

Association Between The Point-Rating System Used for Oral Health and The Prevalence of Gram-Negative Bacilli in Hematological Inpatients: A Prospective Observational Study

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Abstract

Background: Gram-negative bacteremia is a major cause of death among hematology inpatients who require heavy-dose chemotherapy and hematopoietic stem cell transplantation. Gram-negative bacillus (GNB) is more likely to be detected when the oral health is poor. However, there is a dearth of studies on the relationship between oral assessment and prevalence of GNB in hematology inpatients.

Methods: This prospective study aimed to evaluate the relationship between the original point-rating system for oral health examinations (point-oral exam) and the prevalence of GNB in hematology inpatients at the hematology ward of the Yamanashi University Hospital. GNB was detected by cultivating samples from the sputum and blood of each patient.

Results: A total of 129 subjects underwent a medical checkup and point-oral exam. The sputum and blood culture results of 55 patients were included in this study. The total points of patients positive for GNB ($n = 25$, 45.5%) were significantly higher than those who were negative for GNB (total score: median, 25th, 75th, percentile; 6 (4, 7) vs 2 (1, 4); $p = 0.00016$). Based on the receiver operating characteristic analysis, a cutoff score of 5 proved to be most useful to detect GNB.

Conclusion: An oral evaluation with a cutoff value of 5 or higher in the point-oral exam might indicate the need for a more thorough oral management to prevent the development of systemic infections from GNB.

Introduction

The maintenance of oral hygiene and the presence of 20 or more natural teeth have been associated with dental and periodontal diseases as well as serious systemic diseases, such as postoperative pneumonia [1–3]. Reductions in the incidence of pneumonia and the associated mortality rate as a result of good oral healthcare have been reported previously [4–6].

Hematology inpatients are susceptible to severe and life-threatening infections, such as febrile neutropenia and bacterial pneumonia from dental focal infections, due to the adverse effects of hematopoietic stem cell transplantation (HSCT) and high-dose chemotherapy. Meyer et al. reported that more than 20% of HSCT patients developed bloodstream infections and nearly 10% developed bacterial pneumonia [7]. Prior to HSCT, high-dose chemotherapy is associated with increased susceptibility to oral mucositis, which is associated with poor clinical and economic outcomes [8]. Oral mucositis is a gateway for bacterial invasion through damaged mucosa, and oral care before transplantation is known to prevent oral mucositis [9]. In previous clinical studies, dental focal infections have been reported as a common cause of febrile neutropenia in hematological cancer patients [10–13]. Furthermore, poor oral hygiene increases the number of Gram-negative bacilli (GNBs), the causative agents of periodontal disease; aspiration of GNB-contaminated saliva can lead to the development of nosocomial pneumonia [5, 14–18]. Furthermore, Gram-negative bacteremia is a leading cause of illness and death among hematology inpatients, after HSCT [19, 20]. On the other hand, Yamagata et al. reported that dental treatment to control infected areas and sources of infection with proper cleaning procedures prior to HSCT treatment

resulted in the absence of any dental infections in the patients after transplantation [21]. Moreover, oral management significantly decreased the incidence of oral adverse events (oral mucositis) during treatment before and after HSCT in inpatients with hematopoietic neoplasms [22, 23].

Despite strong evidence of the role of oral health in the prevention of life-threatening infections in hematology inpatients, an association between oral health assessments and the detection of GNB in hematology inpatients has not been reported so far. Therefore, this prospective study was conducted to identify the association between oral health examination, based on an original point-rating system, and the detection of GNB from the sputum and blood cultures of hematology inpatients.

Methods

Study design and participants

This prospective study comprised hematological inpatients aged ≥ 18 years who underwent an adequate medical checkup at the Yamanashi University Hospital from June 21, 2016, to March 27, 2018.

After providing informed consent, the patients were required to undergo a point-oral exam within 1–2 weeks of admission to the hematology department. The height, weight, and blood test results of the hospitalized patients were recorded at the beginning of the point-oral exam. Data concerning sex, age, body mass index (BMI), white blood cell, hemoglobin (Hb), C-reactive protein (CRP), ALT (alanine aminotransferase), creatinine, and albumin were collected from each patient. Results of the sputum and blood culture tests, obtained within 3 months after the oral evaluation, were used in the study. The participants were followed up for approximately 3 years (up to December 2020) and assessed for associations between the presence of GNB and the point-oral exam/hematological disease-related mortalities. This prospective observational study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Yamanashi University Hospital (No R02431). The patients were free to withdraw from the study at any time.

