OPEN ACCESS

GAVIN PUBLISHERS

International Journal of Experimental and Clinical Research

Tsubata Y, et al. Int J Exp Clin Res: IJEACR-133.

Research Article

DOI: 10.29011/IJEACR-133. 000033

NAMPT Activity Before Cancer Chemotherapy may be Useful in Predicting Severe Neutropenia

Yukari Tsubata[1,4], Ryosuke Tanino[1,4], Mika Nakao[1,4], Tamio Okimoto[1,4], Chikamasa Yamashita[2,4], Hiroshi Ohtsu [3,4], Takeshi Isobe[1,4*]

[1]Department of Internal Medicine, Division of Medical Oncology and Respiratory Medicine, Shimane University, School of Medicine, Shimane, Japan

[2]Department of Pharmaceutics & Drug Delivery, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Tokyo, Japan

[3]Leading Center for the Development and research of Cancer Medicine, Graduate School of Medicine, Juntendo University, Tokyo, Japan

[4]MEXT (the Ministry of Education, Culture, Sports, Science and Technology)'s Promotion Plan for the Platform of Human Resource Development for Cancer Project, Japan

[*]**Corresponding author:** Takeshi Isobe, Department of Internal Medicine, Division of Medical Oncology & Respiratory Medicine, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan. Tel/Fax: +81853202580; Email: isobeti@med.shimane-u.ac.jp; ytsubata@med.shimane-u.ac.jp

Citation: Tsubata Y, Tanino R, Nakao M, Okimoto T, Yamashita C, et al. (2018) NAMPT Activity Before Cancer Chemotherapy may be Useful in Predicting Severe Neutropenia. Int J Exp Clin Res: IJEACR-133. DOI: 10.29011/IJEACR-133. 000033

Received Date: 22 September, 2018; **Accepted Date:** 01 October, 2018; **Published Date:** 05 October, 2018

Abstract

Aim: Febrile neutropenia, a fatal side effect, can be appropriately treated if we can predict its risk of development before starting chemotherapy. The Nicotinamide Phosphoribosyltransferase (NAMPT) activity and Sirtuin 1 (SIRT1) gene expression have been reported to be essential for identifying the neutrophils mediated by granulocyte colony-stimulating factor.

Methods: We measured the activity of the NAD^+-SIRT 1 pathway and Vitamin B3 (VB3) levels in cancer patients and examined correlations with grade 4 neutropenia due to chemotherapy.

Results: A total of 21 chemo-naïve small and non-small cell lung cancer patients were enrolled. We obtained blood samples before the first treatment, 2 and 4 days after starting treatment, at the nadir, and before starting the second course of treatment. NAMPT activity before starting treatment was significantly lower in the group with grade 4 neutropenia (N=9) than in those without neutropenia (N=12) (0.69 vs 1.63 ng/mL, p=0.003). SIRT1 gene expression and plasma concentrations of VB3 did not significantly differ between these two groups. On comparison with healthy volunteers, NAMPT activity was significantly higher in cancer patients, however no significant differences were noted between the grade 4 neutropenia group and grade 0-3 neutropenia group.

Conclusion: Our finding suggests that NAMPT activity before treatment may be a predictive factor for neutropenia with cancer chemotherapy. Further studies will be needed to confirm the role of the NAD^+-SIRT1 pathway in cancer patients receiving chemotherapy, and evaluate the association between NAMPT activity and patient background.

Keywords: NAMPT activity; Neutronenia; SIRT1 gene expression

Introduction

Neutropenia with cancer chemotherapy frequently causes treatment delays, dose reduction, and Febrile Neutropenia (FN) [1,2]. Several studies have examined the proportion and prevalence of neutropenia and FN with various types of cancers [3-5], and confirmed the patient risk factors for FN are elderly age, advanced disease state, poor performance status or nutritional sta-

tus, and presence of medical comorbidities [6]. However, little is known about the predictive markers for severe neutropenia, thereby hampering the ability to estimate the disease severity before starting chemotherapy. Scokowa et al. reported the Nicotinamide Phosphoribosyltransferase (NAMPT) activity and the Sirtuin 1 (SIRT1) gene expression-noticing NAD^+-SIRT1 pathway to be essential for differentiating the neutrophils mediated by Granulocyte Colony-Stimulating Factor (G-CSF) in healthy individuals and in individuals with congenital neutropenia [7].

