Proposed type of manuscript

Original article

Title

Involvement of oral bacteria and oral immunity as risk factors for chemotherapy-induced fever with neutropenia in patients with hematological cancer

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The running title

Risk factors for chemotherapy-induced fever with neutropenia

Abstract

The aim of this study is to investigate the association between chemotherapy-induced fever with neutropenia less than 1,500/μL (FwN) and oral bacteria and/or oral immunity in patients with hematological cancer. Thirty-two patients with hematological cancer were enrolled in the study. Secretory immunoglobulin A (sIgA) in saliva and the anaerobic bacteria in tongue coating of each subject was assessed before the first chemotherapy. Eleven subjects had an onset of FwN and 21 subjects did not during the observation periods. It was revealed by the cox-proportional hazard model analysis that the levels of sIgA were low (HR 0.98, $p<0.05$) and the rate of *Fusobacterium nucleatum* [*F. nucleatum* count per total bacterial count (%)] was high (HR 1.65, $p<0.05$) in patients with FwN onset. Using ROC curve analysis, the optimal cut-off point based on the AUC in the *F. nucleatum* / sIgA ratio was 0.023, and this model had a 78.4% probability ($p<0.01$). The risk of FwN onset was also significantly higher among the group of ≥ 0.023 *F. nucleatum* / sIgA ratio (HR 66.06, $p<0.01$). These results suggest that the rate of *F. nucleatum* and the levels of sIgA at baseline might be related to FwN onset as risk factors.

Keyword

Fever with neutropenia, Hematological cancer, Risk factor, Secretory immunoglobulin A, *Fusobacterium nucleatum*

Introduction

It has been reported that adverse events observed in cancer chemotherapy were caused by a combination of many complex factors. Among them, febrile neutropenia (FN) is one of the frequent adverse events of hematological cancer chemotherapy. Neutrophils, a type of white blood cell, take in pathogens that invade the body, digest and degrade them with enzymes released from granules in the cytoplasm. When chemotherapy is performed on patients with hematological cancer, bone marrow function decreases, and neutropenia is observed about seven days after drug administration. Notably, there is a high risk of fever due to susceptibility to infection during neutropenia [1].

FN is defined as a condition in which the axillary temperature is 37.5°C (or oral temperature 38°C) or higher. Also, the number of neutrophil count is less than 500 cells/μL or is less than 1,000/μL with a predicted decline to 500/μL within 48 hours [2]. In particular, it has been reported that cancer chemotherapy with strong bone marrow suppression, such as in the treatment of hematological cancers, increases the likelihood of FN becoming severe, leading to death [3]. On the other hand, there is a risk that local infections due to oral mucositis may progress to serious infections [4]. Granulocytopenia, which occurs at the same time as oral mucositis, increases the risk of bacteremia and sepsis. Gram-negative bacilli, the normal bacterial flora in the mouth, can spread hematogenously from the site of the ulcer and can cause local or systemic infection. Anaerobic bacteria represented by periodontopathogenic bacteria existing in the oral cavity are also assumed to be the infection source in those cases.

There were few studies regarding the association between FN development and the oral cavity. It has been reported that professional oral care significantly reduced the

incidence of FN [5] and that *Fusobacterium nucleatum*, a periodontopathogenic bacteria, was the cause of septic patients with FN [6]. However, the details of the relationship between the onset of FN and oral bacteria are still unknown, and it has not yet been clarified in many ways as to what kinds of microorganisms are actively involved in its progress. Professional oral care management has been widely performed to prevent adverse oral events in patients with hematological cancers on intensive chemotherapies. However, the therapeutic efficacy of professional oral care approaches to prevent the incidence of FN remains elusive.

On the other hand, it has been demonstrated that secretory IgA (sIgA) plays an important role as the "first line of defense" of the oral mucosal surface as an antibacterial factor in saliva. It was reported that the amount of sIgA and saliva volume was reduced by chemotherapy for patients with breast cancer [7]. Furthermore, Karolewska et al. reported that the amount of sIgA decreased during chemotherapy for children with acute leukemia, and sIgA levels in patients with oral mucositis were lower than that in patients without oral mucositis [8]. Thus, cancer treatment also affects oral immunity.

The purpose of this study was to 1) investigate the incidence of fever with neutropenia (FwN) among adverse events in hematological cancer patients receiving chemotherapy, 2) to analyze the association with FwN and oral bacteria such as anaerobic microorganisms and other factors in the oral cavity, especially, sIgA, and 3) to propose predictive indices highly relevant to FwN onset.

