Association between the point-rating system used for oral health and the prevalence of pneumonia-causing bacteria in malnourished patients

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Abstract. Despite the importance of oral care in the prevention of aspiration pneumonia, the association between oral hygiene and the prevalence of pneumonia-causing bacteria has not yet been determined. The present study is a cross-sectional study aimed at determining the association between the original point-rating system used during oral examinations (the prompt non-invasive oral assessment) and the prevalence of pneumonia-causing bacteria in a population of hospitalized patients with malnutrition. The nutrition support team cared for 61 patients; 6 were excluded as they were not eligible. Bedside analyses were conducted using the point-rating system. The findings were analyzed to determine the association between the prompt non-invasive oral assessment and the detection of pneumonia-causing bacteria. Patients who tested positive for pneumonia-causing bacteria (n=13) received significantly higher total and hygiene item scores than those who tested negative (n=42) [median (25th, 75th percentile), total score, 6 (4, 7) vs. 3 (1, 5), P=0.02; hygiene score, 2 (1, 3) vs. 1 (0, 2), P=0.02]. In the receiver operating characteristic analysis, a total oral assessment cut-off score of 4 was identified as optimal for detecting pneumonia-causing bacteria. Additionally, a multivariable analysis revealed a high odds ratio for the presence of pneumonia-causing bacteria in patients with poor oral hygiene (odds ratio, 2.09; 95% CI, 1.04 to 4.22). Thus, the present study demonstrates that the prompt non-invasive oral assessment is a simple and effective tool for detecting pneumonia-causing bacteria in hospitalized patients.

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Abbreviations: NST, nutrition support team; AP, aspiration pneumonia; BMI, body mass index; ROC, receiver operating characteristic

Key words: assessment, cross-sectional study, nutrition, oral hygiene, pneumonia

Introduction

Oral bacteria have been associated not only with dental and periodontal diseases, but also with serious systemic diseases (1,2). Oral hygiene is essential, particularly for hospitalized patients who are undernourished. This is due to the fact that in immunosuppressed patients, poor oral hygiene is more likely to result in aspiration pneumonia (AP), which is caused by the aspiration of foreign materials into the bronchial airway (3-8). AP is among the most common causes of mortality among elderly Japanese individuals and nursing homes residents, and is most often caused by the pathogenic bacterium, *Streptococcus pneumoniae* (9). Previous studies have found that the quantity of aspirated bacteria is a major factor in the development of pneumonia (4,10). Moreover, in high-risk patients, such as those who are bedridden, have dysphagia or other eating disorders, and/or are immunosuppressed, the oral cavity may comprise a reservoir of pathogens that potentially cause AP.

Oral hygiene, including professional oral care, reduces bacterial levels in the oral cavity (9,11). In the elderly, daily oral care has been shown to decrease the frequency of fever and pneumonia-related mortality (8). Oral health assessment, management and care are of particular importance for groups of patients that require nutritional support team (NST) intervention, including those with head and neck cancer, and those who are malnourished, are older and are frail (12,13). Manabe *et al* reported that sputum suctioning, the deterioration of swallowing function, dehydration and dementia were

risk factors for AP in older adults; however, they did not evaluate the effects of oral assessment on the risk of AP (14).

Despite strong literature-based evidence highlighting the role of oral health in the prevention of AP, no published study to date, to the best of our knowledge, has investigated the association between the point-rating systems used during oral health assessments and the detection of pneumonia-causing bacteria. Therefore, the present study aimed to investigate a novel oral assessment system that is simpler than existing versions. To this end, a cross-sectional study was conducted to identify the association between oral health examination based on an original point-rating system and the detection of pneumonia-causing bacteria.

Patients and methods

Study population. A total of 61 patients requiring NST intervention who were hospitalized at Kanazawa University Hospital between January 1, 2013, and June 30, 2013, were possible candidates for the present study. Data on sex, body mass index (BMI), and hemoglobin, albumin and transthyretin levels were obtained from each patient. In total, 6 patients were excluded if they were too ill to allow the collection of the required data. The present study was performed in accordance with the Declaration of Helsinki with the approval of the both of the Institutional Review Board of Kanazawa University Hospital (approval no. 2441-2) and Yamanashi University Hospital (approval no. 1746). A trial registration number was also obtained (UMIN000030913). Informed consent was obtained from all study participants. Patients were free to withdraw from the study at any time.

