Uniform International Method to Measure Cervical Length: Are We There Yet?

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Mini-Summary

What does this study add to current knowledge?
• The method of cervical length measurement significantly impacts the incidence of short cervical length.

What are the main clinical implications?
• We demonstrated that different techniques of measurement influence the estimated incidence of women designated as high risk for preterm birth and receiving treatment. We advocate awareness for the method being used in clinical trials and cohort studies.

Keywords
Singletons · Preterm birth · Cervical length · Prediction

Abstract
Introduction: Cervical length is an important predictor of spontaneous preterm birth. So far, the best way to measure cervical length has not been established. We aimed to compare the incidence of short cervical length between three methods of cervical length measurement with and without the inclusion of cervico-isthmic complex (CIC) (six methods in total) and to determine the positive predictive value for spontaneous preterm birth. Material and Methods: We performed a prospective single-center cohort study in women with a singleton pregnancy between August 2014 and December 2018. During the routine fetal anomaly scan (18–22 weeks), women were offered transvaginal ultrasound for cervical length measurement to screen for the risk of spontaneous preterm birth. Each cervix was measured in six different ways: single-line, two-line, and tracing method between the internal and external os of the cervix with and without CIC. We evaluated the predictive value of the different measurements for spontaneous delivery before 37 weeks using positive predictive values. Results: Our final study population comprised 1,691 women. The overall rate of preterm birth <37 weeks was 8.0% (4.6% spontaneous, 3.4% iatrogenic preterm birth). The mean gestational age at cervical length measurement was 19+6 weeks. The different measuring techniques resulted in significant different cervical lengths, showing a maximum difference of >8 mm between the techniques (41.04 mm [SD 7.1] with one-line without CIC and 49.18 [SD 9.05] mm with trace with CIC) with an
incidence of short cervical length below <25 mm ranging from 0.4% to 1.1% (p = 0.18). The positive predictive values for spontaneous preterm birth <37 weeks ranged from 42.9% to 20.0%. **Conclusion:** Different measurement methods for cervical length resulted in statistically significant differences in measured cervical length. Depending on the chosen cut-off this translates to different incidences of short cervical length and influences the number of women designated as high risk for preterm birth and receiving treatment. For interpretation and comparability between (inter-) national studies, it is important to adequately report on the employed technique. Future research should focus on determining the optimal measuring technique and a universal method of measurement.

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**Introduction**

Measurement of cervical length has become an essential tool in assessing the risk of (threatened) preterm birth and currently numerous studies are being performed worldwide to evaluate the most effective treatment strategy in women with a short cervix. However, the percentage of women with a short cervical length differs per study. This is mainly explained by the use of different cut-offs throughout the world ranging from ≤15 mm [1, 2] to ≤35 mm in singletons and 38 mm in multiples [3–6]. In addition, the prevalence of a short cervix also seems dependent on the population and country studied. Grobman et al. [5] as well as Van der Ven et al. [4] studied nulliparous women using a cut-off of 30 mm reporting prevalence of short cervix in the USA of 10.3% versus 2.2% in the Netherlands.

Whether these differences are true differences or differences in the method of measuring the cervix remains unclear. Greco et al. [7] already pointed out that it is important to distinguish the endocervical canal from the isthmic complex. They hypothesized that previous studies did not find an association between a short cervix in the first trimester and spontaneous preterm birth because sonographers wrongly include the isthmus [7].

Different initiatives have been undertaken to promote uniform measurement of the cervical length. Both the International Society of Ultrasound in Obstetrics and Gynecology and Fetal Medicine Foundation published practical advice online about measuring the cervical length [8, 9]. They advised using a straight line acknowledging the fact that a straight line will underestimate the cervical length in the curved cervix, and advice avoiding inclusion of the isthmus in the cervical length measurement.

However, studies about cervical measurement rarely describe in enough detail the method they use to measure the cervix [10–13]. Moreover, there is not always enough adherence to criteria for measurement [14, 15]. Although it seems intuitive that a short cervix is always straight and therefore the method of measurement (one-line, two-line, or tracing with or without adding the cervico-isthmic complex [CIC]) should not influence the predictive ability, this was not previously examined.

We decided to compare six methods of cervical length measurement (three methods with and without CIC) to determine whether the method of measuring influenced the prevalence and predictive ability of short cervical length for preterm birth. In addition, we evaluated which method of measurement was used in current literature concerning cervical length and preterm birth.

