Kobe J. Med. Sci., Vol. 58, No. 5, pp. E128-E137, 2012

Significance of the Neutrophil Myeloperoxidase Index in Patients with Atherosclerotic Diseases

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Received 20 November 2012/ Accepted 17 December 2012

Key words: MPXI, Plasma myeloperoxidase concentration, Atherosclerosis, Ischemic heart diseases, Arteriosclerosis obliterans

The pattern of changes in the neutrophil myeloperoxidase (MPO) levels in various atherosclerotic conditions was analyzed by assessing the mean myeloperoxidase index (MPXI), which is calculated during the routine complete blood count (CBC) performed using the flow-cytochemistry blood autoanalyzer ADVIA120/2120 (Siemens), and plasma MPO concentrations. MPXI values of ischemic heart disease (IHD) patients did not differ from those of healthy volunteers. However, MPXI values of IHD patients with arteriosclerosis obliterans (ASO) (–6.1 ± 1.8) were significantly lower than those of IHD patients without ASO (0.8 ± 0.5). In contrast, the MPO values in IHD patients with ASO were significantly elevated. In subjects without IHD, while the MPXI values in mild cases of ASO (Fontaine's stages I/II, 3.4 ± 0.8) were significantly higher than those of healthy volunteers (0.4 ± 0.4), the values of those with severe ASO (stages III/IV, 0.3 ± 0.8) were significantly lower than those of mild cases. However, when ASO patients developed IHD, the MPXI values dramatically decreased (stages I/II, –7.3 ± 1.9; stages III/IV, –5.2 ± 1.6). These results indicate that MPXI is elevated in mild, but not in severe, ASO cases, and that MPXI decreases dramatically when ASO patients develop IHD. MPXI may constitute an informative independent biomarker for diagnosis and follow-up of IHD complicated by ASO.

In response to inflammatory events, activated neutrophils release myeloperoxidase (MPO), catalyzing the synthesis of a number of reactive species, including hypochlorous acid, chloramines, tyrosyl radicals, and nitrogen dioxide. While the synthesis of oxidants is employed by the host in defense against microorganisms, it also causes substantial oxidative damage at sites of inflammation. This includes coronary atherosclerotic lesions that cause ischemic heart disease (IHD) (13, 16, 20), where activated neutrophils infiltrate the fissured and thrombosed atherosclerotic coronary lesions (10), and subsequently degranulate, leading to accumulation of MPO in injured tissues (4).

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MPO-synthesized reactive species catalytically consume nitric oxide (NO), which presumably causes reduced local bioavailability of NO and impairs NO-dependent vasodilatation (6, 16). MPO has been implicated in both the oxidation of low-density lipoprotein, an early event in atherosclerosis (3, 16), and in high-density lipoprotein dysfunction (11, 16, 17). MPO is also implicated in the activation of metalloproteinases, leading to plaque destabilization and susceptibility to rupture (16). Thus, MPO has an important role in augmenting inflammation at coronary atherosclerotic lesions and in the development of IHD.

While little is known about the role of MPO in vascular lesions other than coronary atherosclerosis, it has been reported that MPO released by activated neutrophils plays a substantial role in systemic vasculitis with positive MPO-anti-neutrophil cytoplasmic antibodies (ANCA). The underlying mechanisms appear to be similar to those of the coronary arterial diseases (15).

The blood autoanalyzer ADVIA120/2120 (Siemens) calculates the mean myeloperoxidase index (MPXI) during an automated process of the white blood cell (WBC) differentials. The MPXI values, which are usually unrecognized in clinical practice, may be a useful supplementary indicator in the diagnoses of certain pathophysiological conditions. For instance, the MPXI increases in a few hematological malignancies, including chronic and acute myeloid leukemia (8, 14) and in megaloblastic anemia and megaloblastosis (7). During anticancer chemotherapy, the MPXI increases in the prenadir phase and decreases at the nadir phase (1). We have also previously reported that MPXI is low in bacterial sepsis, unchanged in viral and tuberculous diseases, and high in other bacterial infections (19).

The MPXI in activated neutrophils may be dependent on the net effect of azurophil degranulation, which results in lowered MPXI, and the stimulated synthesis of MPO in response to inflammation, which increases MPXI. Thus, MPXI can be attenuated, unchanged, or elevated in response to specific disease conditions and activities. Therefore, MPXI may show specific patterns that are distinct from plasma MPO or other biomarkers of inflammation, and further investigation of the associations between MPXI and specific pathological conditions may yield interesting findings.

It is known that plasma MPO concentration is elevated in parallel with coronary atherosclerosis, and high plasma MPO is reportedly associated with both the severity of coronary lesions and the prognosis of patients (2, 9, 12, 19, 20). However, nothing is known about MPXI changes in patients with atherosclerotic diseases. In this study, we provide evidence that MPXI is not correlated with IHD, MPXI is elevated in milder arteriosclerosis obliterans (ASO) cases but not in severe cases, and that MPXI decreases dramatically when IHD develops in ASO patients.