Evaluation of the oral status and data collection (blood/sputum culture test)

Oral evaluations were performed at the bedside and scored by a visiting dental hygienist and a dentist. To ensure minimum discrepancy among the internal evaluators, the final scoring was approved by the dentist (the corresponding author) after consultation with the person who scored the original point-rating system for oral health examinations at the bedside.

Blood and sputum samples and oral assessment findings at baseline were collected within 1 month after admission to the hematology department. Additionally, two sets of blood cultures (aerobic and anaerobic) and sputum cultures were collected from the patients whenever a systematic infection was suspected, based on clinical symptoms such as fever (> 37.5 °C), cough, sputum production, dyspnea, chest pain, or the appearance of new infiltrates on chest radiography images during hospitalization, or

when the general condition of the patient deteriorated. For hematopoietic stem cell transplant patients, blood cultures were performed regularly every week after transplantation. A positive result was determined if the presence of both leukocytes and GNB was detected during sputum examination or GNB was detected in the blood culture, within 3 months after oral evaluation. Oral assessments were performed by a dentist who was blinded to the results of the laboratory tests. The noninvasive oral assessment scores were evaluated and recorded by the same dentist using an oral health assessment panel (Fig. 1). Eilers' Oral Assessment Guide is one of the most well-known oral health assessment methods and is frequently used by many institutions [24]. However, this system is not suitable for hematology inpatients who are immunocompromised or depressed because it is laborious to perform owing to a large number of evaluation items, including the swallowing assessment. An oral evaluation method that was as noninvasive and quick as possible was required for the hematology inpatients. Therefore, the point-oral exam was created by referring to other available systems, such as Eilers' Oral Assessment Guide and the revised Oral Assessment Guide, as described in our previous study [25, 26]. The four important evaluation items in the point-oral exam were hygiene, xerostomia, mucositis, and occlusion [27–32]. Each item was scored as follows: 0, excellent; 1, slight dysfunction; 2, moderate dysfunction; and 3, severe dysfunction. The classifications of hygiene, xerostomia, and mucositis were made according to the Oral Health Assessment Tool and Eilers' Oral Assessment Guide [24]. Occlusion was added as a new assessment item according to Eichner's classification; Iimura et al. reported that the maximal bite force was associated with a 3-year survival in the elderly [33, 34]. The total scores, defined as the sum of the individual item scores, ranged from 0 (best oral health) to 12 (worst oral health). Importantly, previous studies have shown that the detection rate of pneumonia-causing bacteria and GNB is higher in point oral examinations with 4 or more points [25].

The following Gram-negative bacteria, which are known to be strongly involved in the development of febrile neutropenia and serious systemic infections, were included in the study: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter cinaedi*, *Campylobacter* spp., and *Fusobacterium* spp. [16, 19, 20]. The patient was initially instructed to gargle several times with water, after which the sputum was collected into a sterile Petri dish to avoid contaminating the saliva and dental plaque. Moreover, Geckler's classification of macroscopic sputum findings and Miller and Jones' classification of microscopic findings were employed to avoid collecting sputum samples contaminated with saliva and upper respiratory tract secretions [35, 36]. The M1 class sputum—based on the Miller & Jones' classification—which is viscous and consists mostly of saliva, was excluded [36]. Alternatively, good-quality sputum, based on Geckler's classification (classes 3, 4, and 5), was collected [35]. The samples were delivered to the laboratory immediately after collection and were subjected to Gram staining and isolation according to standard techniques. Three types of agar plates were used, namely, bromothymol blue lactose, sheep blood, and chocolate. The globally adopted BacT/ALERT® microbial detection system (bioMérieux, Marcy l'Etoile, France) is used for blood culturing [37]. Blood culture bottles were placed into a BacT/ALERT® instrument upon arrival at the laboratory and were incubated at 37 °C for 7 days [37]. Bacterial identification was conducted 24–48 h after subculturing. Positive bacterial culture findings were confirmed by the presence of colonies on the agar

plates and identified using the MicroScan WalkAway system (Beckman Coulter, Pasadena, CA, USA). A neutral third party at our hospital's clinical microbiology laboratory performed the bacterial analyses while blinded to the oral health assessment data, according to the method described previously by Ogihara et al. [38].