Material and Methods

1. Subjects

Thirty-three patients aged 22 to 86 years old (20 males and 13 females) whose oral cavity status were checked and who received their first chemotherapy for hematological cancer at Tokushima University Hospital from April 2012 to December 2016 were initially enrolled in the study. Before enrollment, the participants were informed about the methods and objectives of the study, and they provided written informed consent.

2. Clinical parameters

Diagnosis and treatment procedure of hematological cancer, the chemotherapeutic drug used, administration of antibiotics and/or G-CSF for the prevention or treatment of infections, the systemic conditions including diabetes mellitus were obtained from the patient's medical record. The period of time including the presence of fever, and results of the blood test, such as the value of serum albumin and neutrophil count (or the white blood cell count), were also obtained. In this study, FwN was set as a fever of unknown origin (an axillary temperature of 37.5°C or higher) with neutropenia less than 1,500/μL to widely analyze the patients enrolled. FN is defined as a fever of unknown origin with neutropenia, a neutrophil count of less than 500/μL, and an axillary temperature of 37.5°C or higher based on the Japanese Society of Medical Oncology guidelines [9]. The day of the onset of FwN was also confirmed from the patient's medical record. Observation periods were decided 28 days from the first day of the chemotherapy. Also, the smoking habits of the patients were recorded at baseline.

3. Assessment of samples of the saliva and the tongue coating

Saliva and tongue coating samples from each subject were assessed before the first chemotherapy. Unstimulated whole saliva from each subject was collected using a

sterilized cotton swab kit (Salikids®; Saersted, Vümbrecht, Germany) before lunch. The cotton roll was placed into the oral cavity of each subject for 3 min, then the roll was returned into the Salikids® tube, and was centrifuged at 3,000 x g for 2 min at 4°C. The resulting aliquots were divided into analytical tubes and were kept in a deep freezer at -80°C until use.

The levels of salivary protein were measured using Protein Assay Kit (Bio-Rad, Hercules, CA, USA) according to the method of Bradford [10] with BSA as a standard. The levels of sIgA of saliva were measured using an enzyme immune-assay kit (EIA s-IgA test, Medical & Biological Laboratories, Nagoya, Japan) according to the manufacturer's instructions. Both of the levels of salivary protein and sIgA were evaluated as the total amount per minute secretion.

Bacterial Counter ™ (Panasonic, Osaka, Japan) was used to assess the number of bacteria in tongue coating according to the manufacturer's instructions. Tongue coating samples were collected using a sterile 5mm-diameter cotton stick by swabbing the tongue dorsum three times from back to front (approx. 2-cm-long swabbing motions). Samples were suspended in 5 ml of distilled water in disposable cups and bacterial quantification with Bacterial Counter ™ was performed. Thereafter, the samples were dispensed into vials and kept at -80°C until used.

Tongue coating samples were also used to quantify the number of three periodontopathogenic bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Campylobacter rectus*) by quantitative PCR as previously reported by Moriyama et al [11]. The MiniOpticon system (Bio-Rad Laboratories, Hercules, CA, USA) with SYBR Green I dye was used for the quantitative PCR analysis. The primers used for the quantitative PCR have been previously described [12]. A standard curve was generated

based on the known number of *F. nucleatum* ATCC 23726, *P. gingivalis* ATCC 33277, and *C. rectus* ATCC 33238. Ten-fold serial dilutions of bacterial standards were prepared and each extracted DNA was used. The concentrations of each organism in tongue coating samples were calculated from the number of copies of the target sequence.

4. Statistical analysis

Data were analyzed using the software IBM SPSS Statistics ver. 26 (SPSS Japan Inc. Tokyo). The difference between the two groups with baseline was assessed using the chi-square test or Fisher's exact test for categorical data, and the Mann-Whitney U test for the other data. Covariates related to FwN onset was evaluated by the method of the cox-proportional hazard model. The items of the values of $p<0.05$ were used for the receiver operating characteristic (ROC) curve analysis for FwN onset.

The ROC curve is the plot that displays the full picture of the trade-off between the sensitivity (true positive rate) and (1- specificity) (false positive rate) across a series of cut-off points. The area under the ROC curve (AUC) is considered an effective measure of the inherent validity of a diagnostic test. Total AUC is a single index for measuring the performance of a test [13]. The larger the AUC, the better is the overall performance of the diagnostic test to correctly identify FwN subjects and non-FwN subjects.