The NST intervened in the nutritional management of each patient in response to a request by the attending physician and in the event that albumin levels decreased to ≤3.0 g/dl. The NST held weekly clinical conferences aimed at providing better nutritional supplies and care to patients with nutrition deficiency and at ensuring the administration of necessary clinical examinations and treatments to improve patients' overall nutritional conditions.

Oral status evaluation, and blood and sputum test data collection. The body heights and weights, BMIs and blood test results of the enrolled patients were recorded at the onset of the NST intervention. The participants underwent routine blood and sputum culture testing together with a bedside oral assessment conducted by a dentist. Sample and oral assessment findings were collected within the first or second week following the onset of NST intervention. Additionally, blood cultures (two sets, aerobic and anaerobic) and sputum cultures were drawn from patients each time pneumonia was suspected, based on clinical symptoms such as fever, cough, sputum production, dyspnea, chest pain, or the appearance of new infiltrates on chest radiography images during hospitalization. The causative bacteria were estimated from the results of sputum cultures and blood cultures. Data used to detect pneumonia-causing bacteria were tracked and updated for all patients.

Oral health assessments were performed by a single dentist who was blinded to the results of the laboratory tests, including the pneumonia-causing bacterial culture tests, and who evaluated and recorded the prompt non-invasive oral assessment scores using our original oral health assessment panel (Fig. S1). Eilers' Oral Assessment Guide is among the most well-known oral health assessment methods and is used frequently at other institutions (15,16). However, this system requires the assessment of multiple components, rendering it more time-consuming, and as such, it was found to be too complicated and intolerable for patients in poor general conditions. Moreover, ward rounds often did not provide sufficient time for the NST bedside evaluations of several components. Therefore, the present study devised a simple oral health assessment method that was developed from other available systems including Eilers' Oral Assessment Guide and the revised oral assessment guide (17). In the Eilers' Oral Assessment, 4 evaluation items were considered to be important: Hygiene, xerostomia, mucositis and occlusion (18-23). The 4 evaluation items were each scored as follows: 0, excellent; 1, slight dysfunction; 2, moderate dysfunction; and 3, severe dysfunction. For example, a score of 0 points for 'hygiene' indicated a clean oral cavity without plaque, whereas a score of 3 points indicated a highly insanitary oral cavity with large amounts of plaque and food residues. The classifications of hygiene, xerostomia and mucositis were according to the Oral Health Assessment Tool and the Eilers' Oral Assessment Guide (15,24,25). Occlusions were defined usingEichner's classification (26,27). The total scores were defined as the sum of individual item scores, and ranged from 0 (best oral health) to 12 points (worst oral health). The scores were then used by the NST for patient assessment.

The following 8 species that have been strongly associated with the incidence of pneumonia were targeted (4,28-31): Methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Escherichia coli*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. For sputum culture, the patient was initially instructed to gargle with water several times, after which sputum was collected into a sterile Petri dish to avoid contaminating the saliva and dental plaque. Moreover, to avoid collecting sputum samples contaminated with saliva and upper respiratory secretions, the Geckler's classification of macroscopic sputum findings and the Miller & Jones' classification of microscopic findings were employed to exclude unsuitable specimens (32,33). Per the Miller classification, M1 class sputum that is viscous and consists mostly of saliva was excluded (33). Furthermore, good quality sputum specimens were selected as per the Geckler classification (classes 3, 4 and 5) (32,33). The sample was delivered to the laboratory immediately after collection and subjected to Gram staining and isolation as per standard techniques. Three types of agar plates were used: Bromothymol blue lactose, sheep blood, and chocolate. The globally adopted BacT/ALERT® microbial detection system (bioMérieux) for blood culturing was used (34). Blood culture bottles were placed in a BacT/ALERT® instrument upon arrival at the laboratory and incubated at 37˚C for 7 days (34). Upon the detection of growth, Gram staining and sub-culturing were performed on the 3 aforementioned types of agar plates using standard techniques. Bacterial identification was conducted 24-48 h following sub-culture (34). Positive bacterial culture findings were confirmed by the presence of colonies on the

Table I. Demographic and clinical data of the patients.