Receiver operating characteristic analysis to set the cutoff point for the point-oral exam

Receiver operating characteristic (ROC) analysis was performed to determine the cutoff values for separating the detection and non-detection groups, and survivors and deaths, respectively.

Statistical analyses

The χ^2 or Fisher's exact test was used to compare categorical data, and the Mann–Whitney U test was used for continuous data. Fisher's exact probability test was used to determine significant differences in the scores for the point-oral exam between the groups in terms of each of the four components. In the multivariable analysis, logistic regression was applied to adjust for potential confounding factors. The two-tailed significance level was set at $p < 0.05$. Statistical analyses were performed using the SPSS 27 software (SPSS, Chicago, IL), except for analyzing the ROC curve, which was performed using EZR (Saitama Medical Center, Jichi Medical University, Japan), a graphical user interface for R (The R Foundation for Statistical Computing) [39].

Results

Baseline characteristics of the subjects

Figure 1 shows the flowchart for the selection of the study subjects. A total of 129 hematology inpatients (age, ≥ 18), who received a medical checkup with point-oral exam at the Yamanashi University Hospital, were considered as possible candidates for the present study. Among them, 55 patients who underwent both sputum and blood culture tests were included, whereas 74 patients were excluded due to incomplete data regarding the bacterial cultivation (Fig. 2). Table 1 summarizes the baseline characteristics of the 55 patients included in this study. The mean age of the patients was 64 years, and 65% of them were males. No significant intergroup differences in the blood test results were observed. The most common underlying disease was malignant lymphoma ($n = 14$; 25.5%), followed in descending order by multiple myeloma ($n = 13$), acute myelocytic leukemia ($n = 12$), myeloid dysplasia ($n = 6$), lymphoid neoplasm ($n = 5$), pancytopenia ($n = 3$), chronic myelocytic leukemia ($n = 1$), and aplastic anemia ($n = 1$). The most common treatment provided was high-dose chemotherapy ($n = 32$; 58.2%), followed by autogenetic hematopoietic stem cell transplant ($n = 18$), allogenic heterotopic transplantation stem cell transplant ($n = 2$), transfusion therapy ($n = 2$), and palliative therapy ($n = 2$).

Table 1
Demographic and clinical data of participants.

Variable	Detection Group (n = 25) (mean ± SD)	Non-detection Group (n = 30) (mean ± SD)	P value
Sex (Men, n [%])	20 (80%)	16 (53%)	0.088 [†]
Age (years)	65.9 ± 13.2	63.3 ± 13.3	0.47 [§]
Body mass index (kg/m ²)	22.95 ± 4.18	22.11 ± 3.18	0.48 [§]
WBC (/μL)	10.61 ± 19.00	10.71 ± 21.9	0.99 [§]
Hb (g/dL)	8.94 ± 2.14	9.45 ± 2.5	0.33 [§]
CRP (mg/dL)	5.24 ± 10.14	3.16 ± 4.7	0.43 [§]
ALT (IU)	29.36 ± 24.0	33.04 ± 62.2	0.78 [§]
Creatinine (mg/dL)	1.04 ± 0.93	0.95 ± 0.83	0.56 [§]
Albumin (g/dL)	3.45 ± 0.73	3.53 ± 0.83	0.38 [§]
Categorical variables are expressed as numbers and %: sex			
Continuous variables are expressed as mean ± standard deviation			
[†] p value by chi-squared test			
[§] p value by Mann-Whitney U-test			
Detection Group: Detection of Gram-negative bacilli			
Non-detection Group: Non-detection of Gram-negative bacilli			

Patients from whom at least one of the GNBs was cultured formed the “detection group” (n = 25; 45.5%), while the remainder formed the “nondetection group” (n = 30; 54.5%). No significant intergroup differences in the blood test results or demographic data (sex, age, and BMI) were observed (Table 1).

Incidence of isolates on blood culture

Out of the 55 subjects, 15 (27.3%) presented with bacteria in the blood culture tests; among them, 5 (5/15, 33.3%) had GNB, 9 (9/15, 60%) had Gram-positive bacteria, and the remaining 1 patient (1/15, 6.7%) had *Candida* spp. The most frequently detected bacterial species was *Corynebacterium* spp. (3/15; 20%), followed by *Escherichia coli* (2/15; 13%).