**Material and Methods**

**Study Design**

We performed a prospective cohort study among consecutive women with a singleton pregnancy who visited the fetal ultrasound department of Amsterdam UMC, location Academic Medical Center (AMC), for their routine fetal anomaly scan between January 1, 2014, and December 31, 2018. Data for this study were collected using an Astraia database. Astraia is a local registry that is used for collection of all sonographic data and pregnancy outcomes.

**Inclusion and Exclusion Criteria**

Both nulliparous and parous women with a singleton pregnancy were included. If cervical length was measured multiple times during pregnancy, only the cervical length measured during the fetal anomaly scan between 18 and 22 weeks of gestation was included. Amsterdam UMC not only is a tertiary referral center but also has a regional function for the surrounding local midwifery practices, leading to a heterogeneous population, including a large number of low-risk women and women with a preexisting medical condition or at increased risk for pregnancy complications.

Exclusion criteria were congenital abnormalities or antepartum fetal death. Moreover, women were excluded if the cervical length measurement was not performed or if the outcome of pregnancy was unknown or the onset of labor was not specified (i.e., spontaneous rupture of the membranes, spontaneous contractions, induction, and elective cesarean section).

**Data Collection**

Prior to the ultrasound, all women received an intake by a certified sonographer, during which details on general and obstetric history, cigarette smoking, alcohol or drug abuse, maternal body mass index, and method of conception (spontaneous, in vitro fertilization, ovulation induction, or intracytoplasmic sperm injection) were recorded. Gestational age was generally based on first-trimester crown-rump length. In case of late booking the estimated due date was based on second-trimester biometry, in concordance with the national guideline [16].
During the routine fetal anomaly scan, performed by a certified sonographer, all women were offered a cervical length measurement to screen for an increased risk of preterm birth. Women were free to decline this measurement. All women participating in prenatal screening in the Netherlands implicitly give informed consent to collect pregnancy outcomes. Moreover, women undergoing a scan in our center also provide written informed consent to use their data for research. All measurements and pregnancy and delivery characteristics were stored in the ASTRAIA database. The Medical Ethical Committee confirmed that an official approval for this study was not required (W17_449). After extracting data from the ultrasound registry, all data were anonymized. Cervical length measurements were performed by transvaginal ultrasound using a GE Voluson E8 or E10 using a RIC5-9-D 4D endovaginal probe (4–6 MHz).

Women had an empty bladder and were placed in a dorsal lithotomy position. The endovaginal probe was placed in the anterior fornix of the vagina and the cervical canal was visualized from the external ostium (contact point anterior and posterior lip of the cervix) to the internal ostium (were the cervical mucosa ends). A longitudinal view was obtained. The cervix should fill approximately 50%–75% of the image. Excessive pressure by the probe should be avoided. The examination took approximately 3–5 min and afterward the shortest measurement was recorded. The CIC was identified as previously described by Greco et al. [7].

Each cervix was measured in six different ways: single-line, two-line, and trace between the internal and external ostium, with and without CIC. In some women, the CIC was not visible, in this case, the measurements with and without CIC were identical (Fig. 1).

All included women were followed until delivery. Follow-up of the pregnancies was retrieved by questionnaires that were handed out routinely after the 20-week fetal anomaly scan. In addition, we checked the electronic patient charts of the AMC for any missing data. Preterm birth was defined as a delivery before 37 completed weeks of gestation and categorized by spontaneous (spontaneous rupture of the membranes or contractions) or iatrogenic (induction of labor or elective cesarean).

**Statistical Analysis**

Baseline characteristics, details of cervical length measurements, and pregnancy outcomes were presented with summarizing statistics. Continuous variables were presented as means with standard deviations or medians with interquartile ranges, as appropriate. Categorial or dichotomous variables were presented as the number and percentage of the study population.

Differences in baseline characteristics, details of cervical length measurements, and pregnancy outcomes were compared between women with preterm births before 37 weeks and term births using an independent samples t test for normally distributed continuous variables, a Mann-Whitney U test for non-normally distributed continuous variables, and a χ² test for categorial or dichotomous variables. Distribution of the cervical length measurement was assessed by histograms. Positive predictive value was calculated for cervical length below 35, <30, <25, <20, and <15 mm.

