#### ORIGINAL ARTICLE



# Progression of non-carious cervical lesions: 3D morphological analysis

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#### Abstract

Objectives This longitudinal study aimed to investigate morphologically and quantitatively the progression of non-carious cervical lesions (NCCLs) using a confocal laser scanning microscope (CLSM) and replica models.

Materials and methods The samples examined comprised sets of replicas annually obtained from 83 lesions in 16 participants over 3 to 5 years. All lesions were visually categorized as wedge-shaped, saucer-shaped, or mixed-shaped lesions. CLSM images of the replicas were analyzed in terms of axial depth, occlusogingival width (height) in the buccolingual cross-section, and estimated volume using a custom code of the image analysis software to estimate the progression of the NCCLs over time. The morphological characteristics of the NCCLs were also objectively divided into three groups according to the depth to height ratio (D/H ratio). Fisher's exact test and the Cochran-Armitage trend test were used for statistical analysis.

Results Saucer-shaped lesions progressed mainly in height, whereas wedge-shaped lesions increased both in height and depth. Annual progression in depth and volume significantly increased as the D/H ratio increased. More than half of the NCCLs with a small D/H ratio progressed 50 μm or more in height, whereas none of them progressed more than 50 μm in depth. Annual progression in depth significantly increased as the lesion depth at baseline increased.

Conclusions Progression patterns significantly differed between NCCLs of different shapes. Most NCCLs progressed slowly in depth regardless of their shape. Moreover, NCCLs may progress through active and inactive stages.

Keywords Non-carious cervical lesions  $\cdot$  Longitudinal study  $\cdot$  Progression  $\cdot$  Morphology  $\cdot$  Three-dimensional analysis  $\cdot$  Confocal laser scanning microscope

Clinical relevance Annual monitoring rather than restoration is recommended, especially for shallow NCCLs.

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### Introduction

A non-carious cervical lesion (NCCL) is a hard tissue defect at the cementoenamel junction in the absence of dental caries [[1](#page-7-0)]. Generally, NCCLs are classified into two types according to their morphological features  $[1-6]$  $[1-6]$  $[1-6]$  $[1-6]$  $[1-6]$ . One is a wedged-shaped NCCL with a sharp, V-shaped internal line angle at the deepest portion of the lesion. The other is a saucer-shaped lesion with a rounded C- or U-shaped internal angle. Although earlier studies suspected that these lesion shapes were caused by specific factors [[7](#page-7-0), [8\]](#page-7-0), it has since been commonly accepted that their initiation and progression have a multifactorial etiology  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$  $[1-3, 5, 6, 1]$ [9](#page-7-0)–[12\]](#page-8-0). Unfortunately, the relative contributions of the various etiological factors remain unclear.

A systematic review [\[2](#page-7-0)] reported that the prevalence of NCCL ranged from 0.8 to 85.7%. This wide variation is probably caused by the difficulty in standardizing NCCL diagnosis and classification, and sample characteristics

(e.g., size, age, and location). However, many studies have shown that the prevalence and incidence of NCCLs increase with age [[2,](#page-7-0) [3](#page-7-0)]. Elderly populations with many teeth are increasing; therefore, the opportunity for treatment or management of NCCLs will likely increase to be similar to that of root caries.

NCCLs are often restored because of the need to not only replace lost tooth tissue, but also manage hypersensitivity and poor esthetics, as well as prevent further loss of tooth tissues [\[13](#page-8-0), [14\]](#page-8-0). However, questionnaire-type studies have revealed wide variations in dentists' clinical decisions  $[15–17]$  $[15–17]$  $[15–17]$ . This is probably because there are no generally accepted and specific guidelines regarding when and how these defects should be restored [[13,](#page-8-0) [14\]](#page-8-0). Several long-term clinical trials, which were conducted in university dental hospitals, have shown that current adhesives demonstrate good clinical performance over 5 years [[18](#page-8-0)–[21\]](#page-8-0). In contrast, a questionnaire survey conducted among 337 general practitioners in Brazil found that more than 90% were of the opinion that resin composite restorations would not last for more than 5 years [[22\]](#page-8-0).