Variable	Detection group (n=13) (mean ± SD, range)	Non-detection group (n=42) (mean ± SD, range)	P-value
Sex [male, n (%)]	7 (54%)	23 (55%)	0.95
Age (years)	63.5±17.1 (29,92)	60.9±17.4 (17,90)	0.63
Body mass index (kg/m^2)	18.9±4.2 (13.5,25.0)	19.7±4.4 (10.4,28.1)	0.48
Hemoglobin (g/dl)	10.1±2.4 (7.2,14.1)	10.4±2.0 (6.9,13.7)	0.59
C-reactive protein (mg/dl)	1.8±2.4 (0.2,7.8)	3.6±4.9 (0,17.9)	0.79
Albumin (g/dl)	2.7±0.7 (1.7,3.8)	3.2±1.1 (1.5,7.4)	0.14
Transthyretin (g/dl)	16.8±8.3 (3.0,31.0)	18.6±9.9 (2.0,37.0)	0.61

Continuous variables are expressed as the means (standard deviation, interquartile range). Categorical variables are expressed as numbers.

agar plates. A neutral third party at the Clinical Microbiology Laboratory at Kanazawa University Hospital performed the bacterial analyses while blinded to the oral health assessment data. Statistical analyses were performed by professionals blinded to both the oral assessment and laboratory data. These procedures ensured a blinded, cross-sectional study.

Statistical analyses. Welch's t-test was used to determine significant differences in clinical parameters between the detection and non-detection groups. The Kolmogorov-Smirnov test was used to confirm that the distribution of each clinical parameter in the detection and non-detection groups was parametric. Fisher's exact test was used to determine significant differences in prompt non-invasive oral assessment scores between the groups in terms of each of the 4 components. Differences in categorical variables were assessed using the Chi-squared test. The associations were expressed as P-values and odds ratios with 95% confidence intervals (CIs). In the multivariable analysis, logistic regression was applied to adjust for potential confounding factors. Receiver operating characteristic (ROC) analysis was used to determine the cut-off point for separating the detection and non-detection groups. All statistical analyses were performed using the SPSS 23 software (SPSS, Inc.). Differences were considered statistically significant at a P-value <0.05.

Results

In total, 61 patients were candidates for the present study. Among these, 6 patients were excluded; specifically, 3 of these 6 patients refused bedside oral assessments due to mental diseases, such as major depression and severe anorexia, 1 patient was unable to complete a sputum test due to a severe breathing disorder, and the remaining 2 patients had undergone total gastrectomy and hepatic transplantation; however, they had not recovered sufficiently in order to be assessed for a dentist's examination. Therefore, the prompt non-invasive oral assessment was applied to 55 patients who were hospitalized for the following reasons: Hematological disease (mainly leukemia), 17 patients who received oral care and management in the department of oral and maxillofacial surgery at the time of admission; pneumonic disease (mainly lung cancer), 14 patients; psychiatric diseases, 8 patients; cardiovascular disease, 5 patients; gastroenterological disease, 3 patients (2 with hepatitis C and 1 with Crohn's disease); skin disease, 3 patients; laryngeal cancer: 2 patients; uterine cancer: 1 patient; spinal tumors, 1 patient; and upper arm sarcoma, 1 patient.

Patients from whom at least 1 of the aforementioned 8 bacterial species were cultured comprised the 'detection group' (n=13), while the remainder comprised the 'non-detection group' (n=42). No significant inter-group differences were observed with respect to the blood test results or demographic data (e.g., age, sex and BMI) (Table I). The sputum-cultured pneumonia-causing bacteria in the detection group are shown in Table SI. Notably, *E. coli* and *H. influenzae* were not detected in any of the patients. The number of patients with only Gram-negative bacilli (patient nos. 20, 26, 27, 36, 39 and 41) was higher than that of patients with only Gram-positive cocci (patient nos. 44, 45 and 50). The group in which only Gram-negative bacilli (n=6) were detected had lower albumin levels than the group in which only Gram-positive cocci (n=3) were detected (mean serum albumin levels were 2.37 and 3.58 g/dl, respectively) (Table SI).

A blood culture test was performed on 33 (60%) patients. Among these, pneumonia-causing bacteria were detected in 4 (12%) patients, and the bacteria were the same as those found in the sputum (data not shown).