To obtain the optimal cut-off points, the method uses the square of the distance between the point (0, 1) on the upper left-hand corner of ROC space and any point on ROC curve ie. $d^2= (1\text{-sensitivity})^2 + (1\text{-specificity})^2$, then the square of this distance is minimized [13]. The effectiveness of the optimal cut-off value obtained from the ROC curve analysis was evaluated by the method of the cox-proportional hazard model.

Statistical significance was set at $p < 0.05$.

5. Ethics

The Ethics Committee of Tokushima University Hospital approved this study (protocol approval number 1563).

Results

1. Baseline characteristics of FwN subjects and non-FwN subjects

Among the 33 subjects, one patient was excluded from this study because of fever and insufficient samples at baseline. The primary diseases of hematological cancer of the subjects in this study are shown in Table 1. By investigating the 32 subjects (mean age 60.2±15.4 years), 75% were diagnosed as having malignant lymphoma. All of the subjects did not have severe stomatitis at baseline.

Table 2 summarizes the participant characteristics according to the status of FwN onset such as demographic variables, clinical parameters at baseline, and regimen for chemotherapy. It was revealed that 11 subjects had an onset of FwN (FwN subjects) and 21 subjects did not (non-FwN subjects) during observation periods (maximum 28 days). It was observed that the age of the FwN subjects was significantly younger than those of the non-FwN subjects. Also, a significant difference was observed in the levels of sIgA between the two groups ($p<0.05$).

Upon investigation of oral bacteria at baseline, the rate of *F. nucleatum* [*F. nucleatum* count per total bacterial count (%)] was shown to have the tendency to be higher in the FwN subjects than those in the non-FwN subjects ($p =0.088$), whereas the rate of *P. gingivalis* and *C. rectus* did not show any tendencies.

In contrast, results showed a relationship between salivary components and the subsequent onset of FwN. The salivary volume per minute did not differ between the two groups. The levels of sIgA were significantly lower in FwN subjects than in non-FwN subjects ($p < 0.05$). No significant difference in the levels of salivary protein was observed between the two groups.

2. Factors related to FwN onset during chemotherapy

As shown in Table 2, no relation of FwN onset was observed in the items of antibiotics and G-CSF. The relationship between the presence of moderate neutropenia (<1,500 /μL) and the onset of FwN during the observation period were investigated. The rate of moderate neutropenia during chemotherapy in the FwN subjects and in the non-FwN subjects was 11 of 11 patients and 15 of 21 patients, respectively. No significant difference was observed between the two groups (Fisher's exact test, $p=0.071$). The difference in age between the subjects with moderate neutropenia and the subjects without neutropenia was also investigated. There was no significant difference in age between the two groups (Mann-Whitney U test, $p=0.29$).

3. Multivariable analysis by the cox-proportional hazard regression model depicting FwN onset

The results of the cox-proportional hazard regression analysis adjusted for the covariates are shown in Table 3. Covariates of both the levels of sIgA and the rate of *F. nucleatum* [*F. nucleatum* count per total bacterial count (%)] showed a significant effect on FwN onset after adjusting for potential confounding effects of other selected factors such as gender, age, smoking habit, diabetes mellitus, albumin, use of G-CFS and

regimens of the study. It was revealed that the levels of sIgA were low (HR 0.984, 95%CI: 0.970-0.999, p = 0.038) and the rate of *Fusobacterium nucleatum* [*F. nucleatum* count per total bacterial count (%)] was high (HR 1.647, 95%CI: 1.071-2,008, p = 0.017) in patients with FwN onset.

4. ROC curve analysis and the optimal cut-off value

Regarding the ROC curve, the farther the curve is to the upper left corner of the graph, the better the items are in predicting FwN onset. As shown in Fig. 1 and Table 4, based on the AUC, the model using the levels of sIgA and the rate of *F. nucleatum* had a 74.5% probability and 68.8% probability of predicting FwN onset, respectively. In addition, the model using the *F. nucleatum* / sIgA ratio had a 78.4% probability (p = 0.003). Subsequently, the optimal cut-off value was determined in concordance with the FwN onset based on the AUC. As shown in Table 4, the optimal cut-off point based on the AUC in the *F. nucleatum* / sIgA ratio was 0.023, which had a sensitivity and specificity of 0.72 and 0.79, respectively.