Incidence of isolates on sputum culture

Bacteria were detected in the sputum cultures of all 55 patients; 20 patients (45.5%) had GNB, and the remaining 35 (54.5%) had Gram-positive bacteria. GNB strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus* spp., *Escherichia coli*, *Enterobacter* spp., and *Stenotrophomonas maltophilia* were detected. However, in some patients, the cultures could not be identified in detail.

Scores for the point-oral exam in the detection and nondetection groups

As shown in Table 2, the total scores for the point-oral exam were significantly higher in the detection group than in the nondetection group (median [25th, 75th percentile]; total score, 6 [4, 7] vs. 2 [1, 4]; $P = 0.0002$). Similarly, the scores for the hygiene, xerostomia, and mucositis categories were significantly higher in the detection group (Table 2). The 75th percentile of the mucositis score demonstrated a low score of 1 in the detection and nondetection groups. However, the scores for the category of occlusion did not significantly differ between the two groups (Table 2).

Table 2
Score of category between Detection group and Non-detection group.

Score of category (range)	Detection Group (n = 25) Median (25th, 75th percentile)	Non-detection Group (n = 30) Median (25th, 75th percentile)	<i>P</i> value
Total point (0–12)	6 (4, 7)	2 (1, 4)	0.0002*
Hygiene (0–3)	2 (1, 2)	1 (0, 1)	0.014*
Xerostomia (0–3)	1 (1, 2)	0 (0, 1)	0.001*
Mucositis (0–3)	1 (1, 1)	0 (0, 1)	0.004*
Occlusion (0–3)	1 (1, 2)	0 (0, 1)	0.051
Detection Group, detection of Gram-negative bacilli;			
Non-detection group, non-detection of Gram-negative bacilli			
<i>P</i> value by Fisher's exact test			
* Statistically significant ($p < 0.05$)			

Cutoff points for the total points in the point-oral exam

The cutoff points for the total scores in the point-oral examinations were determined as 5 for both the detection and nondetection groups/survivors and deceased. The values exhibited high sensitivity and high specificity at 72.0% and 86.7%, respectively (Fig. 3). In the follow-up until December 2020, 24

patients (43.6%) died due to blood-related diseases, while the remaining 31 patients (56.4%) survived. As shown in Fig. 4, the cutoff values used to distinguish between the surviving and deceased patients had lower sensitivity and lower specificity (58.3% and 74.2%, respectively) than those used to determine between the detection and nondetection groups.

Risk for detection of GNB in the multivariate logistic regression model

A multivariable analysis revealed that the odds ratio for the prevalence of GNB was nearly 11 times higher in patients with poor oral health (total points of 5 or more) than in patients with good oral health (total points of less than 4; Table 3).

Table 3
Risk for detection of gram negative bacillus by status of oral assessment in multivariate analysis.

Parameter		Odds ratio	95% CI	P-value
Hygiene	Poor (category 2 or 3)	1.94	0.40–9.28	0.41
	Good (category 0 or 1)	1.0 (reference)		
Xerostomia	Poor (category 2 or 3)	1.09	0.18–6.47	0.92
	Good (category 0 or 1)	1.0 (reference)		
Total Points	Poor (points \geq 5)	10.95	2.02–59.34	0.006*
	Good (points < 4)	1.0 (reference)		
Abbreviation: SE, standard error; CI, confidence interval.				
* Statistically significant ($p < 0.05$)				

Discussion

This study prospectively investigated the associations between the prevalence of GNB and the point-oral exam among 55 hematology inpatients at a hospital. The prevalence of GNB was higher in patients with poor oral health (score of 5 or more in the point-oral exam) than those with good oral health (score of 4 or less). The results suggest that a value of 5 or higher is effective in identifying the presence of GNB, whereas a value of less than 4 might indicate the absence of GNB. To the best of our knowledge, this is the first study to focus specifically on the association between GNB detection rates and oral health scores.

The point-oral exam was categorized into four items (hygiene, xerostomia, mucositis, and occlusion) to evaluate its association with the prevalence of GNB. In the present study, the GNB detection group had poorer oral hygiene and xerostomia status compared with the GNB nondetection group. However, no

significant difference in occlusion was observed between the two patient groups. Additionally, the evaluation items for mucositis could not be specified from this study because the 75th percentile of the score of xerostomia was low (1) in the detection and nondetection groups, making it difficult to distinguish between them based on the scoring, although there was a significant difference in scoring between the detection and nondetection groups.

