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QUARTERLY

Monitoring of urine nitric oxide (NO) related substrates and immunological competence in hematological malignancy★

Junko Tanaka✉, Kunio Koshimura, Michihiro Tsumori, Yoshio Murakami and Yuzuru Kato

First Division, Department of Medicine, Shimane Medical University, Izumo, Japan

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It has been reported that concentrations of neopterin in the urine are changed according to the host immunological conditions. In the present study, we measured urinary concentration of neopterin in patients with malignant hematological disorders and investigated the relationship between urinary neopterin levels and laboratory indices for cellular immunity. Urine neopterin levels were correlated with serum sIL-2R levels in the patients with malignant lymphoma, and inversely correlated with lymphocyte reactivity with ConA in the patients with acute myelocytic leukemia. However, no significant correlation was observed between urine neopterin levels and lymphocyte reactivity with phytohemagglutinin and pokeweed mitogen, CD4/8 ratio, $CD56^+16^+$ subset or serum IFN-γ levels. In the patients with malignant lymphoma, parallel changes in serum sIL-2R and urine neopterin were observed. The presented results suggest that urine neopterin levels are related to the activation of T cells in malignant lymphoma.

Recently, it has been revealed that nitric oxide (NO) is produced by T cells or macrophages [1–3], suggesting that NO is associated with cellular immunity. Tetrahydrobiopterin (BH_4), a cofactor for NO synthase (NOS), is produced when the cells involved in cellular immunity are activated [4, 5]. Furthermore, it has been reported that urine concentrations of neopterin, an intermediate product of BH_4, changes according to immunological conditions of the host [6, 7]. These results suggest that NO and BH_4 are involved

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✉Address for all correspondence to Junko Tanaka, First Division, Department of Medicine, Shimane Medical University, 89-1 Enya-cho, Izumo 693-8501, Japan; phone: (81) 853 20 2184; fax: (81) 853 23 8650; e-mail: jtanaka@shimane-med.ac.jp

Abbreviations: AML, acute myelocytic leukemia; BH_4, tetrahydrobiopterin; ConA, concanavalin A; ML, malignant lymphoma; MM, multiple myeloma; NO, nitric oxide; NOS, NO synthase; NP, neopterin; PHA, phytohemagglutinin; PWM, pokeweed mitogen; WBC, white blood cells.

in cellular immunity. In the present study, we measured urinary concentrations of neopterin in patients with malignant hematological disorders and investigated the relationship between urinary neopterin levels and laboratory indices for cellular immunity.

EXPERIMENTAL PROCEDURES

Urine samples for neopterin (NP) assay were collected from two patients with malignant lymphoma (ML), three patients with acute myelocytic leukemia (AML) and two patients with multiple myeloma (MM) who were hospitalized in Shimane Medical University Hospital. Among these patients, autoperipheral blood stem cells transplantation was carried out in Cases 1 and 6. Urine samples were collected at the time of admission, pre- and post-chemotherapy, and pre- and post-transplantation. To avoid the influence of inflammation, urine samples were not collected when patients were assumed to have inflammation as diagnosed by fever and increased serum C-reactive protein. Urine sample was centrifuged at 3000 r.p.m. for 10 min. The supernatant was stored at −80°C until assayed. Urinary neopterin levels were measured according to the method of Hibiya *et al.* [8]. Briefly, an aliqout of the urine supernatant (120 μl) was added to 60 μl of 6 M HCl and boiled at 100°C for 2 h. Then the sample was lyophilized and reconstituted with 120 μl of 50 mM ammonium phosphate (pH 3.0). The mixture was centrifuged at 4200 r.p.m. for 10 min. The supernatant was applied on HPLC with fluorescent detection (excitation: 350 nm, emission: 455 nm). As indices for cellular immunity, lymphocyte reaction with phytohemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM) were estimated using JIMRO Fluorometric Blastformation Test (Japan Immunoresearch Laboratories Co., Ltd., Takasaki, Japan), that is measured amount of nucleic acid of reacted lymphocyte by lectin (PHA, ConA and PWM). CD4/8 ratio and $CD56^{+}16^{+}$ cell subset were measured using flow cytometry. Soluble IL2 receptor (sIL2R) levels in the serum measured in SRL Inc. Laboratory. Serum interferon γ (IFN-γ) levels were estimated using ELISA assay kit (Amersham Pharmacia Biotech, Tokyo, Japan).