Several longitudinal studies have demonstrated the progression of NCCLs over time [[4,](#page-7-0) [8,](#page-7-0) [10,](#page-7-0) [23\]](#page-8-0). The shape and size of NCCLs may play a significant role in their progression. In addition, it is important for dentists' decision-making for NCCL treatment to provide information about the direction and the process of progression. However, these issues were not taken into consideration in those studies that focused on the relationship between lesion progression and etiologic factors. To address this, a pilot study morphologically and quantitatively investigated the progression of NCCLs in 10 sets of NCCL epoxy resin replicas, obtained from 6 out of 26 participants [\[24](#page-8-0)]. A two-dimensional (2D) analysis was conducted using swept-source optical coherence tomography. Although the wedge-shaped lesions appeared to show a greater progression in depth compared to the saucer-shaped ones, a statistical analysis was not performed due to insufficient sample size.

In general, three-dimensional (3D) analysis seems to be more accurate and effective than is 2D analysis. A confocal laser microscope (CLSM) in conjunction with image analysis software makes 3D analysis of replicas possible. Furthermore, measurement with a CLSM may be more accurate than that with optical coherence tomography because of its higher magnification and resolution [\[25](#page-8-0)]. The purpose of this study was to perform a 2D and 3D analysis of the progression of NCCLs using stone casts of the NCCLs. The null hypotheses for this study were that (1) there is no relationship between the shapes and progression patterns (direction, rate, and process) of NCCLs and (2) lesion size has no effect on the progression rate.

#### Materials and methods

# Preparation of NCCL replicas for CLSM image scan

A total of 26 participants with relatively shallow NCCLs were recruited at Nagasaki University Hospital by one of the authors (SK) between 1999 and 2005. The participants were either unaware of the NCCLs or were aware of them but did not want them to be restored as they did not have any symptoms or esthetic complaints. If the participants asked for the NCCL to be restored during the follow-up visit, they were restored immediately. Nevertheless, the specimens obtained until then were used for measurements and analysis. This study was approved by the Ethics Committee of Nagasaki University School of Dentistry (No. 20). The purpose and the research protocol, as well as the expected benefits and possible discomforts, were explained to the participants, and written informed consent was obtained from all participants. After careful removal of debris attached to the tooth surfaces, a silicone impression (Exafine putty type, injection type: GC, Tokyo, Japan) of the NCCLs was taken, and then an improved dental stone (Fuji Rock: GC) was poured into it. Stone casts were obtained every year for up to 5 years for each participant. These were transferred from Nagasaki University to Tokyo Medical and Dental University after the inter-university collaboration was approved by the respective institutional review boards (No. 14070391 for Nagasaki University and No. D2017-059 for Tokyo Medical and Dental University).

Eighty-three sets of casts with clear margins were obtained from 16 participants at baseline and after 3 to 5 years and used for CLSM (VK-X150, KEYENCE, Osaka, Japan) analysis (Fig. [1a](#page-2-0)). Prior to CLSM image scanning, the gingival margins of the casts were trimmed to allow proper visualization of the NCCLs.

#### CLSM image capture of NCCL replicas

The cast was fixed to a mold prepared with a self-curing resin (Ostron, GC) and placed on the stage of the CLSM so that the objective lens could capture the entire image of the NCCL (Fig. [1b](#page-2-0)). Casts prepared from the same participant at each observation timepoint were placed in the same orientation as accurately as possible.

#### Analysis of CLSM images of NCCL replicas

A typical 2D CLSM image of a NCCL is shown in Fig. [1c.](#page-2-0) The CLSM captured the longitudinally sectioned image of the NCCL. The white cross-sectional outline reflects the lesion surface, and the NCCL was characterized in terms of its axial depth (hereafter referred to as "Depth") and occlusogingival width (hereafter referred to as "Height") in the buccolingual cross-section at the mesiodistal center of the lesion width.

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<span id="page-2-0"></span>

Fig. 1 Photograph and schemas of the CLSM system and image capturing. a Overview of CLSM; b NCCL with mold; c CLSM crosssectional image captured at the center of the lesion width. NCCLs were

characterized in terms of Depth and Height. An angle formed by two line segments, ab and ac, was defined as the internal line angle

Maximum mesiodistal width (hereafter referred to as "Width") was also determined in the 3D image obtained using the CLSM (Fig. 2). An image analysis software (Multi File Analyzer, VK-H1XM, KEYENCE) was used to handle the raw data of the CLSM images. In this study, the Depth, Height, Width, and estimated volume (hereafter referred to as "Volume") values were employed as representative data for a single NCCL sample. The progression rates for the four parameters of the lesions were determined by the differences between the dimensions at baseline and end of follow-up. According to the annual progression, lesions were classified into three groups for the sake of convenience: inactive ( $\leq$ 50 μm), moderate (50–100 μm), and active  $(\geq 100 \mu m)$  for 2D analysis; inactive  $(\leq 0.1 \mu m^3)$ , moderate  $(0.1-0.5 \text{ mm}^3)$ , and active  $(\ge 0.5 \text{ mm}^3)$  for 3D analysis.