Bacteria colonize in various parts of the oral cavity, including the dental plaque and the tongue. However, pathogenic bacteria, such as GNB, are rarely detected in the oral cavity of healthy individuals. On other hand, the frequencies of GNBs are reported to be high in hospitalized patients. Furthermore, patients with aspiration pneumonia and those who are medically compromised are known to have increased levels of GNBs in their oral cavity and pharynx [14]. In the study by El-Solh et al. [40], GNBs were the most predominant organisms (49%) detected in bronchial samples from 67 patients with AP, followed by anaerobes (16%) and *Streptococcus aureus* (12%). In the present study, the detection rate of GNB was high at 45.5%, which was consistent with the results of previous studies[40, 41].

It has been reported that hospitalized patients, especially those with leukemia and bone marrow transplants, have higher rates of oral and pharyngeal GNB. Moreover, in one study, enteric microorganisms identified as *Klebsiella* (42.7%), *Enterobacter* (18.8%), and *Pseudomonas* (15.6%) were isolated from 62.2% of leukemia patients compared to 28% of controls [42, 43]. In some studies, the prevalence of GNB in the oropharyngeal flora correlated best with the clinical severity of the disease and motility in hematological inpatients [44–46]. Similarly, in the present study, ROC analysis suggested that a score of 5 or more on the oral assessment was associated with higher mortality due to the hematological disease.

This study may show that maintaining good oral hygiene and a moist oral mucosa might improve the condition of the oral cavity, a potential reservoir of GNB [47, 48], and reduce the retention of GNB in high-risk, hospitalized, hematological patients. Previous studies have shown that poor oral hygiene and xerostomia are closely related to increased bacteria, including GNB, in the oral cavity [49, 50]. On the other hand, according to the study by Senpuku et al., oral management (including hygiene and professional care) that involves the elimination of pneumonia-causing bacteria and fungi could diminish the risk of developing systemic diseases [51]. These points of view suggest that maintaining the cleanliness and moisture of the oral cavity should prevent the development of systemic complications in hospitalized patients with hematological diseases, such as those requiring high-dose chemotherapy for leukemia and bone marrow transplantation. In our previous study, we reported that the group of patients with good nutritional status had a better oral health status and lower GNB prevalence than those with poor nutritional status [25]. In the current study, GNB was frequently detected in eight out of ten malnourished patients who required strict nutritional management (data not shown).

One of the limitations of this study is that we did not use molecular biology techniques, such as polymerase chain reaction, to identify the species of the bacteria. In particular, the metabolic profiling of saliva should be adopted to detect and quantify the pathogens because this method can detect a higher

proportion of various bacterial infections in the oral cavity than fluid cultures of the bronchoalveolar lavage, without causing any distress to the patient [52, 53]. Furthermore, culturing of these organisms in a future study is necessary to determine their sensitivity to various antibacterial agents and for application in the clinical setting. Although careful sputum sampling was conducted to reduce the risk of oral bacterial contamination in the present study, the possibility of contamination leading to inaccurate results cannot be ignored. A large-scale, multicenter, prospective cohort study is required to confirm the findings of this study and to ensure interobserver reliability.

The strength of the present study was that data management and analyses were performed by an independent biostatistician and a health information manager to exclude evaluator subjectivity. The evaluation method used herein could be easily implemented and evaluated by nontrained nurses if the assessment item of occlusion, which may require evaluation by a dentist, is excluded. Occlusion might not be an important item to evaluate since no significant difference in occlusion was noted between the detection and nondetection groups. However, the importance of each item should be determined by increasing the number of cases and conducting an inter-rater reliability test for the point-oral exam.

In conclusion, the present study demonstrated an association between the prevalence of GNB and oral health assessment in hematological inpatients. The prevalence of GNB was evidently higher in patients with poor oral health (score, ≥ 5 on the point-oral exam) than in those with good oral health (score, ≤ 4). Thorough oral management should be considered as early as possible in patients with a total score of ≥ 5 , before full-scale treatment of the main disease.

Abbreviations

GNB: Gram-negative bacilli

AP: Aspiration pneumonia

HSCT: hematopoietic stem cell transplantation

BMI: Body mass index

ROC: Receiver operating characteristic

Declarations

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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.

AM and RI corrected the manuscript and collected all data.

HY participated in the design of the study and performed statistical analyses.