RESULTS

Immunological indices and urine NP levels of the patients are shown in Table 1. Serum sIL-2R levels of the patients with ML were much higher than those of the patients with AML and MM, and beyond the healthy control estimated in the Laboratory. Other immunological indices were within healthy control range and not different among the patients with ML, AML and MM. Urine NP levels did not differ among the patients with ML, AML and MM. Urine NP levels were correlated with serum sIL-2R levels in the patients with ML, but not AML or MM (Fig. 1). Regardless of the disease, urine NP levels varied markedly between samples from the same patient. The NP levels were inversely correlated with lymphocyte reactivity with ConA in the patients with AML (Fig. 2). No significant correlation was observed between urine NP levels and lymphocyte reactivity with PHA and PWM, CD4/8 ratio, $CD56^{+}16^{+}$ subset or IFN-γ levels. In the patients with ML, urine NP levels varied parallel to the serum sIL-2R levels (Fig. 3). In contrast, no relationship was observed between the profile of urine NP levels and lymphocyte reaction with ConA in the patients with AML.

DISCUSSION

Urine NP levels were remarkably elevated at some collection points in many patients beyond the healthy control levels [9]. Since NP is altered by immunological disorders [6, 7], it is

Table 1. Immunological data of patients with ML, AML and MM

	WBC (μ/ml)	PHA	ConA	PWM	sIL-2R (U/ml)	CD4/8J±t (μ/ml)	$CD56^+16^+$cell (μ/ml)	IFN-γ (pg/ml)	NP (nmol/mgCr)
Case 1 ML (62 y.o. F)									
after 2 courses of chemotherapy and recovery from bone marrow suppression	3700	1.3	1.3	1.3	3680	0.2		<0.6	16.83
after 3 courses of chemotherapy and recovery from bone marrow suppression	4700	2.0	1.6	1.5	2930	0.5	8.2	<0.6	20.50
after 3 courses of chemotherapy and bone marrow suppression state	500	2.0	2.7	2.5	1470	0.5	18.8	<0.6	12.23
after high dose chemotherapy and before PBSCC	2900	1.8	2.2	1.6	2990	0.6	15.2	<0.6	14.41
before PBSCT	300							<0.6	10.52
after PBSCT	100				1150	0.7	7.9	5.01	11.09
after PBSCT and recovery from bone marrow suppression	2600				812	0.1	7.8	1.0	14.08
follow up	1800								10.59
Case 2 ML (60 y.o. M)									
after chemotherapy and recovery from bone marrow suppression	8900				501				1.01
after chemotherapy and recovery from bone marrow suppression	2800	1.5	1.4	1.1	617	0.4		<0.6	3.31
after chemotherapy and recovery from bone marrow suppression	2700	1.3	1.2	1.6	617	0.3	3.3	<0.6	1.40
terminal	3700				2410			<0.6	5.89
Case 3 AML (33 y.o. F)									
relapse	3300	3.6	3.6	1.7	185	0.8	1.3	<0.6	0.42
after re-induction therapy and bone marrow suppression state	100							<0.6	1.11
after re-induction therapy and recovery from bone marrow suppression	1400	2.5	3.9	1.8	191	1.5	0.7	<0.6	1.13
after re-induction therapy and bone marrow suppression state	400	6.1			352	1.7	0.1	<0.6	26.38
after re-induction therapy and recovery from bone marrow suppression	1400							<0.6	0.40
after re-induction therapy and bone marrow suppression state	1660	2.9	2.7	1.9	237	0.9	3.4	<0.6	20.60