Fig. 2 Intra-oral photographs of representative NCCL in three different shapes, and their 2D and 3D images obtained using CLSM. Clinical pictures and CLM images are rotated 180 degrees for clarity. a Intraoral view of a saucer-shaped lesion; b 3D CLSM image of the saucershaped lesion at baseline; c 2D CLSM image of the saucer-shaped lesion at baseline; d 3D CLSM image of the saucer-shaped lesion after 4 years; e 2D CLSM image of the saucer-shaped lesion after 4 years; f intra-oral view of a wedge-shaped lesion; g 3D CLSM image of the wedge-shaped

lesion at baseline; h 2D CLSM image of the wedge-shaped lesion at baseline; i 3D CLSM image of the wedge-shaped lesion after 4 years; j 2D CLSM image of the wedge-shaped lesion after 4 years; k intra-oral view of a mixed-shaped lesion; l 3D CLSM image of the mixed-shaped lesion at baseline; m 2D CLSM image of the mixed-shaped lesion at baseline; n 3D CLSM image of the mixed-shaped lesion after 4 years; o 2D CLSM image of the mixed-shaped lesion after 4 years

<span id="page-3-0"></span>The morphological features of the 83 NCCLs were assessed by visual inspection of the stone models and categorized into wedge-shaped and saucer-shaped lesions based on the curvature of the lesion walls and the presence of a clear line angle. Figure [2](#page-2-0) exhibits intra-oral pictures of representative NCCLs in three different shapes, and their 2D and 3D images obtained using the CLSM. NCCLs that did not fit these criteria were classified as mixed-shaped lesions. When direct evaluation was difficult, CLSM cross-sectional images were used to assist the classification. With respect to the wedge-shaped lesions, an internal line angle, which was defined as the vertex angle formed by two line segments (ab and ac), was measured (Fig. [1c\)](#page-2-0). The measurements and classification of the NCCLs were performed by the first author. The morphological characteristics of the NCCLs were also objectively divided into three groups according to the ratio of Depth to Height (D/H ratio): <0.25 (small), 0.25–0.5 (medium), and > 0.5 (large). The D/H ratios were calculated from values obtained at the end of follow-up.

# Statistical analysis

All analyses were conducted using the statistical software JMP® Pro 15 (SAS Institute Inc., Cary, NC, USA). The Kruskal-Wallis and Dunn's post-hoc tests were used for the comparison of baseline dimensions among the three-shaped lesions. The annual progression of the two NCCL shapes was compared with the Fisher's exact test. The 83 NCCLs were divided into three groups using an ordinal scale of small, medium, and large, on the basis of the baseline Height, Depth, and Volume values. The reference values were set such that the numbers of lesions in the three groups were as similar as possible. Subsequently, the Cochran-Armitage trend test was used to analyze the association of annual progression with lesion size. Similarly, the association of annual progression with the D/H ratio was also analyzed. Bonferroni correction was applied to both tests to counteract the problem of multiple comparisons, with adjustment to a significance level of 0.017.

#### Results

The 83 NCCLs were categorized into 23 wedge-shaped, 48 saucer-shaped, and 12 mixed-shaped lesions (Table 1). Eight out of the 16 participants (mean age: 59, age range at baseline: 42–70 years) were male. All but one participant had more than one lesion. Five participants had only one lesion shape; four had saucer-shaped lesions, and one had wedge-shaped lesions. The remaining 10 participants had two or more lesions. The dimensional characteristics of the respective NCCL shapes at baseline are summarized in Table 2. The Kruskal-Wallis and Dunn's post-hoc tests revealed that the saucer-shaped lesions had a significantly smaller mean depth than did the mixedTable 1 Shape distribution of NCCL in each participant