SO is responsible for supervising bacteria culture and identification.

KN and KK reviewed and edited the manuscript.

KK and KU are responsible for supervising the clinical trial, supervising the interpretation of the data, and drafting the manuscript.

All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Yamanashi University Hospital (approval number R02431). 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 Appendix Figure 1 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" in Appendix Figure 1 have been reprinted with permission from "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 the Department of Dentistry, National Cancer Center Hospital. Appendix Figure 1 is reprinted with permission from the *International Journal of Functional Nutrition* (<https://doi.org/10.3892/ijfn.2020.8>).

Competing interests

The authors declare no conflicts of interest.

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Figures

Category	Numerical and descriptive ratings (Prompt noninvasive oral assessment)			
	0 Excellent	1 Slight dysfunction	2 Moderate dysfunction	3 Severe dysfunction
Hygiene	 Clean	 Slight local debris	 Moderate local debris	 General debris, decay
Xerostomia	 Watery	 Thick or Ropy	 Little	 Absent
Mucositis	 Pink, moist	 Slight red or coated, no ulcers	 Moderate red or coated, ulcer Sight ~ moderate pain	 Ulcer with bleeding Sever pain
Occlusion	4 occlusal supporting zones (OSZs)	2-3 OSZs	1 OSZ	No OSZs

Figure 1

Each of the four evaluated items (“hygiene,” “xerostomia,” “mucositis,” and “occlusion”) received a score ranging from 0 (excellent assessment) to 3 (extremely poor assessment). The total score ranged from 0 (excellent oral health) to 12 (worst oral health). Figure 1 is reprinted with permission from the International Journal of Functional Nutrition (<https://doi.org/10.3892/ijfn.2020.8>.)

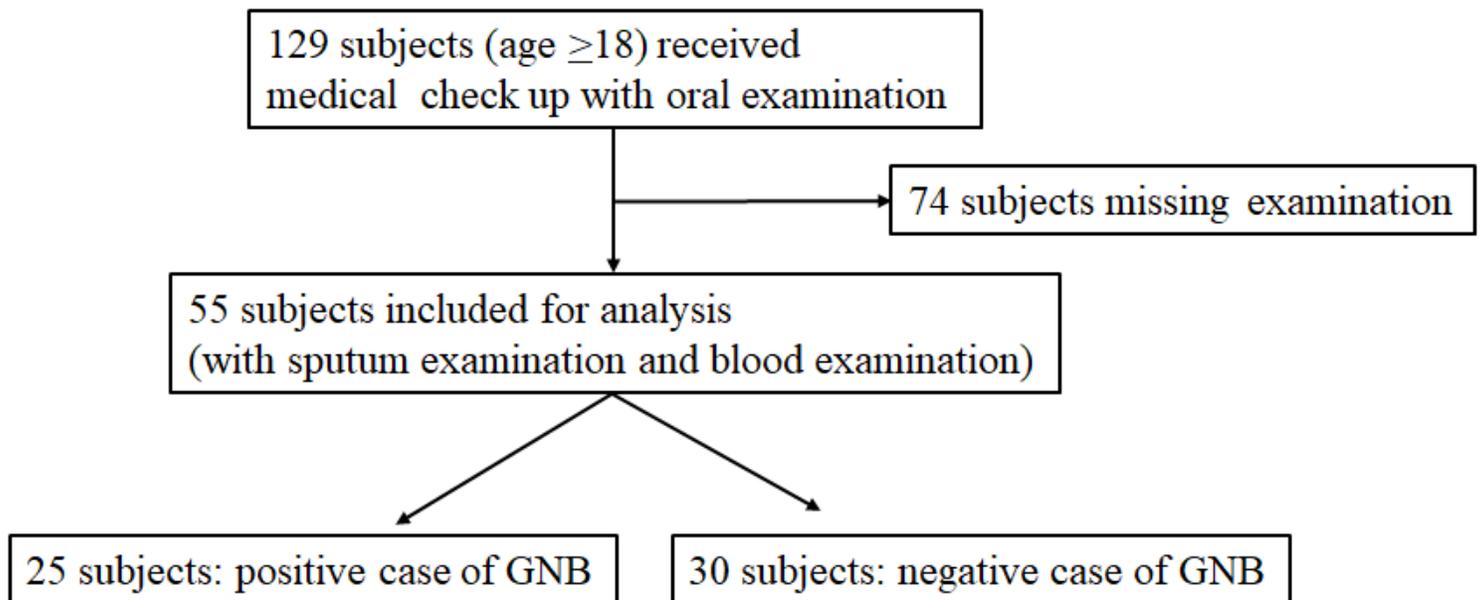


Figure 2

Flowchart of the study design.