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Case 4 AUL (55 y.o. M)									
onset	5000	1.0	1.0	1.0	433	4.8	2.6	<0.6	22.20
after re-induction therapy and bone marrow suppression state	400	1.1	1.1	1.1	457	5	2.6	<0.6	10.79
after consolidation therapy 1 and recovery from bone marrow suppression	3550	1.7	1.8	1.9	447	1.5	17.6	<0.6	28.25
after consolidation therapy 2 and recovery from bone marrow suppression	2710		2.6	1.3	586	2.5	13.2		20.30
after consolidation therapy 3 and recovery from bone marrow suppression	2830	1.5	2.5	1.7	576	2.9	10.8	<0.6	17.80
Case 5 AML (66 y.o. M)									
after maintenance therapy 5 and recovery from bone marrow suppression	6600	1.2	1.1	1.0	521	3.4	7.6	<0.6	30.20
after maintenance therapy 6 and recovery from bone marrow suppression	5540	1.3	1.5	1.3	485	4.2	4.7	<0.6	13.50
Case 6 MM *(35 y.o. M)*									
after chemotherapy and recovery from bone marrow suppression	5700	2.0	1.9	1.0	535	1.1	15.3	<0.6	3.34
before high dose chemotherapy, due to PBSCC	7200	3.6	2.8	2.0	318	1.3	7.7	<0.6	0.55
after high dose chemotherapy and before PBSCC	12900	1.8	1.3	1.2	443	1.8	4.3	<0.6	0.94
after PBSCC	3800	4.0	2.8	2.1	290	1.0	7.5	<0.6	1.30
after chemotherapy and recovery from bone marrow suppression	3300	4.2	3.6	2.0	251	1.5	19.9	0.7	1.29
follow up	3200								1.24
before high dose chemotherapy, due to PBSCT	3500	21	2.1	1.4	259	0.9	9.4	4.05	24.30
after PBSCT	8420	1.8	1.2	1.1	583	0.2	8.6	1.15	30.50
Case 7 MM (Plasmacytoma) (68 y.o. M)									
after tumorectomy	2500	2.4	2.5	1.8	293	3.2	11.4	<0.6	4.17
after chemotherapy and recovery from bone marrow suppression	6000	2.0	1.2	1.4	451	2.8	15.2	<0.6	5.71
after chemotherapy and recovery from bone marrow suppression	2650	2.6	2.4	2.8	421	1.5	16.5	<0.6	2.39

possible that the marked variation of urine NP levels may be due to the immunological conditions of the host. Serum sIL-2R is considered to be a marker for T cell activation [10]. The significant elevation of sIL-2R in the patients with ML indicates that T cell activation was triggered by the pathological state of ML. Thus the correlation between urine NP and serum sIL-2R in the patients with ML suggests that urine NP is released from activated T cells. The parallel variation of urine NP with serum sIL-2R in the patients with ML raised

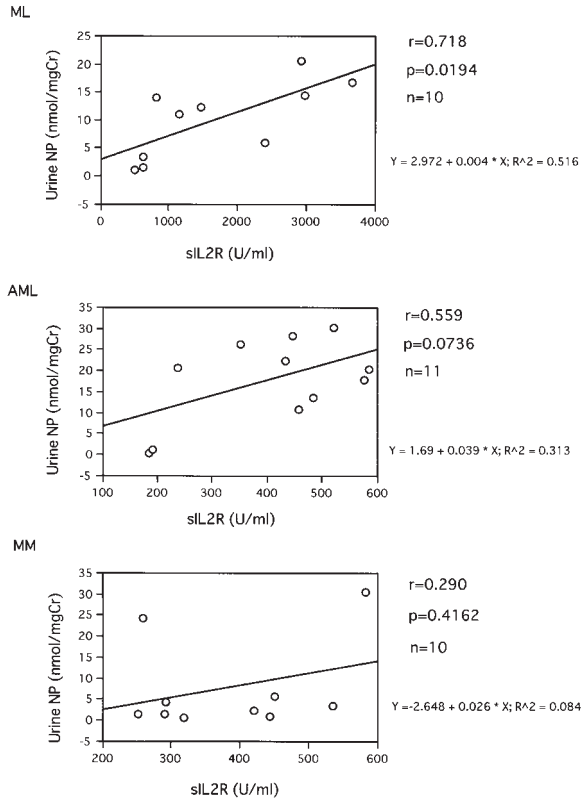

Figure 1. Correlation between urine neopterin (NP) and serum sIL-2R in patients with malignant lymphoma (ML), acute myelocytic leukemia (AML) and multiple myeloma (MM).