5. The hazard of FwN onset based on *F. nucleatum* / sIgA ratio

The results of the cox-proportional hazard regression analysis adjusted for the covariates are shown in Table 5. Covariates of *F. nucleatum* / sIgA ratio showed a significant effect on FwN onset after adjusting for potential confounding effects of other selected factors. The risk of FwN onset was significantly higher among the group of ≥ 0.023 *F. nucleatum* / sIgA ratio compared with the group of <0.023 (HR 66.062. 95% CI: 3.645 - 1197.37, p = 0.005).

The treatment of patients with acute myelogenous leukemia is often associated with

severe long-term neutropenia, which may cause disease bias. Therefore, we reevaluated a cox-proportional hazards regression analysis without patients with myelogenous leukemia. However, those with ≥ 0.023 *F. nucleatum* / sIgA ratio remained to be a significantly high-risk population of FwN onset (Supplemental Table 1).

Figure 2 shows the cumulative hazard probability curves of FwN event stratified by gender and age with the proportional hazard model using the *F. nucleatum* / sIgA ratio (cut-off point: 0.023). Apparent higher FwN events were detected among the subject's group of ≥ 0.023 *F. nucleatum* / sIgA ratio.

Discussion

In this study, we focused on blood cancer patients with reduced immunity and investigated the relationship between FwN development and oral bacteria, in which some of the microorganisms are strongly involved in FN development [14]. It is reported that about 500 kinds of oral bacteria exist in the oral cavity and that there are 1.0 x10^8 bacteria per 1mL of saliva [15]. Our study revealed that *F. nucleatum* might be a possible risk factor of chemotherapy-induced FwN in patients with hematological cancer. This organism is a gram-negative obligate anaerobic bacillus, and a type of periodontopathogenic bacterium resident in the human oral cavity [16]. Furthermore, *F. nucleatum* plays a central role in the formation of dental plaque and possesses the ability to co-aggregate with a large number of bacterial species [17]. In particular, it is abundant not only in subgingival plaque but also in supragingival plaque. As plaque matures, the environment in plaque changes, and *F. nucleatum* increases [18]. This organism is also frequently isolated from tongue coating regardless of periodontal condition [16, 19]. It was reported by Gibbons et al. [20] that among the distribution of oral bacteria, the proportion of *Fusobacterium* found at the surface of tongue dorsum was 1%. An average of 0.6% of *F. nucleatum* is detected in the saliva of healthy subjects [21]. The results of this study showed that the median of *F. nucleatum* (%) in the FwN subjects was 2.7%, and it was high when compared to those of non-FwN subjects (0.2%).

The reason for this phenomenon is unclear. An explanation to account for this observation is that it will be related to the characteristics of blood cancer, such as malaise, nausea, and vomiting, resulting in insufficient brushing. This might change the bacterial flora in the oral cavity including the severity of tongue coating. *F. nucleatum*

may possess the ability to co-aggregate and attract other anaerobic bacteria. These bacteria possess an endotoxin which is one of the causes of fever. Also, these bacteria, including *F. nucleatum*, induces apoptotic cell death in peripheral blood mononuclear cells and polymorphonuclear cells as having an immunosuppressive role [16, 22]. These abilities of *F. nucleatum* might cause FwN development.

It was demonstrated that *F. nucleatum* is linked to systemic diseases. For example, this organism is known to be among the bacterium causing aspiration pneumonia [23]. Han et al. reported that *F. nucleatum* may induce premature and term stillbirths in an in-vitro study and reviewed the epidemiological and mechanistic evidence of the role of *F. nucleatum* in adverse pregnancy outcomes [24]. It was also reported to be related to colorectal cancer [25] and esophageal cancer [26]. It was also reported to cause bacteremia over a 10-month observation period [6].

It was not possible to observe the detailed status of oral mucositis during chemotherapy in this study whereas the condition of each subject at baseline was observed. It was reported that severe oral mucositis also affected the onset of FN [4]. Oral mucositis often appears during chemotherapy. The oral mucosa appears to be weak even with slight oral mucositis. Most patients receiving chemotherapy have the side effects of myelosuppression, therefore, it is considered that the oral environment easily causes bacterial translocation, which is an invasion of bacteria due to physiological abnormality. It has been reported that it is possible for *F. nucleatum* to invade the human oral epithelial cells [27] and that the FadA adhesin/invasin conserved in *F. nucleatum* is a key virulence factor [28]. Also, most patients undergoing chemotherapy have side effects of myelosuppression. Therefore, this organism may also invade blood circulation, cause infection, then cause fever. It was reported that these obligate anaerobic bacteria