The treatment of healthy individuals with high doses of vitamin B3 (nicotinamide; VB3), a substrate of NAMPT, induced neutrophilic granulocyte differentiation [7]. We therefore hypothesized that the NAD^+-SIRT1 pathway might be controlled in cancer patients who developed severe neutropenia after starting chemotherapy. An appropriate response to febrile neutropenia, which is a fatal side effect, can be ensured at the risk of developing neutropenia can be predict before starting chemotherapy.

In order to identify the predictive factors of neutropenia and suggest preventative and therapeutic regimens for severe neutropenia. We herein analyzed the predictive value of NAMPT activity, SIRT1 gene expression, and VB3 levels for neutropenia in cancer patients, with concurrent measurements in healthy volunteers. This study was supported by efforts from the promotion plan for the platform of human resource development for cancer.

Materials and Methods

Patients

A total of 21 chemo-naïve small and non-small cell lung cancer patients newly diagnosed at Shimane University Hospital were enrolled between October 2013 and August 2014. The eligibility criteria for the chemotherapy regimen were cisplatin and pemetrexed (±bevacizumab), etoposide, docetaxel, vinorelbine or irinotecan, carboplatin and paclitaxel (±bevacizumab), etoposide or nab- paclitaxel and docetaxel monotherapy. The major exclusion criteria were obvious bone marrow metastasis and the administration of corticosteroids, except as an antiemetic treatment.

Hematological toxicities were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0, and determined during the first course of chemotherapy. This study was reviewed and approved by the relevant institutional review boards of the Shimane University (IRB number: 1347), and written informed consent was obtained from all patients and healthy volunteers prior to participation.

Analysis of the NAD^+-SIRT1 Activity

We obtained blood samples from 21 patients before the first course chemotherapy, 2 and 4 days after starting chemotherapy, at the nadir, and before starting the second course of chemotherapy. We analyzed Complete Blood Cell Counts (CBCs), including number of neutrophils. The plasma NAMPT activity was measured by ELISA (Visfatin EIA Kit; Cayman Chemical, Ann Arbor, MI, USA), and VB3 levels were measured by bioassay (SRL, Tokyo, Japan). The mononuclear cell fraction was obtained from a density gradient using a cell preparation tube (BD Vacutainer® CPT; Becton, Dickinson and Company, NJ, USA). Reverse transcription polymerase chain reaction was carried out to measure SIRT1 gene expression at each point. We also sampled plasma and mononuclear cell fractions to assess NAMPT activity and SIRT1 gene expression in 19 healthy volunteers.

Statistical Analysis

All data were assessed using Student's t-test. P-values <0.05 were considered to indicate a statistically significant difference. All analyses were conducted using the SPSS software program version 19 (IBM, Okinawa, Japan).

Results

Patient Characteristics

The patient characteristics are summarized in (Table 1). Twenty-one patients with a median age of 64 years were enrolled in this study. Almost 70% were male. The histological classifications were 3 SCLCs and 18 NSCLCs, and clinical or pathological stage was adjuvant setting (stage IIA-IIIA) in 6 and recurrent or metastatic in 15. Nine patients had grade 4 neutropenia. (Figure 1) shows the change in the number of neutrophils after chemotherapy. The neutrophil count temporarily rose under the influence of a corticosteroid 2 to 4 days after starting therapy, before subsequently reaching its nadir.