All analyses were performed using R version 3.6.1 [17] and IBM SPSS statistics 25 [18]. A p value of <0.05 was considered to indicate statistical significance.

**Results**

From January 1, 2014, to December 31, 2018, a total of 1,709 women with complete follow-up had a cervical length measurement in at least three different ways (one-
line, two-line, and tracing without CIC). After excluding cases with the termination of pregnancy \((n = 2)\) or intra-uterine fetal demise \((n = 9)\), 1,698 women were included for further analysis. In 7 women onset of labor \((\text{spontaneous/iatrogenic})\) was unknown, these women were excluded for the prediction of sPTB. Baseline characteristics are shown in Table 1. Mean maternal age was 32.1 years, median body mass index was 24.2 kg/m\(^2\) and 47.1% of the women were nulliparous. Of the total cohort 6% were smoking, compared to 12.6% in the subgroup of women with a preterm birth.

Median gestational age at delivery was 39+4 weeks of gestation. There were 135 women \((8.0\%)\) who delivered before 37 weeks of gestation and 46 women \((2.7\%)\) before 34 weeks (Table 2).

Figure 2 shows the distribution of the different measurement techniques of the cervical length. When comparing the different methods of cervical length measurement, one-line without CIC resulted in the shortest cervix (mean 41.04 mm; SD 7.1), compared to two-line without CIC (43.29 mm [SD 7.36]), and trace without CIC (mean 44.14 mm; SD 7.60). The trace with CIC resulted in the

| Table 1. Baseline characteristics |
|----------------------------------|
| Characteristics                  | Total cohort \((n = 1,698)\) | Preterm birth <37 weeks \((n = 135)\) | Term birth \((n = 1,563)\) | \(p\) value |
| Age                             | 32.1 (5.4)                      | 32.2 (5.9)                       | 32.1 (5.3)                  | 0.83        |
| BMI \((\text{available for } n = 1,655 \text{ women})\) | 24.2 (21.6–28.0)              | 25.3 (21.2–29.0)*             | 24.2 (21.7–28.0)*          | 0.29        |
| Ethnicity \((\text{available for } n = 884 \text{ women})\) | European 656 (74.2)          | 40/56 (71.4)                    | 616/828 (74.4)             |            |
|                                        | African, Caribbean, Afro-American 94 (10.6) | 8/56 (14.3)                     | 86/828 (10.4)              |            |
|                                        | Hindu, Surinam 70 (7.9)        | 2/56 (3.6)                      | 68/828 (8.2)               |            |
|                                        | Indian, Pakistani, Bangladeshi 41 (4.6) | 3/56 (5.4)                      | 38/828 (4.6)               | 0.23        |
|                                        | Asian: Chinese, Korean, Japanese 7 (0.8) | 0/56 (0)                        | 7/828 (0.8)                |            |
|                                        | Other 16 (1.8)                 | 3/56 (5.4)                      | 13/828 (1.6)               |            |
| Parity \((\text{available for } n = 1,607 \text{ women})\) | Nulliparous 757 (47.1)      | 73/124 (58.9)                   | 684/1,483 (46.1)           |            |
|                                        | Primiparous \(>1 \text{ previous birth}\) 850 (50.1) | 51/124 (41.1)                   | 799/1,483 (53.9)           | 0.009       |
| Smoking                              | 100 (6.0)                      | 17 (12.6)                       | 83/1,534 (5.4)             | 0.002       |
| Gestational age at ultrasound scan   | 19.8 (0.68)                    | 19.8 (0.71)                     | 19.8 (0.68)                | 0.97        |

Data are presented as mean (SD), median (IQR) or \(n/N\) (%). BMI, body mass index; IQR, interquartile range. * Available for \(n = 130\) women in preterm birth group and \(n = 1,525\) women in term birth group.

