfactory</td>
<td>3 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>Normal</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>CIN1</td>
<td>1 (3.7)</td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ASC/AGC-H, atypical squamous/glandular cells of undetermined significance, cannot exclude high-grade lesion; ASC/AGC-US, atypical squamous/glandular cells of undetermined significance; CIN, cervical intraepithelial neoplasia (types 1, 2, and 3); HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

a Exact Fisher test P value of .05 for statistical significance. (p<.001).
as an option in primary screening, especially in low-income settings. The findings of the current study endorse this premise because the careHPV test recognized high-grade intraepithelial lesions (CIN3+). The performance indices confirm the hypothesis, with remarkably high sensitivity and negative predictive values obtained for the results of the HPV test, either alone or in association with cytology and visual inspection. However, the specificity was lowest, which could be explained by the presence of HPV not necessarily being associated with the existence of a high-grade lesion or also by the fact that the women who were negative for both tests (molecular and cytology) were not evaluated. Optionally, reflex cytology can be used when HPV testing provides a positive result. The accuracy of careHPV as calculated from the area under the receiver operating characteristic curve demonstrated that despite the low specificity, careHPV could be considered in the detection of hr-HPV and CIN2+, as proposed.

ASC-US is a common diagnosis in CC screening and is reportedly found in 2% to 5% of screened women. This result represents an uncertain alteration that generally ranges from normal to LSIL. HPV testing has frequently been used to assess women who have been diagnosed with ASC-US in an attempt to refer for colposcopy only those women who are positive for hr-HPV.

In the current study, we found that 40.0% of the women with a cytological assessment of ASC/AGC-US (26 women) were positive for hr-HPV; all of these women were referred for colposcopy, but only approximately one-half underwent the procedure. This cytological result was higher than the values obtained in other Brazilian studies conducted in Porto Alegre (southern region), Belém (northern region), Natal (northeast region), and Pelotas (southern region). Among the women who underwent colposcopic examination, 24.3% (27 of 111 women) were diagnosed with normal findings; 36.0% (40 of 111 women) received a diagnosis consistent with a low-grade intraepithelial lesion; 7.2% (8 of 111 women) had a diagnosis consistent with a high-grade intraepithelial lesion; and 32.4% (36 of 111 women) received unsatisfactory diagnoses.

Some guidelines recommend follow-up for women with cytological findings of ASC-US and a negative hr-HPV test. In the current study, women with cytological findings of ASC-US and a negative hr-HPV test were not referred for colposcopy. HPV testing has been indicated for the screening of women at risk of developing cervical lesions due to its higher sensitivity, lower subjectivity, and greater reproducibility compared with cytology. Thus, HPV testing may improve the prevention of cervical precursor lesions to a greater extent than cytology. All of the HSILs observed using cytology were found to be positive for hr-HPV. Among the identified LSILs, 55.3% were positive for hr-HPV. This result is comparable to those found in other studies. One of the limitations of the current study was that the group in whom both primary tests (cytology and molecular tests) yielded negative results, representing 89.1% of all women enrolled (2736 of 3068 women), was not referred for colposcopy or biopsy. If a bias verification method were to be applied to the data from the current study, the sensitivity might decrease and the specificity might increase. A Canadian study recorded a rate of CIN2+ of 0.61% when cytology and HPV test results were both negative. Among the women referred for colposcopy, 66.6% (221 women) did not undergo this examination. On average, the women waited 166.7 days (median, 156.0 days; standard deviation, 69.0 days) for colposcopy examination (range, 3-407 days). A recent study demonstrated that >46% of cases are examined within 180 days after referral and that the majority of invasive cancers are treated after 180 days. The authors concluded that the diagnosis did not differ depending on whether colposcopy was conducted before the positive test or 6 months after it. When a colposcopy is performed earlier, patient anxiety is lower, and follow-up losses can be reduced. Furthermore, invasive lesions that are missed by cytology might be identified. The detection of hr-HPV DNA is important for women with an uncertain cytological diagnosis who require a precise result to be referred for colposcopy or to be followed after treatment of a high-grade lesion. Colposcopy examinations are limited in the public health care systems of developing countries for several reasons, including a lack of expert professionals or equipment and suboptimal preservation of equipment. This situation affects the interval between referral and colposcopy and the confirmation of lesions by biopsy. Treatment is consequently postponed to ameliorate patient anxiety and fear. Fakokunde et al observed that women who underwent colposcopies after 6 months exhibited less of a tendency to obtain treatment or to develop high-grade lesions compared with a group of women who were examined earlier. The time that elapses between cytological sampling and colposcopy examination varies; however, Pap tests for high-grade lesions are given high
priority. The majority of HPV infections clear spontaneously, and only those infections involving high-risk HPV persist. Because progression toward a malignant lesion takes a relatively long time, an opportunity exists to identify and adequately treat patients with premalignant cervical lesions. The results of the current study have demonstrated that using cytology and HPV testing in combination improves the specificity of the results, thereby enhancing the probability of detecting severe HPV-induced cervical lesions. These results also demonstrated that the careHPV test is accurate for primary screening in rural and remote areas. If performed within MUs, the careHPV test has the potential to improve the detection of cervical precursor lesions in underserved settings.\(^{13,49,50}\)

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careHPV kits, equipment, preservative liquid medium, consumable materials, and conical brushes were kindly provided by Qiagen. These materials were used for research only. The authors designed the study, analyzed the results, and wrote the article independently. Complementary materials were subsidized by Barretos Cancer Hospital.

**CONFLICT OF INTEREST DISCLOSURES**
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**AUTHOR CONTRIBUTIONS**
Adriana T. Lorenzi: Validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, and visualization. José Humberto T. Fregnani: Conceptualization, methodology, formal analysis, data curation, writing—review and editing, visualization, and supervision. Julio César Possatti-Resende: Resources and writing—review and editing. Márcio Antoniazzi: Resources and writing—review and editing. Cristovam Scapulatempo-Neto: Conceptualization, methodology, validation, resources, and writing—review and editing. Stína Syrjänen: Writing—review and editing. Luisa L. Villa: Writing—review and editing. Adhemar Longatto-Filho: Conceptualization, methodology, writing—original draft, writing—review and editing, visualization, supervision, project administration, and funding acquisition.

**REFERENCES**