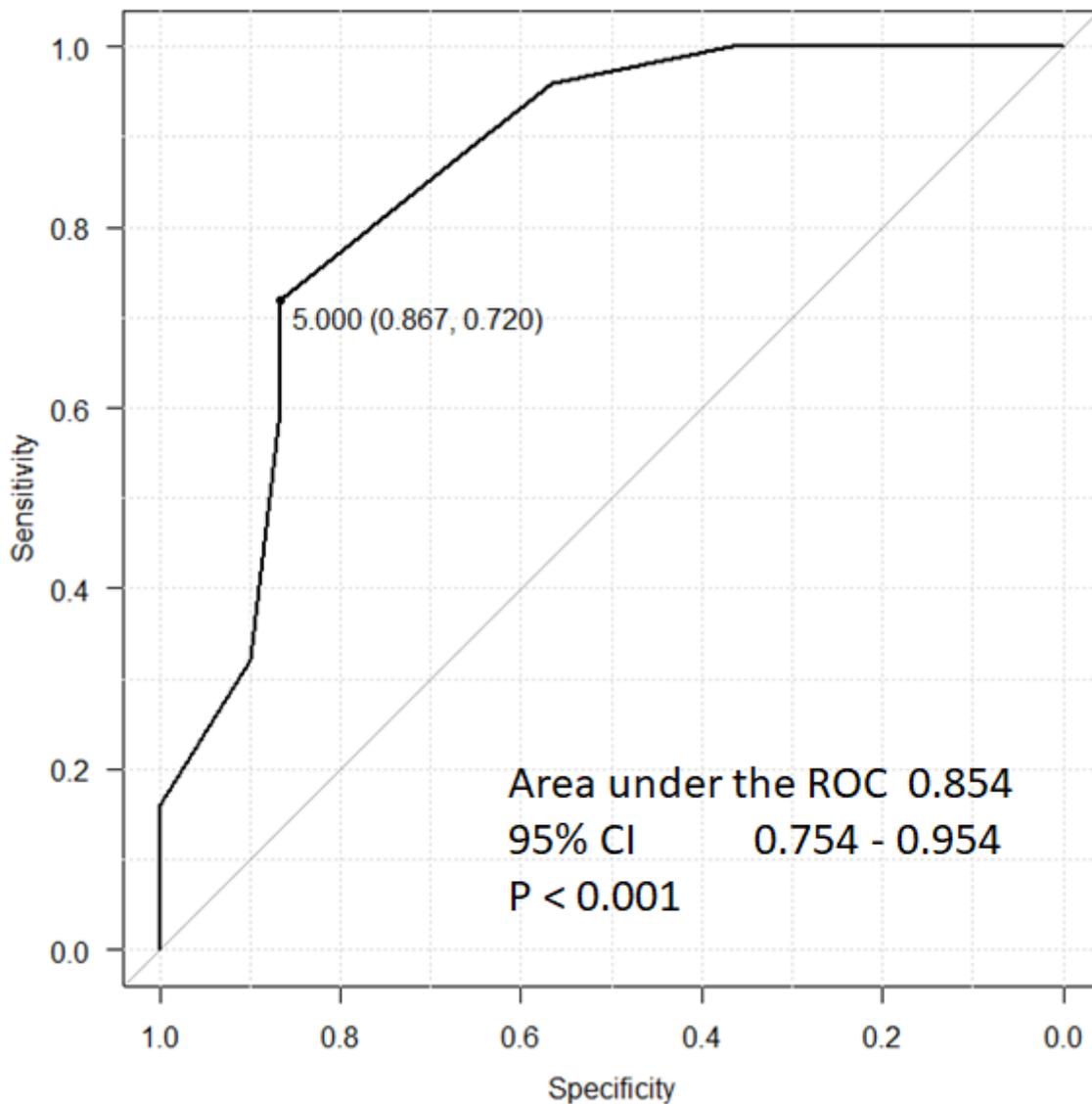


Figure 3

In the receiver operating characteristic (ROC) analysis, the score for the point-oral exam that classified a patient as being at risk for the prevalence of GNB was 5, with a sensitivity and specificity of 72.0% and 86.7%, respectively. The area under the ROC curve was 0.854, indicating moderate accuracy.