| Table 2. Pregnancy outcomes |
|-----------------------------|
| Characteristics             | Total cohort \((n = 1,698)\) | Preterm birth <37 weeks \((n = 135)\) | Term birth \((n = 1,567)\) | \(p\) value |
| Gestation at delivery, weeks | 39+4 (38+3 to 40+3)          | 35+3 (33+1 to 36+3)              | 39+5 (38+5 to 40+4)        | <0.001      |
| Preterm birth <34 weeks     | 46 (2.7)                     | 46 (31.4)                       | 0 (0)                      | <0.001      |
| Onset of labor \((\text{available for } n = 1,496 \text{ women})\) | Spontaneous 993 (66.4)     | 78/128 (60.9)                    | 915/1,368 (66.9)           | 0.17        |
|                                        | No labor or induction* 503 (33.6) | 50/128 (39.1)                   | 453/1,368 (33.1)           |            |
| Outcome                     | Alive 1,690 (99.5)            | 127 (94.1)                      | 1,563 (100)                | <0.001      |
| Birthweight, g \((\text{available for } n = 1,617 \text{ women})\) | 3,325 (2,965–3,695) | 2,430 (1,595–2,820)*             | 3,375 (3,035–3,720)*       | <0.001      |

Data are presented as median (IQR) or \(n/N\) (%). * Available for \(n = 130\) women in preterm birth group and \(n = 1,487\) women in term birth group. * Induction of labor or elective cesarean section.
longest measurement (mean 49.18 mm; SD 9.05), followed by two-line with CIC (mean 48.27 mm; SD 8.89) and one-line with CIC (mean 45.82 mm; SD 8.29) (Table 3). The different cut-offs of cervical length (<35, <30, <25, <20, and <25 mm) resulted in a great variety in the incidence of a "short" cervix. For cervical length <30 mm incidence ranged between 4.7% and 2.7%, predictive value ranged between 15.4% and 20.7% (Table 3). For cervical length <25 mm, this incidence ranged between 1.1 (one-line without CIC) and 0.4 (two-line with CIC). The positive predictive value for spontaneous preterm birth varied between 20.0% and 42.9%; however, the number of patients with a preterm birth is limited. For cervical length <15 mm incidence was low.

In Table 4, we listed the studies concerning cervical length and preterm birth. Six out of 13 studies used 1 line without CIC and two out of 13 studies used either one-line or two-line without CIC. The method of measurement was not described in 4 out of 13 studies. The incidence of short cervical length varied widely depending on the population and the cut-off used (ranging from 19.6% <30 mm in a population with previous preterm birth to 0.15% ≤10 mm in a mixed population).

Discussion

Our main finding is that the method of cervical length measurement significantly influences the incidence of short cervical length. In addition, we showed that the positive predictive value of short cervical length for sPTB was dependent on the method of measurement. We report that the method of measurement being used in clinical trials or cohort studies is not always specified.

The cervical length measurements included in this study were all performed by certified sonographers that were trained to detect the isthmic part of the cervix. We recruited a large heterogeneous population of women that visited the antenatal ultrasound department of our hospital. Our hospital is a tertiary referral center for pregnant women and serves as a regional ultrasound center for surrounding midwifery practices.

A limitation of this study that it is a single-center study. Women visiting the AMC for their antenatal checks more often have a complex obstetric or medical history. Moreover, the clinic is located in an urban area where more women with a non-Caucasian ethnicity live. It is known that risk of preterm birth is higher in rural areas and non-
Table 3. Incidence of short cervical length for the different types of measurement and positive predictive value for spontaneous preterm birth <37 weeks (sPTB <37 weeks)

| Type of measurement | Cervical length, mm, (SD) | <35 mm, n (%) | PPV sPTB, (%) | <30 mm, n (%) | PPV sPTB, (%) | <25 mm, n (%) | PPV sPTB, (%) | <15 mm, n (%) | PPV sPTB, (%) |
|---------------------|---------------------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|
| 1 line without isthmus | 41.04 (7.1) | 265 (15.6) | 27 (10.2) | 80 (4.7) | 13 (16.3) | 19 (1.1) | 4 (21.1) | 7 (0.4) | 3 (42.9) |
| 2 line without isthmus | 43.29 (7.36) | 138 (8.1) | 15 (10.9) | 52 (3.1) | 8 (15.4) | 15 (0.9) | 4 (26.7) | 5 (0.3) | 2 (40.0) |
| Trace without isthmus | 44.14 (7.60) | 127 (7.5) | 13 (10.2) | 47 (2.9) | 9 (17.0) | 15 (0.9) | 3 (20.0) | 6 (0.4) | 3 (50.0) |
| 1 line with isthmus | 45.82 (8.29) | 109 (6.4) | 13 (11.9) | 41 (2.4) | 7 (17.1) | 9 (0.5) | 3 (33.3) | 6 (0.4) | 3 (50.0) |
| 2 line with isthmus | 48.27 (8.89) | 63 (3.7) | 9 (14.3) | 33 (1.9) | 6 (18.2) | 7 (0.4) | 3 (42.9) | 5 (0.3) | 2 (40.0) |
| Trace with isthmus | 49.18 (9.05) | 60 (3.5) | 10 (16.7) | 29 (1.7) | 6 (20.7) | 8 (0.5) | 3 (37.5) | 6 (0.4) | 3 (50.0) |