The total prompt non-invasive oral assessment scores were significantly higher in the detection group than in the non-detection group [median (25th, 75th percentile), total score, 6 (4, 7) vs. 3 (1, 5), P=0.02; Table II]. Similarly, the hygiene item scores were significantly higher in the detection group [median (25th, 75th percentile), hygiene score, 2 (1, 3) vs. 1 (0, 2), P=0.02; Table II]. By contrast, the xerostomia, mucositis and occlusion scores did not significantly differ between the detection and non-detection groups [median (25th, 75th percentile), xerostomia score, 1 (0, 2) vs. 1 (0, 2), P=0.49; Table II]; mucositis [0 (0, 1) vs. 0 (0, 0), P=0.18] and occlusion [2 (1, 3) vs. 1 (0, 2), P=0.06] (Table II). Mucositis consistently received the lowest scores among the 4 components.

When the participants were divided into a good hygiene group (category 0, clean; and 1, slight local debris; n=36) and the poor hygiene group (category 2, moderate local debris; and 3, general debris; n=19), according to the departments in which they had been hospitalized, hematology inpatients

Table II. Score of each category between the detection and non-detection group.

Score of each category (range)	Detection group (n=13) Median (25th, 75th percentile)	Non-detection group (n=42) Median (25th, 75th percentile)	P-value
Total point (0-12)	6 (4,7)	3 (1,5)	0.02[a]
Hygiene (0-3)	2 (1,3)	1 (0,2)	0.02[a]
Xerostomia (0-3)	1 (0,2)	1 (0,2)	0.49
Mucositis (0-3)	0 (0,1)	0 (0,0)	0.18
Occlusion (0-3)	2 (1,3)	1 (0,2)	0.06

[a]P<0.05.

Table III. Association between prevalence of pneumonia-causing bacteria and clinical variables.

	Pneumonia-causing bacteria			
Variables	Positive (n=13) (%)	Negative (n=42) (%)	Total	P-value
Age (years)				
65≥	6 (26.1)	17 (73.9)	23	0.72
<65	7 (21.9)	25 (78.1)	32	
Sex				
Male	7 (23.3)	23 (76.7)	30	0.95
Female	6 (24)	19 (76)	25	
Hospital department				
Hematology	1 (5.9)	16 (94.1)	17	0.038[a]
Other	12 (31.6)	26 (68.4)	38	
Hygiene				
Clean (category 0)	2 (10)	20 (90)	22	0.048[a]
Slight local debris (category1)	4 (29)	10 (71)	14	
Moderate local debris (category2)	2 (20)	8 (80)	10	
General debris (category 3)	5 (56)	4 (44)	9	

Data are expressed as numbers (%). [a]P<0.05.

had a significantly higher ratio of good cases than the other departments, as demonstrated by the following data. Of the 17 hematology department inpatients, 15 (88%) were good hygiene cases. By contrast, of the 38 inpatients of other departments, 21 (55%) were good hygiene cases (P=0.018). The pneumonia-causing bacteria detection rate was significantly higher among patients hospitalized in departments other than hematology (P=0.038) and among those with poor hygiene (P=0.048) (Table III). Additionally, a multivariable analysis revealed a high odds ratio for the presence of pneumonia-causing bacteria only in patients with poor hygiene (odds ratio, 2.09; 95% CI, 1.04 to 4.22) (Table IV).

As shown in Fig. 1, a cut-off point of >3.5 corresponded to the highest numerical Youden index value (130.7). As scores were calculated as whole numbers (0-12), the clinical cut-off point was set at 4. This value exhibited a high sensitivity and high negative predictive value (77 and 89%, respectively), with a relatively low specificity and low positive predictive value (55 and 35%, respectively). Finally, ROC analysis validated the values of ≥4 and <4 as discriminatory of patients with and without pneumonia-causing bacteria, respectively.

Discussion

A previous systematic review demonstrated that good oral health care could reduce the incidence of pneumonia by 40% and the associated mortality rate by 10% (35). Additionally, a previous study found that oral health care reduced the incidence of ventilator-associated pneumonia in patients on ventilatory support (36). Moreover, according to the study by Senpuku *et al*, attention to oral hygiene and professional care that include elimination of pneumonia-causing bacteria and fungi (such as *Pseudomonas* spp. and *Candida albicans*) could diminish the risk of developing systemic diseases (37). Recently, it was reported that in mechanically ventilated patients, bacterial species may migrate rapidly from the mouth and upper airways, and this can contribute to the pathogenesis of pneumonia (4). Taken together, oral cleaning is considered an

Table IV. Risk of detection of pneumonia-causing bacteria by hospital departments or the status of oral hygiene.