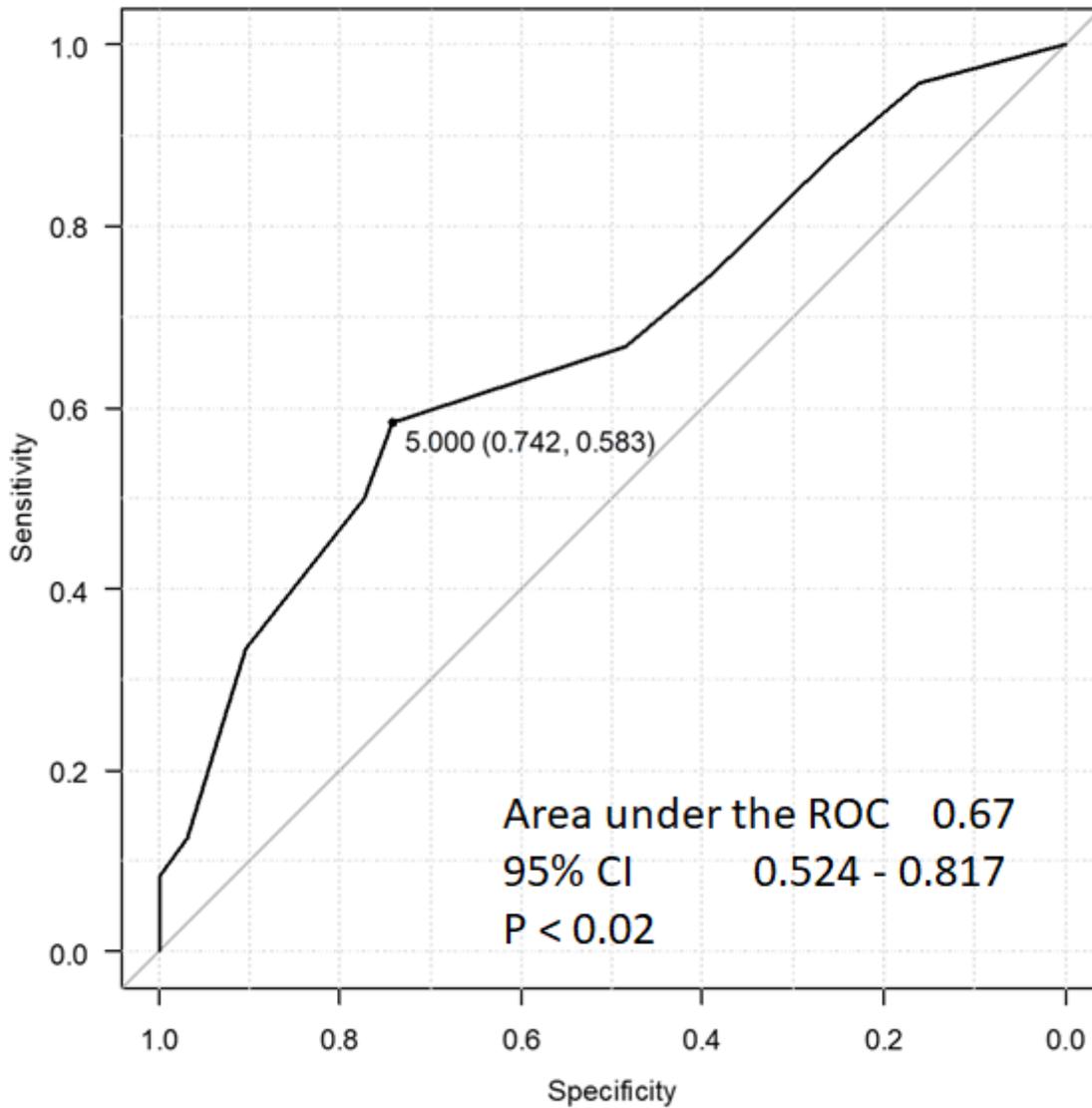


Figure 4

In the receiver operating characteristic (ROC) analysis, the score for the point-oral exam that classified the patient as being at risk for mortality was 5, with a sensitivity and specificity of 58.3% and 74.2%, indicating moderate accuracy.