Table 4. Transvaginal cervical length cut-offs, prevalence, and measurement techniques in singleton gestations

| Study | Year | N | Cut-off, mm | Prevalence, % | Population | Country | Method of measurement |
|-------|------|---|-------------|---------------|------------|---------|-----------------------|
| Kuusela et al. [3] | 2015 | 2,122 | ≤30 | 3.40 | Mixed population | Sweden | One-line without CIC |
| van der Ven et al. [4] | 2015 | 6,233 | ≤30 | 1.40 | Multiparous women without previous PTB | The Netherlands | One-line with CIC |
| van der Ven et al. [4] | 2015 | 5,710 | ≤30 | 2.20 | Nulliparous women | The Netherlands | One-line with CIC |
| Owen et al. [30] | 2001 | 183 | <30 | 19.60 | Previous PTB | USA | One-line without CIC or two-line without CIC |
| Thangaraj et al. [13] | 2018 | 173 | ≤30 | 8.70 | Nulliparous women | India | Was not described |
| Grobman et al [5] | 2012 | 15,435 | ≤30 | 10.30 | Nulliparous women | USA | One-line without CIC |
| Wulff et al. [31] | 2018 | 3,334 | ≤25 | 0.80 | Mixed population | Denmark | One-line without CIC |
| Kuusela et al. [3] | 2015 | 2122 | ≤25 | 0.50 | Mixed population | Sweden | One-line without CIC |
| Souza et al. [11] | 2020 | 526 | ≤25 | 3.00 | Nulliparous women | Brazil | Was not described |
| Mistry et al. [10] | 2018 | 147 | ≤25 | 1.30 | Nulliparous women | India | Was not described |
| Esplin et al. [32] | 2017 | 8,771 | ≤25 | 2.45 | Nulliparous women | USA | One-line without CIC |
| Facco and Simhan [2] | 2013 | 467 | ≤20 | 5.0 | Nulliparous women | USA | One-line without CIC |
| Wulff et al. [31] | 2018 | 3,334 | ≤20 | 0.30 | Nulliparous women | USA | One-line without CIC |
| Esplin et al. [32] | 2017 | 8,771 | ≤20 | 1.32 | Nulliparous women | USA | One-line without CIC |
| Facco and Simhan [2] | 2013 | 1,237 | ≤20 | 4.90 | Nulliparous women | USA | One-line without CIC |
| Facco and Simhan [2] | 2013 | 1,284 | ≤20 | 2.20 | Nulliparous women without previous PTB | USA | One-line without CIC |
| Orzechowski et al. [33] | 2014 | 1,569 | <20 | 1.10 | Mixed population | USA | One-line without CIC or two-line without CIC |
| Hassan et al. [12] | 2011 | 32,091 | 10–20 | 2.30 | Mixed population | USA | Was not described |
| Facco and Simhan [2] | 2013 | 467 | ≤15 | 3.40 | Mixed population | USA | One-line without CIC |
| Wulff et al. [31] | 2018 | 3,334 | ≤15 | 0.24 | Nulliparous women | USA | One-line without CIC |
| Facco and Simhan [2] | 2013 | 1,237 | ≤15 | 2.10 | Nulliparous women | USA | One-line without CIC |
| Facco and Simhan [2] | 2013 | 1,284 | ≤15 | 0.90 | Nulliparous women without previous PTB | USA | One-line without CIC |
| Fonseca et al. [1] | 2007 | 24,620 | ≤15 | 1.70 | Mixed population | England, Greece, Brazil, Chile | One-line without CIC |
| Wulff et al. [31] | 2018 | 3,334 | ≤10 | 0.15 | Mixed population | Denmark | One-line without CIC |
Caucasian women are at increased risk for spontaneous preterm birth. This results in a relatively high percentage of overall PTB below 37 weeks (8%) in our cohort compared to percentages ranging between 5.5% and 5.8% in the Netherlands national registry (PERINED) [19, 20].