Parameter	Unadjusted odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)[a]	P-value
Hospital department				
Hematology	1.70 (0.085-33.91)	0.73	2.01 (0.09-45.24)	0.66
Other	1.0 (reference)		1.0 (reference)	
Oral hygiene status				
Poor (category 2 or 3)	2.09 (1.04-4.22)	0.038[b]	2.90 (1.23-6.83)	0.015[b]
Good (category 0 or 1)	1.0 (reference)		1.0 (reference)	

CI, confidence interval. [a]Adjusted for age and sex. [b]P<0.05.

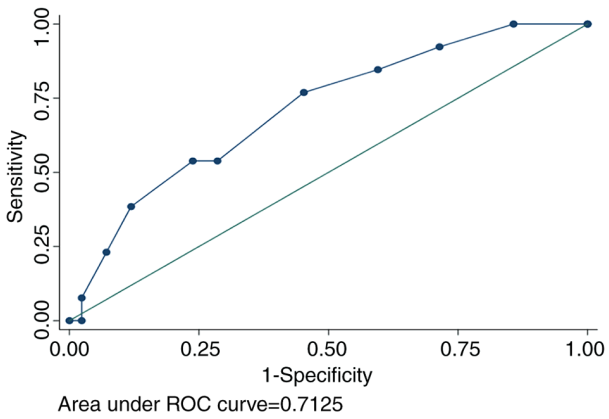

Figure 1. Receiver operating characteristic (ROC) analysis suggested that the prompt non-invasive oral assessment score of ≥4 patients most classified as being at risk of having pneumonia-causing bacteria, with a sensitivity of 77% and a specificity of 55%. The area under the ROC curve was 0.71, indicating moderate accuracy. The cut-off score with the highest Youden index (130.7) was >3.5.

effective tool for the suppression and prevention of AP (38,39). The findings of the present study are consistent with those of these above-mentioned studies.

El-Solh *et al* (40) reported that among 67 patients with AP, bronchial sampling indicated that Gram-negative bacilli were the most predominant organisms (49%), followed by anaerobes (16%) and *S. aureus* (12%). In the present study, Gram-negative bacilli were predominantly detected over Gram-positive cocci, as in previous studies. Pathogenic bacteria, including Gram-negative species, which are not observed in the oral cavity of the normal host, may emerge in hospitalized patients (41). The cleavage of cell-surface fibronectin exposes receptors for Gram-negative rods on underlying airway epithelial cells and is closely associated with host factors, such as acute illness (42). Furthermore, a number of studies have already demonstrated that undernourishment increases the risk of developing AP (43-45). It is reasonable to hypothesize that undernutrition and poor oral hygiene cause inflammation and damage to the airway mucosa, resulting in a high detection rate of Gram-negative bacilli. In fact, the group in which only Gram-negative bacilli were detected in the present study had a poor oral hygiene condition and had significantly lower albumin levels than the group in which only gram-positive cocci were detected (Table SI).

The present study demonstrated that the oral cleaning state differed, depending on the department of hospitalization and that hematology inpatients had a high cleaning ability. This finding may be attributed to the fact that hematology inpatients are routinely referred to the department of oral and maxillofacial surgery at the time of hospitalization for the purpose of preventing severe odontogenic infections that may have occurred when the immune system was weakened by high-dose cancer chemotherapy. Moreover, patients who are hospitalized for pulmonary diseases are likely to have unclean oral cavities due to the long-term use of endotracheal intubation and ventilation through the oral cavity. In addition, oral hygiene management may be difficult for otolaryngology inpatients as the oral cavity is close to or included in the surgical wound.

Adequate salivary flow may prevent the accumulation of pneumonia-causing bacteria, and can thus maintain oral health while regulating the oral microbiome. However, the xerostomia scores did not significantly differ between the detection and non-detection groups in the present study. The standardization of non-stimulated saliva collection and the time of its collection should be performed in future research so that the evaluation of xerostomia detection is not based solely on visual findings, which are likely to be inaccurate (46,47). The detection group in the present study tended to receive worse occlusion scores than the non-detection group, although this difference was not significant. To evaluate chewing ability, laborious tests, such as checking the rate of disintegration of items, such as gummy jelly are required. However, the aim of the present study was to promote a non-invasive and relatively prompt examination. Therefore, the present study evaluated chewing ability based on the Eichner index of occlusion, which indicates a significant association with masticatory functions, such as maximum biting force and occlusal contact area in elderly Japanese individuals (26,27). Flores-Orozco *et al* reported that being underweight and having a low body fat percentage were associated with a long chewing cycle duration and masticatory lateral asymmetry even in young adults with natural dentition (48), suggesting that chewing and swallowing may be decreased in malnourished patients. Similarly, it has been reported that tooth loss is associated with dysfunctional mastication, a risk of aspiration and increased malnutrition, including diabetes (49-51). Furthermore, Furuta *et al* reported