F, female; M, male

Table 2 Baseline lesion dimensions according to the shape of NCCLs

Variable	Lesion shape	Measurements at baseline			
		Mean	SD	Minimum	Maximum
Height (mm)	Saucer	2.15	0.80	0.90	4.60
	Wedge	1.75	0.75	0.40	3.35
	Mixed	2.40	1.15	0.80	4.85
Depth (mm)	Saucer	0.35	0.30	0.00	1.35
	Wedge	0.45	0.30	0.10	1.40
	Mixed	0.60	0.25	0.25	1.25
Width (mm)	Saucer	4.05	1.30	1.40	7.30
	Wedge	4.35	1.80	1.30	9.70
	Mixed	4.80	1.15	1.90	6.05
Volume $\text{(mm)}^3$ )	Saucer	4.0	3.5	0.5	15.5
	Wedge	4.5	2.5	0.5	10.0
	Mixed	6.0	3.0	2.5	11.5
$D/H$ ratio*	Saucer	0.15	0.10	0.00	0.55
	Wedge	0.35	0.30	0.05	1.00
	Mixed	0.35	0.25	0.15	0.80

SD, standard deviation

\*D/H ratio was calculated from values obtained in the last year of the measurement

The Kruskal-Wallis test revealed that the saucer-shaped lesions had a significantly smaller mean depth than did the mixed-shaped lesions

shaped lesions. The mean value of the internal line angles of wedge-shaped lesions was 101° (range, 52°–149°).

There were no significant differences in the progression of Height and Width between the saucer- and wedgeshaped NCCLs (Tables 3 and [4](#page-5-0)). With respect to Depth, the proportion of lesions in the active stage was significantly higher among the wedge-shaped NCCLs than among the saucer-shaped NCCLs (Table [5](#page-5-0)). The saucer-shaped lesions progressed mainly in terms of Height, whereas the wedgeshaped lesions increased in terms of Height and Depth. Most NCCLs showed slow progression in terms of Depth, regardless of their shape.

Regarding the classification of NCCLs according to the D/H ratios, 57 lesions had a D/H ratio less than 0.25 (saucer: 40, wedge: 12, mixed: 5); 15 had a D/H ratio of 0.25– 0.5 (saucer: 6, wedge: 6, mixed: 3); and 11 had a D/H ratio greater than 0.5 (saucer: 2, wedge: 5, mixed: 4). The annual progression in Depth and Volume significantly increased as the scale of the D/H ratio increased (Fig. [3](#page-6-0)). However, no significant relationship was found between the annual progression in Height and the D/H ratio. More than half of NCCLs with a small D/H ratio progressed 50 μm or more in Height, whereas none progressed more than 50 μm in Depth. A very strong positive relationship  $(r = 0.865)$  was found between the ratio of the annual increase in Depth to the annual increase in Height and the D/H ratio (Fig. [4\)](#page-6-0). The annual progression in Depth significantly increased as the scale of Depth at baseline increased (Fig. [5](#page-7-0)). Although the proportion of inactive lesions with volume loss appeared to be significantly greater when the Volume at baseline was small, the Cochran-Armitage trend test did not show a significant association between lesion size and progression in Volume.

### **Discussion**

Shape classification by visual inspection is subjective, but it has the advantage that it can be immediately carried out based on the presence of the internal line angle and the curvature of the lesion walls. In contrast, the D/H ratio can objectively express the morphological features of

"relatively shallow and wide" or "narrow and deep" lesions, although it cannot depict the curved surface of the defect walls or the whole image of the defect. Furthermore, since the D/H ratio is a continuous variable, it is possible to closely examine the relationship between the NCCL shapes and progression patterns, as shown in Fig. [4](#page-6-0). NCCLs with D/H ratios greater than 0.5 always have an internal line angle of 90° or less.