	N=21
median age ,years (range)	64(55-86
Gender Male / Female	15/6
Type of cancer (SCLC/NSCLC)	3/18
Clinical orpathological stage (IIA/IIB,IIIA/IV)	2/2/2/15
ECOG performance status (0/1/2/3)	9/9/2/1
Chemotherapy regimen (N) CDDP/VNR (6), CDDP/PEM/BEV (5),DTX monotherapy(3), CDDP/GEM (3), CDDP/VP-16(2), CBDCA/PTX/BEV (1) , CBDCA/VP-16 (1)	
Abbreviations: SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; CDDP, cisplatin; VNR, vinorelbine; PEM, pemetrexed; BEV, bevacizumab; DTX, docetaxel; GEM, gemcitabine; VP-16, etoposide; PTX, paclitaxel; CBDCA, carboplatin	

Table 1: Patient characteristics.

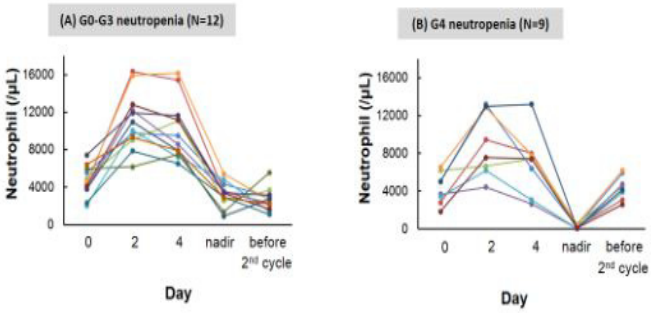

Figure 1: Neutrophil count after chemotherapy. The neutrophil count of grade 0-3 neutropenia patients (A) and grade 4 neutropenia patients (B). Data represented each patient in the line of the different color.

Assessment of NAD$^+$-SIRT1 activity

NAMPT activity at before starting chemotherapy was significantly lower in the group with grade 4 neutropenia (N=9) than in those without neutropenia (N=12) (0.69 vs 1.63 ng/mL, p=0.003) (Figure 2). With regard to SIRT1 gene expression, while a notable increase in expression was observed at the neutrophil nadir in the G0-3 neutropenia group, no significant differences were noted between this group and the grade 4 neutropenia group (Figure 3). The plasma concentrations of VB3 did not significantly differ between these two groups (data not shown). On comparison with healthy volunteers, the NAMPT activity was significantly higher in cancer patients, but no statistical differences were noted between the grade 4 neutropenia group and healthy volunteers (Table 2).

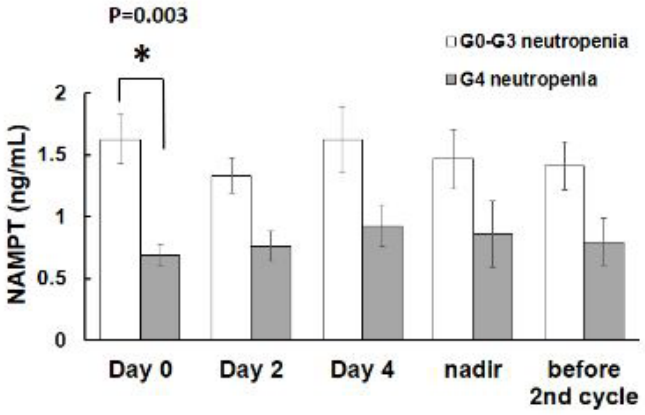

Figure 2: NAMPT activity before and after cancer chemotherapy. Data shown mean±SE. All data were assessed using Student's t-test.