have accounted for 3.4% of bacteremia in neutropenic patients [29]. It is considered that anaerobic bacteria containing *F. nucleatum* can cause acute infection during myelosuppression, and cause bacteremia easily. Both the bacterial translocation of *F. nucleatum* and its invasive ability into oral epithelial cells might cause infection in the blood circulation in patients with neutropenia due to cancer chemotherapy. However, *F. nucleatum* was not detected in blood cultures in FwN patients in this study. *F. nucleatum* is a well-known fastidious anaerobe to be hardly cultivated. Hence, this organism is frequently missed in routine culture conditions employed by hospital laboratories [28]. the detection of this anaerobic bacterium using the real-time PCR method should be implemented in the risk evaluation of FN onset in the future.

In our previous observational study, professional oral health care (POHC) was effective for the prevention of severe oral mucositis in patients undergoing autologous hematopoietic stem cell transplant with blood cancer undergoing chemotherapy [30]. Also, POHC significantly reduced the incidence of FN in patients treated with allogeneic bone marrow transplantation [5]. Taken together, POHC given by dentists and dental hygienists for patients with blood cancer before and after chemotherapy is very important for the prevention of FN onset.

In the duration of neutropenia after chemotherapy, patients with malignant lymphoma had a relatively high risk of developing FN, with the incidence of FN ranging from 12 to 23% [31]. The FwN onset among patients with malignant lymphoma in this study were 20.8%, which is within the range reported above. In contrast, it was reported that elderly people are at a higher risk for FN onset [14]; however, an unexpected result was obtained in this study. FwN onset caused by myelosuppression was also found in young subjects. One of the reasons may be that some of the patients with FwN had already

weakened resistance such as those with acute leukemia. Further study is needed to clarify these results by increasing the number of subjects.

In this study, we also measured sIgA levels in saliva. As a result, the levels of sIgA was significantly lower in the FwN subjects. The reason why the levels of sIgA were lower in the FwN group in this study is unknown. Sun et al. have reported that patients suffering from malignant tumors had a lower level of sIgA than healthy subjects. Also, the level of sIgA in the hematopoietic system tumor was significantly lower than that in other malignant tumors [32]. It is presumed that the sIgA level before cancer chemotherapy was related to the subsequent risk of FwN onset.

Immunologic defense in the mouth is mediated by a complex system including bioactive molecules such as antibodies and other proteins in saliva. Although lysozyme is present at a high concentration in saliva and possesses a bactericidal activity, it is less effective against dental plaque-producing bacteria [33]. Defensins are produced by human epithelial cells and active against bacteria, fungi, and enveloped viruses; however, their production levels are low [34]. The relation of these molecules to FN has not been reported.

On the other hand, IgA is the most abundant antibody isotype found in the body and plays an important role in the immune responses at mucosal surfaces. The main role of sIgA antibodies in the oral cavity might be to prevent the colonization of pathogenic microorganisms [35]. Secretory IgA does not only prevent adhesion of bacterial cells and viruses to mucosal surfaces but also prevent submucosal invasion by stopping antigens in the mucosal layer. It also has the effect of eliminating the active enzyme and toxin produced by bacteria [35]. It was reported that *F. nucleatum* possessed the ability to secrete serine proteases, and the 65 kDa serine protease was found to digest the

α-chains of immunoglobulin A [36], as the result, the degradation of sIgA may help the evasion of the immune system of the host by the bacteria [16]. Therefore, the lower levels of sIgA in saliva with a high rate of *F. nucleatum* may lead to reduced protection of oral mucosa in addition to the reduced immune system, as the side effect of chemotherapy. One possible explanation for chemotherapy-induced FwN is that these phenomena led to a decrease in the number of neutrophils as an outcome and the onset of fever.

Multiple factors are involved in FwN onset, the endpoint of this study. Oral cavity immunity and microbial pathogenic virulence are among the predominant oral environmental factors of FwN onset. Therefore, we have devised a protocol to measure both sIgA and periodontopathic bacteria in this study. The cox-hazard analysis in Table 3 revealed the significant involvement of sIgA and *F. nucleatum* as independent related factors of FwN onset. Therefore, we thought that it was possible to predict FwN risk by focusing on sIgA and *F. nucleatum*, as the factor of host defense and attacks by the pathogen, respectively. From the results of the ROC curve analysis, *F. nucleatum* / sIgA ratio could be applied as predictive indices highly relevant to FwN onset. It was demonstrated that some of the items such as age, nutritional status, complications, type of cancer, type of anticancer drug and degree of gastrointestinal tract/oral mucosal damage, have been shown as risk factors, epidemiologically [14]. In this study, *F. nucleatum* / sIgA ratio (0.023) was confirmed to be useful as a cut-off line for the 4-week observation period even if the biases such as age, sex, and serum albumin, were considered. These findings may be effective in the future treatment of FwN, in addition to the determination of G-CSF prophylactic administration [37], which is currently considered to be effective in reducing the incidence rate of FwN.