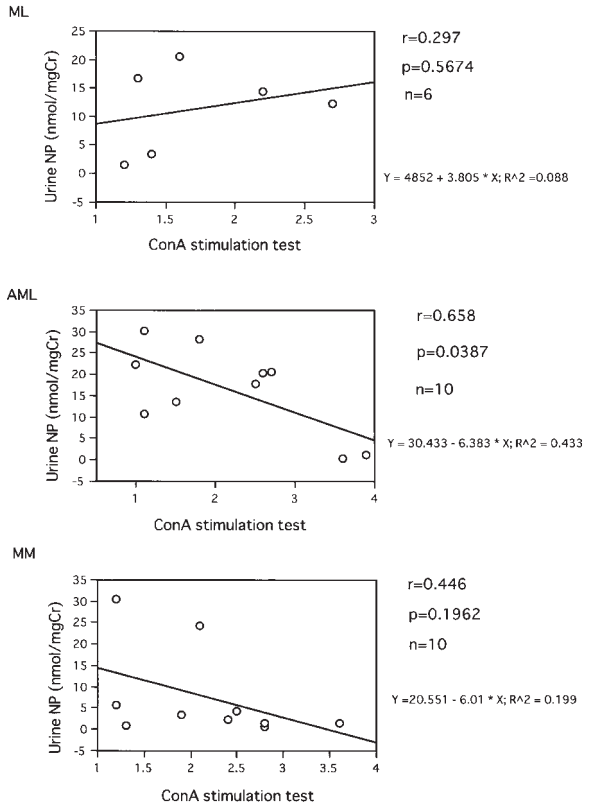

Figure 2. Correlation between urine neopterin (NP) and lymphocyte reaction with ConA in patients with malignant lymphoma (ML), acute myelocytic leukemia (AML) and multiple myeloma (MM).

the possibility that urine NP can be an index for T cell activation in the patients with ML.

In the patients with AML and MM, no correlation was observed between urine NP and se-

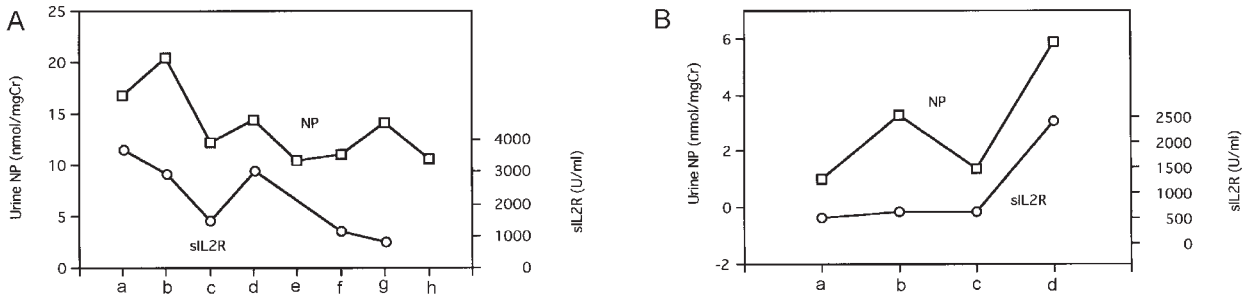

Figure 3. Profile of urine neopterin and serum sIL-2R in patients with multiple myeloma (ML).

A. Case 1: a, after 2 courses of chemotherapy and recovery from bone marrow suppression; b, after 3 courses of chemotherapy and recovery from bone marrow suppression; c, after 4 courses of chemotherapy and bone marrow suppression state; d, after high dose of chemotherapy and before PBSCC; e, before PBSCT; f, after PBSCT; g, after PBSCT and recovery from bone marrow suppression; h, follow up. **B**. Case 2: a, after chemotherapy and recovery from bone marrow suppression; b, after chemotherapy and recovery from bone marrow suppression; c, after chemotherapy and recovery from bone marrow suppression; d, terminal.

rum sIL-2R. In these patients, sIL-2R levels varied within the normal range. Thus it is assumed that urine NP levels are not as sensitive as the level of sIL-2R to T cell activation. The lymphocyte reaction with ConA is also a marker for T cell activation. From our data, the inverse correlation between urine NP and lymphocyte reaction with ConA in the patients with AML cannot be easily explained. Since no relationship was observed between the profile of urine NP and lymphocyte reaction with ConA in the patients with AML, urine NP is possibly affected by some factors other than T cell activation in AML. Taken together, these results suggest that urine NP level is correlated with the activation of T cells in some hematological disorders.

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