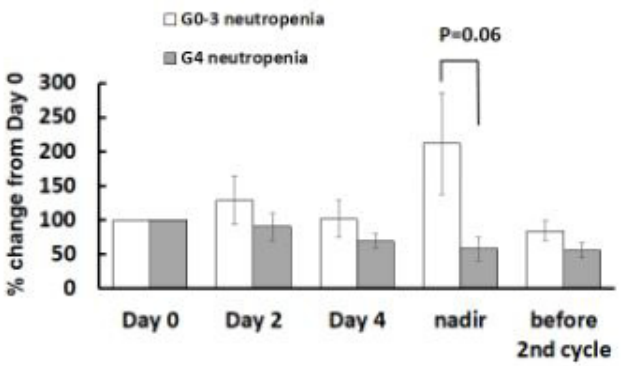

Figure 3: SIRT1 gene expression before and after cancer chemotherapy. Data shown mean±SE. All data were assessed using Student's t-test.

Group	NAMPT(ng/ml), mean±SE
Healthy volunteers (N=19)	0.90±0.08
cancer patients (N=21)	1.27±0.17
G0-3 neutropenia (N=12)	1.63±0.20
G4 neutropenia (N=9)	0.69±0.20
stage IIA-IIIA(adjuvant setting,N=6)	0.85±0.16
stage IIB-IV(metastatic,N=15)	1.48±0.23

Table 2: Comparison of NAMPT activity between lung cancer patients and healthy volunteers.

Discussion

This is the first report to examine the association between the NAD$^+$-SIRT1 pathway and neutropenia in cancer patients. The purpose of this study was to clarify the role of the NAD$^+$-SIRT1 pathway in risk of developing neutropenia after starting cancer chemotherapy. We demonstrated the following three points. First, the NAMPT activity was significantly lower in severe neutropenia patients before starting chemotherapy than in those without neutropenia. Second, the SIRT1 gene expression among patients with grade 0 to 3 neutropenia markedly increased in the nadir phase. Third, the NAMPT activity in advanced lung cancer patients was higher than in healthy volunteers.

NAMPT is a key biosynthetic enzyme converting nicotinamide to nicotinamide adenine mononucleotide, the main source of NAD$^+$[8]. An elevation of the NAMPT and NAD$^+$ levels in extracel-

lular and intracellular (myeloid cells) environments promotes the overexpression and activation of SIRT1 [8]. SIRT1 activation may therefore stimulate increased binding of these factors to promoters of genes encoding G-CSF and G-CSF receptor. NAD^+-dependent SIRTs regulate a variety of biological responses, such as the stress response, metabolism, aging and cell differentiation [8].

Given our present findings, we hypothesize that NAMPT induces a G-CSF autoregulatory loop via the following sequential mechanism: The decrease in the neutrophil number after starting chemotherapy induces activation of the NAD^+-SIRT1 pathway controlled by NAMPT. This positive autoregulatory system proceeds relatively smoothly in cancer patients with NAMPT activity before chemotherapy and it also recognizes an over expression of SIRT1 in the nadir phase. On the other hand, the production of NAD^+ is late or absent in patients with a low NAMPT activity who may develop severe neutropenia without recognizing any over expression of SIRT1.

A number of human malignant diseases have been shown to overexpress NAMPT, including lung, esophageal, and cervical cancers as well as lymphoma [9-13]. Further, several previous studies have reported that SIRT1 overexpression was associated with a poor prognosis in cancer patients. For example, Li et al. showed that SIRT1 expression in lung adenocarcinoma tissue as assessed using immunohistochemistry was significantly associated with a high pathological stage [9,10]. Similar findings were noted in the present study, as SIRT1 expression measured using mononuclear cells, was found to be high in lung cancer patients, particularly those in stage IV, compared with healthy volunteers. Given that the SIRT1 expression decreased in patients under adjuvant conditions with completely resected lung cancer, severe neutropenia may thus develop more easily under adjuvant conditions than in patients with metastasis.

Two limitations associated with the present study include the small size of the study population and the varied patient background. Further studies with larger, more homogeneous patient populations are needed to confirm whether or not the NAMPT activity and SIRT1 expression represent useful predictive markers of severe neutropenia. In addition, the utility of VB3 prophylaxis in preventing neutropenia and the role of SIRT1 gene expression stimulation in promoting cancer progression are unclear and warrant further examination.