A limitation of this study is the small number of patients studied. It was the inability to obtain data regarding the number of oral bacteria and the levels of sIgA in subjects with the same type of cancer and/or regimens during the observation periods. Patients with acute myelogenous leukemia are generally accepted to most likely develop FwN because of the higher intensity of chemotherapies and accompanied severe neutropenia. Therefore, we reevaluated and confirmed the importance of the cut-off point, 0.023 of the *F. nucleatum* / sIgA ratio, in the prediction of FwN in those excluding myelogenous leukemia patients (Supplemental Table 1). However, further validation is needed according to cancer types and/or therapeutic regimens.

In this study, it was possible to evaluate moderate neutropenia (<1,500 /μL) from the medical records of all subjects whereas it was not for severe neutropenia (<500 /μL). However, it is crucial to evaluate the severity of neutropenia regarding the value and the duration for the analysis of FN occurrence. Therefore, further study is needed to consider deeply the relationship between *F. nucleatum*/ sIgA ratio and FN occurrence by adding the data of the severity of neutropenia.

Furthermore, oral mucositis during the observation period and other bacteria related to oral infection are also required for analysis. It is also necessary to increase the number of subjects in future studies, to investigate the involvement of oral bacterial infections including *F. nucleatum* in blood, to obtain several kinds of clinical data including oral condition during the observation period, and to verify the existence of a causal relationship.

Conclusion

By investigating the association of chemotherapy-induced FwN, oral bacteria and oral

immunity, it was revealed that the rate of *F. nucleatum* and the levels of sIgA at baseline might be related to FwN onset, and are risk factors. Also, *F. nucleatum* / sIgA ratio (<0.023) can be considered as a predictor of FwN onset.

Acknowledgments

We would like to thank Dr. Kumiko Kagawa, Hirokazu Miki, and Shiro Fujii, medical doctors at the Tokushima University Graduate School of Biomedical Sciences, who provided valuable support in this study. We also would like to thank Dr. Omar Marianito Maningo Rodis, Tokushima University Graduate School of Biomedical Sciences, who provided support in amending the manuscript. This study was supported by JSPS KAKENHI Grant Number 19K10432 and 19K08839 from the Japan Society for the Promotion of Science.

Statement of Author Contributions

The authors' contributions are as follows: Yuka Sogawa performed all experiments and drafted the paper. Makoto Fukui, Shingen Nakamura, Kimiko Sogabe, Ryohei Sumitani, and Masami Yoshioka obtained the clinical data from subjects and performed some of the experiments. Masahiro Abe contributed to the design and drafting of the paper. Daisuke Hinode designed, coordinated the study, and supervised in drafting the paper. All authors reviewed the paper critically for content and approved it for submission.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Figure legends

Fig. 1 ROC curve for the rate of *F. nucleatum*, the levels of sIgA and *F. nucleatum* / sIgA ratio as a predictor for FwN onset

Fig. 2. The Kaplan-Meier curves showing the cumulative hazard of FwN onset

Table 1 Type of hematological cancer of subjects in this study

	Total subjects (n=32)	
Type	n	%
Malignant lymphoma	24	75.0
Multiple myeloma	3	9.4
Acute myelogenous leukemia	3	9.4
Chronic myelogenous leukemia	1	3.1
Adult T-cell leukemia/lymphoma	1	3.1