Although the incidence of wedge-shaped and saucershaped lesions show a wide range of distribution, previous studies have reported that wedge-shaped lesions generally occur more often than do saucer-shaped lesions [[11](#page-8-0), [26,](#page-8-0) [27\]](#page-8-0). In this study, however, the percentage of wedgeshaped lesions was about half of that of saucer-shaped lesions. This may be due to differences in criteria for diagnosis and classification, such as participants' age and location. There also seems to be a selection bias since two-thirds of the NCCLs involved in this study were relatively shallow (less than 0.5 mm) defects that did not need to be restored. In particular, shallow saucer-shaped lesions are not likely to be detected by visual inspection and an examination with a dental explorer in an epidemiological study. The dimensional characteristics of the respective shapes summarized in Table [2](#page-3-0) are supported by other studies in which saucershaped lesions had a greater mean height and width and a smaller mean depth than did wedge-shaped lesions [\[11\]](#page-8-0). In contrast, Hur et al. reported that wedge-shaped lesions had a greater mean height and depth than did saucer-shaped lesions [\[1](#page-7-0)]. From the viewpoint of the D/H ratio, the mean value (0.36) of the wedge-shaped lesions seems to be comparable to the ratio (0.34) estimated from another study [\[11\]](#page-8-0). In addition, the internal line angles of wedge-shaped lesions ranged from 52° to 149° (mean: 101°). This suggests that the presence of an apparent line angle is more critical for shape classification by visual inspection than is the degree of the internal line angle.

There were significant differences in the progression patterns between the shapes of NCCLs. The saucer-shaped lesions progressed mainly in height (the occlusogingival direction), whereas the wedge-shaped lesions increased both in height and depth (the buccolingual direction). In addition, there was a very strong positive relationship between the ratio

Table 3 Annual progression in Height according to the shape of NCCLs



Fisher's exact test with Bonferroni correction revealed no significant differences according to the shape of NCCLs

<span id="page-5-0"></span>Table 4 Annual progression in Width according to the shape of NCCLs



Fisher's exact test with Bonferroni correction revealed no significant differences according to the shape of NCCLs

of the annual increase in Depth to the annual increase in Height and the D/H ratio. According to the dimensional characteristics associated with the NCCL shapes described above, these progression patterns can be expected, since the NCCL shapes reflect the directions and rates of progression. There is also no doubt that etiological factors cause various shapes and affect progression patterns. NCCL is a multifactorial disease involving erosion, abrasion, and abfraction [\[2](#page-7-0), [28](#page-8-0)]. This is supported by the fact that more than 60% of the participants in this study had NCCLs with two or more different shapes and progression patterns (Table [1](#page-3-0)). However, the proportions of the etiological factors for respective NCCLS are still unclear, as reported in systematic reviews [\[2](#page-7-0), [28](#page-8-0)].

A preliminary study was performed to evaluate the precision of this measurement method using the replicas measured in our previous study [\[24\]](#page-8-0), and it demonstrated no significant differences in Height and Depth values between the two studies [[29\]](#page-8-0). The criteria for the activity of progression were based on the results of our pilot study [[24](#page-8-0)]. NCCLs in various stages of progression were involved in this study, and approximately two-thirds were in the inactive stage. NCCLs may progress through active and inactive stages, as seen in a previous study in which progression rates during the first 3 years were significantly lower than those during the subsequent 3 years [[10\]](#page-7-0).

Height at baseline was categorized on an ordinal scale  $\left($ <1.5 mm, 1.5–2.5 mm, and > 2.5 mm) to evenly divide the 83 NCCLs into three groups. Likewise, Depth at baseline was classified on an ordinal scale  $(<0.25$  mm, 0.25 to 0.5 mm, and >0.5 mm). The annual progression in Depth significantly increased as the Depth scale at baseline increased. This is likely because the saucer-shaped lesions, which showed significantly less progression rate in Depth, accounted for 80% of the small Depth scale of  $< 0.25$  mm. A few studies have previously investigated progression in Depth in NCCLs, which seems to be the most critical issue in clinical practice [[8,](#page-7-0) [10\]](#page-7-0). Unfortunately, their results do not serve as useful references due to their small sample sizes and three-grade evaluations with a scale of 1 mm. Regarding the progression in Height, although there has been no direct information, a change in the mean values of longitudinally sectioned abfraction areas from baseline has been reported [[4](#page-7-0)]. The reported increase in abfraction area of  $0.14 \text{ mm}^2$  after 18 months is similar to our result  $(0.11 \text{ mm}^2/\text{year})$ .