In our center women with a previous preterm birth between 16 and 34 weeks were offered 17-OH progesterone and serial cervical length measurements (between 14 and 24 weeks of gestation). In women with a previous preterm birth <34 weeks and a short cervix ≤25-mm cervical cerclage is advised and women are invited to participate in the PC-trial where randomization is performed between cervical cerclage and placement of a cervical pessary [21].

Women without previous preterm birth were offered cervical length measurement during the fetal anomaly scan; if the cervical length is ≤35 mm women are invited to participate in the Quadruple p trial [6]. If the cervix is ≤25 mm and a woman declines randomization, vaginal progesterone is offered based on previous evidence [22].

In our study, we did not report the presence of funneling or cervical debris. A previous study reported that the latter was associated with an increased risk of preterm birth in women with a cervical length below 30 mm [23]. Most recent guidelines published by the International Society of Ultrasound in Obstetrics and Gynecology and Fetal Medicine Foundation advise to measure the cervix in a straight line without CIC; however, we are unaware of a study that shows superiority of the straight line without CIC in prediction sPTB in comparison to other methods of measurement.

The absolute differences of cervical length measurement we found in our study population were approximately 8.0 mm between the shortest (one-line without CIC) and longest method (trace with CIC). This is in line with previous studies that show a difference of up to 7 mm in the one-line and two-line measurement [24]. To improve standardization of data, we, therefore, recommend authors to describe the method of cervical length measurement used in any publications. Cervical length measurements and its cut-offs are important for both clinical practice as well as research.

The technique of measurement influences the number of cervical lengths below a certain cut-off. This results in variety in the number of women being targeted as high risk. With a cut-off of <25 mm using the one-line technique without CIC, 19 women in our study population possibly would have been treated as high risk for preterm birth and only 4 delivered premature, in comparison to 7 women in the group of the two-line technique with CIC were 3 women in this group delivered premature.

In this study, the incidence of a very short cervix (<15 and <10 mm) was very low. The mean cervical length was relatively long (43.29 mm [SD 7.36 mm]), compared to for example the study of Iams et al. [25] (35.2 mm, SD 8.3 mm). This was previously described in other studies in the Dutch population [4, 26].

This paper highlights the importance of a large international cohort study to determine the optimal technique for cervical length measurement. It is only after the determination of the best technique that one can determine the optimal cut-off for a “short” cervix, since a different technique of measurement directly correlates with great variety in the number of women marked as “high risk.” The importance of a standardized technique was also underwritten by the recent paper of Gascón et al. [27]. Once the optimal measurement technique and cut-off of the cervix are determined, it is important to give attention to adherence to the guidelines and training of sonographers and residents [14, 15, 28, 29].

**Conclusion**

We evaluated six different techniques of cervical length measurement for the prediction of spontaneous preterm birth below 37 weeks. We have pointed out that the technique that is used does influence the number of women being pointed out as “high risk” for spontaneous preterm birth. These women might be included in trials or prevention programs although it is not clear if we target the right group.

This paper endorses the importance of further studies that evaluate cervical length measurement techniques. It is only thereafter that we can give an advice on how to measure the cervix in a uniform way.

**Statement of Ethics**

Women were free to decline the offered measurement of the cervix. All women participating in prenatal screening in the Netherlands implicitly give informed consent to collect pregnancy outcomes. This is not a written informed consent. However, women undergoing a scan in our center also provide written informed consent to use their data for research. This study has been granted an exemption from requiring ethics approval by the Medical Ethical Committee of the Amsterdam UMC (W17_449).
Conflict of Interest Statement

B.W.M. is supported by an NHMRC Investigator grant (GNT1176437). B.W.M. reports consultancy for ObsEva, Merck KGaA, iGenomix, and Guerbet.

Author Contributions

B.K., E.P., and M.Z. designed the study, E.K. and M.Z. analyzed the data. M.Z. and B.M.K. wrote the first draft of the manuscript. B.K., B.W.M., E.P., and E.K. critically revised the manuscript, B.M.K. and M.Z. finalized the manuscript.

Data Availability Statement

The data that support the findings of this study are not publicly available due to ethical guidelines. Further information will be available from the corresponding author (M.Z.).

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