Table 2 Participant characteristics according to the status of FwN onset

	Total subjects (n=32)		FwN subjects (n=11)		Non-FwN subjects (n=21)		
Items	n	%	n	%	n	%	p-value§
Gender（male）	19	59.4	7	63.6	12	57.1	1
Regimen							
Anthracycline	27	84.4	10	90.9	17	81.0	0.637
Ifosfamide	1	3.1	0	0.0	1	4.8	1
Cyclophosphamide	25	78.1	8	72.7	17	81.0	0.668
Etoposide	2	6.3	1	9.1	1	4.8	1
Cytarabine	5	15.6	3	33.3	2	9.5	0.31
Antibiotics	27	84.4	11	100	16	76.2	0.138
Diabetes	2	6.3	1	9.1	1	4.8	1
G-CSF	19	59.4	7	63.6	12	57.1	1
Smoking habit (current)	7	21.9	3	33.3	4	19.0	0.668
Items	median	25th, 75th percentile	median	25th, 75th percentile	median	25th, 75th percentile	p-value¶
Age (years)	62	52.8, 71.0	53	45.5, 59.5	68	59.0, 73.0	0.014
Albumin (g/dL)	3.9	3.3, 4.0	3.6	3.1, 4.0	3.9	3.5, 4.2	0.116
sIgA (μg/min)	156.7	64.7, 232.9	53.5	30.7, 156.7	190.7	94.6, 269.9	0.026
Protein (mg/min)	0.642	0.410,1.508	0.515	0.332,0.831	0.700	0.477,1.605	0.177
C. rectus (%)	0.024	0.009, 0.055	0.028	0.012, 0.069	0.021	0.006, 0.045	0.692
F. nucleatum (%)	0.405	0.077, 3.204	2.738	0.573, 5.225	0.233	0.042, 1.036	0.088
P. gingivalis (%)	0	0, 0.023	0	0, 0.050	0	0, 0.010	0.646

§: Fisher's exact test

¶: Mann-Whitney's U test

Table 3 Results from the cox-proportional hazard regression model depicting FwN onset

Covariates		Single variable analysis			Multivariable analysis*		
		HR†	95% CI	p-value	HR†	95% CI	p-value
Gender	Male§						
	Female	0.819	0.240 - 2.798	0.750	2.891	0.238 – 35.096	0.860
Age (year)		0.960	0.928 - 0.993	0.017	0.922	0.913 – 1.040	0.439
Smoking habit	Never or Former§						
	Current	1.336	0.354 - 5.042	0.669	0.306	0.041 - 2.264	0.246
Albumin (g/dL)		0.354	0.115 - 1.088	0.070	0.291	0.047 - 1.788	0.183
sIgA (μg/min)		0.992	0.985 - 0.999	0.031	0.984	0.970 - 0.999	0.038
F. nucleatum (%)		1.102	0.970 - 1.253	0.136	1.467	1.071 - 2.008	0.017
Regimen							
Anthracycline	No§						
	Yes	1.834	0.235 - 14.343	0.563	0.603	0.025 - 14.578	0.755
Cyclophosphamide	No§						
	Yes	0.709	0.188 - 2.673	0.611	1.205	0.077 -18.842	0.894
Cytarabine	No§						
	Yes	2.053	0.541 - 7.790	0.291	4.693	0.698 - 31.538	0.112
Diabetes mellitus	No§						
	Yes	1.241	0.159 - 9.711	0.837	0.475	0.030 - 7.557	0.598
G-CSF	No§						
	Yes	1.241	0.363 – 4.240	0.731	1.046	0.086 - 12.709	0.972

*: Adjusted by gender, age, smoking habit, albumin, *F. nucleatum*, sIgA, diabetes mellitus, G-CSF and regimen.

†: Hazard ration.

§: Reference category.

Table 4 ROC curve analysis regarding FwN onset

Items	AUC †	p-value	cut-off point	Sensitivity	Specificity
sIgA (μg/min)	0.745	0.008	159.446	0.818	0.619
F. nucleatum (%)	0.688	0.069	0.923	0.727	0.714
F. nucleatum / sIgA ratio	0.784	0.003	0.023	0.727	0.792

†: The area under the receiver operating characteristic (ROC) curve.

Table 5 Cox-proportional hazard regression model depicting FwN onset using *F. nucleatum* /sIgA ratio (n=32)

Covariates		Multivariable analysis*		
		HR†	95% CI	p-value
Gender	Male§			
	Female	1.967	0.273 - 14.194	0.502
Age (year)		0.937	0.869 - 1.011	0.094
Smoking habit	Never or Former§			
	Current	0.178	0.018 - 1.742	0.138
Albumin (g/dL)		0.170	0.018 - 1.634	0.125
F. nucleatum /	<0.023§			
sIgA ratio	≥0.023	66.062	3.645 - 1197.37	0.005
Regimen				
Anthracycline	No§			
	Yes	0.297	0.009 - 9.964	0.498
Cyclophosphamide	No§			
	Yes	0.614	0.573 - 23.608	0.784
Cytarabine	No§			
	Yes	3.679	0.020 - 3.681	0.170
Diabetes mellitus	No§			
	Yes	0.273	0.326 - 118.327	0.328
G-CSF	No§			
	Yes	6.215	0.100 - 118.967	0.224

*: Adjusted by gender, age, smoking habit, albumin, [*F. nucleatum*/ sIgA ratio], diabetes mellitus, G-CSF and regimen.