NCCL progression rate may be more accurately evaluated by 3D measurement (volumetric change) of the entire NCCL than 2D cross-sectional measurements (height, depth, and/or area). Technological advances have enabled precise 3D analysis; however, studies on volumetric lesion progression are lacking. The mean annual volume loss in this study was  $0.36$  mm<sup>3</sup>, which was approximately onequarter of the NCCL progression rate  $(1.50 \text{ mm}^3)$  reported by Sawlani et al. [[23](#page-8-0)]. In their study, NCCLs with a depth of 1–2 mm were investigated, whereas the mean depth of NCCLs in this study was 0.42 mm. This large discrepancy may be a reason for the difference in annual progression rates. Contrary to our expectations, lesion size did not significantly influence the annual progression in Volume, probably due to the small size of the NCCLs at baseline (mean size at baseline: 4.48 mm<sup>3</sup>). Alternatively, another explanation is that half of the NCCLs showed little (less than  $0.2 \text{ mm}^3$ ) volume loss.

The small sample size of this study and the presence of multiple NCCLs in a single participant as well as a participant factor may limit the accuracy of the analysis. Nevertheless,

Table 5 Annual progression in Depth according to the shape of NCCLs



Fisher's exact test with Bonferroni correction revealed significant differences in the number of inactive and active lesions between saucer-shaped and wedge-shaped lesions

<span id="page-6-0"></span>

Fig. 3 Annual progression in Height, Depth, and Volume by the D/H ratio. The D/H ratios were divided into three groups on an ordinal scale:  $\leq 0.25$ , 0.25 to 0.5, and  $> 0.5$ . The annual volume progression at baseline was also categorized on an ordinal scale:  $< 0.1$  mm<sup>3</sup>, 0.1 to 0.5 mm<sup>3</sup>, and

> 0.5 mm<sup>3</sup>. The Cochran-Armitage trend test revealed that the annual progression in Depth and Volume significantly increased as the D/H ratio increased  $(p < 0.017)$ 

according to the results of this study, the null hypothesis that there is no relationship between shapes and progression patterns of NCCLs was rejected. The other hypothesis that lesion size has no influence on the progression rates was partially accepted. Although CLSM is a useful tool for 3D morphological analysis of specimens in the laboratory, it cannot be used in clinical sites. One of the authors (SK) has followed up resin composite restorations in NCCLs for more than 12 years. Half of the newly developed non-carious lesions adjacent to the restorations progressed to be clinically unacceptable within a few years after detection, and the other half were almost arrested [\[30](#page-8-0)]. NCCLs in the moderate and active stages should be investigated further to determine the duration of the active stage, and progression rates should be measured at 1-year intervals. A clinical study using digital dentistry, such as computer-aided design/computer-aided manufacturing systems, which have been developing rapidly, is also required to confirm the findings of the present study. In addition, questionnaires on potential etiologic factors, such as dietary, brushing and lifestyle habits, and bruxing and parafunctional activities were simultaneously administered to the participants. The relationship between progression and the above-mentioned etiological factors will be investigated in the future.

Fig. 4 Ratios of annual increase in Depth to that in Height by D/H ratio. Scatter plot of ratios of annual increase in Depth to annual increase in Height by D/H ratio. Taking measurement error into consideration, NCCLs were excluded from the analysis when the annual progression showed less than 1% of their size at baseline. A very strong positive relationship was found between the ratio of annual increase in Depth to that in Height and the D/H ratio (Pearson's correlation coefficient  $[r] = 0.865$ 



<span id="page-7-0"></span>

Fig. 5 Annual progression in Height, Depth, and Volume by baseline size. Volume at baseline was categorized on an ordinal scale:  $\leq$ 3.0 mm<sup>3</sup>, 3.0 to 5.0 mm<sup>3</sup>, and  $>$  5.0 mm<sup>3</sup>. The Cochran-Armitage trend

## Conclusion

Within the limitations of this study, the following conclusions were drawn:

- 1. Wedge-shaped NCCLs progressed in depth and/or height, whereas the saucer-shaped NCCLs mainly progressed in height.
- 2. There were active and inactive stages in the progression, and most NCCLs progressed slowly in depth, regardless of their shape.
- 3. The annual progression in depth and volume significantly increased as the D/H ratio increased.
- 4. The annual progression in depth significantly increased as the lesion depth at baseline increased.

Data and code availability Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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### **Declarations**

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by Nagasaki University (No. 14070391) and Tokyo Medical and Dental University (No. D2017-059).

Consent to participate Informed consent was obtained from all individual participants included in the study.

test revealed that the annual progression in Depth significantly increased as the lesion depth at baseline increased  $(p < 0.017)$ 

Consent for publication Participants signed informed consent regarding publishing their data and photographs.

Conflict of interest The authors declare no competing interests.

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