Conclusion

We herein showed that the NAMPT activity before treatment may be a predictive factor for neutropenia with cancer chemotherapy. The role of the NAD^+-SIRT1 pathway in cancer patients receiving chemotherapy should therefore be examined further in future studies, and the association between the NAMPT activity and patient background should also be evaluated.

Acknowledgements

We thank all of the patients, their families and the volunteers. We also thank Ms. Michiko Horiguchi for assistance and technical advice.

Conflict of Interest

All authors have no conflict of interest to disclose.

Funding

This study was supported by MEXT's Promotion Plan for the Platform of Human Resource Development for Cancer Project.

List of Presentation

This study was presented at ASCO 2016, abstract published only. "A clinical study on activity of NAMPT and SIRT1 gene expression in neutropenia with cancer chemotherapy." J Clin Oncol 34, 2016 (suppl; abstr e21639).

References

1. Bennett CL, Djulbegovic B, Norris LB, Armitage JO (2013) Colony-stimulating factors for febrile neutropenia during cancer therapy. N Engl J Med 368: 1131-1139.
2. Culakova E, Thota R, Poniewierski MS, Kuderer NM, Wogu AF, et al. (2014) Patterns of chemotherapy-associated toxicity and supportive care in US oncology practice: A nationwide prospective cohort study. Cancer Med 3: 434-444.
3. Weycker D, Edelsberg J, Kartashov A, Barron R, Lyman G (2012) Risk and healthcare costs of chemotherapy-induced neutropenic complications in women with metastatic breast cancer. Chemotherapy 58: 8-18.
4. Stokes ME, Muehlenbein CE, Marciniak MD, Faries DE, Motabar S, et al. (2009) Neutropenia-related costs in patients with first-line chemotherapy for advanced non-small cell lung cancer. J Manag Care Pharm 15: 669-682.
5. Lyman GH, Abella E, Pettengel R (2014) Risk factors for febrile neutropenia among patients with cancer receiving chemotherapy: A systematic review. Crit Rev Oncol Hematol 90: 190-199.
6. Smith TJ, Bohlke K, Lyman GH, Carson KR, Crawford J, et al. (2015) Recommendations for the use of WBC growth factors: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 33: 3199-3212.
7. Skokowa J, Lan D, Kumar B, Wang F, Gupta K, et al. (2009) NAMPT is essential for the G-CSF-induced myeloid differentiation via a NAD+-sirtuin-1-dependent pathway. Nature Med 15: 151-158.
8. Shackelford RE, Mayhall K, Maxwell NM, Kandil E, Coppola D (2013) Nicotinamide phosphoribosyltransferase in malignancy: A review. Genes & Caner 4: 447-456.
9. Li C, Wang L, Zheng L, Zhan X, Xu B, et al. (2015) SIRT1 expression is associated with poor prognosis of lung adenocarcinoma. Onco Targets and Therapy 8: 977-984.
10. Grbesa I, Pajares MJ, Martinez-Terroba E, Agorreta J, Mikecin AM, et al. (2015) Expression of sirtuin 1 and 2 is associated with poor prognosis in non-small cell lung cancer patients. PLoS ONE 10: e0124670.
11. Cao B, Shi Q, Wang W (2015) Higher expression of SIRT1 induced resistance of esophageal squamous cell carcinoma cells to cisplatin. J Thorac Dis 7: 711-719.

12. Teramae M, Fukuda T, Wada T, Kawanishi M, Imai K, et al. (2015) Sirtuin 1 expression predicts the efficacy of neoadjuvant chemotherapy for locally advanced uterine cervical cancer. Mol Clin Oncol 3: 73-78.

13. Jang KY, Hwang SH, Kwon KS, Kim KR, Choi HN, et al. (2008) Sirtuin 1 expression is associated with poor prognosis of diffuse large B-cell lymphoma. Am J Surg Pathol 32: 1523-1531.