that having a low number of teeth was associated with dysphagia, whereas wearing dentures contributed to a recovery of swallowing functions (22). Therefore, an unfavorable occlusal status may eventually lead to AP due to the aspiration of bacterially contaminated saliva and food under unsanitary conditions.

In the present study, ROC analysis revealed that a total oral assessment cut-off score of 4 was useful for distinguishing between the detection and non-detection groups. As this cut-off score exhibited both a moderate sensitivity and high negative predictive value (77 and 89%, respectively), the prompt non-invasive oral assessment may be clinically useful for screening patients at risk of developing AP. By contrast, the low specificity and positive predictive value of this cut-off (55 and 35%, respectively) may lead to an increase in the number of false-positive cases. The reason for the large number of false-positive cases may be that some patients did not brush after eating before the oral evaluation. Therefore, oral hygiene may have been rated worse than normal oral hygiene at the time of the oral assessment.

Further research, including a time-series database, is required to determine the associations of blood and sputum culture results with oral status. Although the present study carefully conducted sputum sampling to reduce the risk of oral bacterial contamination, the possibility that oral bacterial contamination could lead to inaccurate sputum culture results should be considered. Moreover, molecular biology techniques, including the metabolomic profiling of saliva should be adopted to detect and quantify pathogens, as it was recently reported that such methods can detect a higher proportion of streptococcal infections in the oral cavity than bronchoalveolar lavage fluid culturing (52,53). Importantly, the evaluation method used herein can be easily implemented and evaluated by nurses, who should be trained in these evaluation methods, and help improve their reliability. Involving nurses would further enhance the utility of the prompt non-invasive oral assessment used herein as a universal clinical and research tool for patients in a poor condition, particularly as this tool may be more practical than traditional oral assessment systems (54-56).

One strength of the present study was that the data management and analysis were performed by an independent biostatistician to exclude evaluator subjectivity. A limitation of the present study is that it was conducted at a single facility, and thereby the findings were subject to possible selection bias as a specific group of patients with malnutrition who had been admitted to a tertiary medical care center were targeted. To ensure inter-observer reliability, future studies should consider the validity of multiple examiners. Furthermore, the small sample size may have resulted in bias, and therefore may have reduced generalizability. A large-scale, prospective cohort study is required to confirm the present findings and validate the prompt non-invasive oral assessment. This may enable the prompt detection of pneumonia-causing bacteria using the system used herein on a broader scale, and facilitate better and more timely treatment.

In conclusion, the present study demonstrated an association between the original point-rating-based oral examination system and the detection of pneumonia-causing bacteria in patients with poor nutrition. Notably, hygiene was the only assessment component associated with the detection of pneumonia-causing bacteria.

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Availability of data and materials

The data used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

KY designed the study, and drafted and wrote the manuscript. TF participated in the design of the study and in the collection of the data. SK participated in the design of the study and its conception. TaT and ToT contributed to interpretation of the findings of the study. HY participated in the design of the study and performed the statistical analyses. AM, ToT and KY corrected the manuscript. AM and KU supervised the interpretation of the data and the drafting of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the Declaration of Helsinki with the approval of the both of the Institutional Review Board of Kanazawa University Hospital (approval no. 2441-2) and Yamanashi University Hospital (approval no. 1746). A trial registration number was also obtained (UMIN000030913). Informed consent was obtained from all study participants. Patients were free to withdraw from the study at any time.

Patient consent for publication

The images presented in Fig. S1 were reprinted with each patient's informed consent. The images were submitted without any identifying information to ensure patient anonymity. Some of the clinical images of 'Mucositis' illustrated in Fig. 1 were reprinted with permission from the 'Manual for the management of individual serious adverse drug reactions' (https://www.mhlw.go.jp/topics/2006/11/dl/tp1122-1l09.pdf) of the Japanese Ministry of Health, Labour and Welfare, and permission from Department of Dentistry, National Cancer Center Hospital.

Competing interests

The authors declare that they have no competing interests.

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