†: Hazard ration.

§: Reference category.

Figure 1

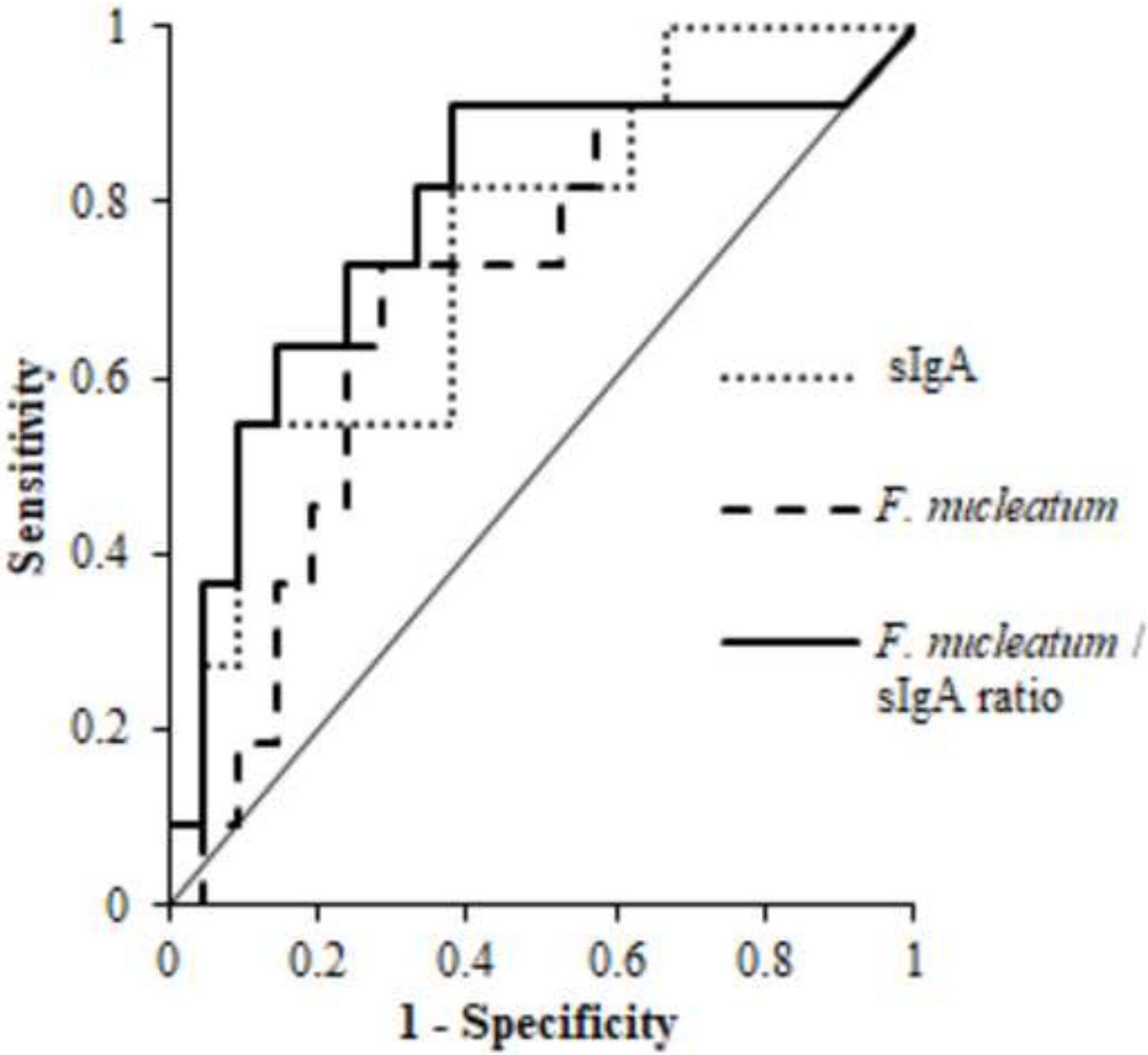

Figure 2