MATERIALS AND METHODS

Patients and normal volunteers

We evaluated 10 healthy volunteers or 20 normal subjects, and 111 consecutive patients with IHD and/or ASO (Tables I, II), whose complete blood counts (CBCs) were obtained from peripheral blood (*ad libitum*) using the ADVIA120/2120 system. Plasma MPO concentrations of healthy volunteers and patients with IHD were also evaluated. For all patients with IHD, blood drawn at the site of coronary arterial lesions during coronary catheterization was also evaluated.

IHD was defined as the presence of ≥50% stenosis in 1 or more coronary arteries during coronary catheterization. The patients' charts were reviewed either prospectively (patients with IHD) or retrospectively (patients with ASO). The cases of IHD included 8 of stable

angina pectoris (SAP), 9 of unstable angina pectoris (UAP), and 12 cases of acute myocardial infarction (AMI) (Table I). ASO was defined as an ankle-brachial blood pressure index (ABI) <0.9 in at least 1 leg. The presence of peripheral arterial lesion(s) was determined by angiography (5). The cases of ASO included 21 at stages I and II, and 22 cases at stages III and IV, according to Fontaine's classification (5) (Table II). The study was performed at the regional central hospital of Awaji Island in central Japan, where most of the island's population is referred. Therefore, the spectrum of diseases treated in this hospital represents the general spectrum of diseases in the regional population. All the studies were performed after the approval by the ethical committee of Kobe University (Japan), and were conducted in compliance with the World Medical Association Declaration of Helsinki.

Assays

The automated blood analyzer ADVIA120/2120 (Siemens) utilizes, in the process of WBC differentials, 4-chloro-1-naphthol as a substrate for the MPO within the granulocytes and black precipitates are produced in these cells. The stained WBCs pass to the flow cell, where light scatter (Y-axis) and absorbance (X-axis) are measured using a tungsten-halogen light source. The MPXI was defined as the deviation from the mean of the X-axis values of neutrophils from an archetypal population (19). Consistency of the MPXI values was assured by daily adjustments with TESTpoint Hematology Control (Siemens) and SETpoint Hematology Calibrator (Siemens). The accuracy and precision of the MPXI values calculated by ADVIA120/2120 were confirmed (19).

For quantitation of plasma MPO concentrations, ELISA was performed using the human serum myeloperoxidase ELISA kit (ImmunDiagnostik AG, Germany). Serum creatine kinase (CK) values were determined by spectrophotometry, using TBA200FR (Toshiba, Japan).

Statistics

The significance of differences between independent means was assessed by the Student's t-test, analysis of variance (ANOVA), or Kruskal-Wallis test. We considered a p value of <0.05 as statistically significant.

Table I. Sex and age of patients with IHDs, and co-morbidity of type 2 diabetes mellitus

	Healthy volunteer	SAP	UAP	AMI
Numbers	10	8	9	12
Sex (male/female)	6/4	6/2	6/3	9/3
Age (years)				
Median (range)	37.0 (22-55)	68.5 (49-86)	68.0 (57-91)	67.5 (39-88)
Mean ± SD	37.3 ± 10.7	68.0 ± 11.5	71.4 ± 11.7	62.5 ± 14.7
Diabetes mellitus	0	4	3	3

Table II. Sex, age and ABI index of patients with ASO, and co-morbidity of type 2 diabetes mellitus

	Normal subjects	ASO		ASO with IHDs	
		Stage I/II	Stage III/IV	Stage I/II	Stage III/IV
Numbers	20	21	22	10	10
Sex (male/female)	12/8	14/7	15/7	7/3	6/4
Age (years)					
Median (range)	51.5 (29-80)	66.0 (44-82)	68.0 (49-85)	73.0 (58-96)	65.0 (58-83)
Mean ± SD	52.5 ± 14.1	63.2 ± 10.7	65.8 ± 11.1	75.9 ± 9.8	69.8 ± 9.4
ABI '(Mean ± SD)	1.10 ± 0.08	0.74 ± 0.20	0.68 ± 0.31	0.68 ± 0.10	0.61 ± 0.15
Diabetes mellitus	0	6	7	4	5

RESULTS

MPXI and plasma MPO of peripheral blood in IHD

We have previously reported that while MPXI values exhibit a regular distribution among different individuals (range, –8.70 to 8.40), the MPXI values for each individual are intrinsically stable and constant at different time points (19). Therefore, a small change in the MPXI value in an individual may be significant and suggest a specific pathophysiological condition, even if the new value is within the abovementioned normal range.

Since previous reports have suggested elevated plasma MPO in IHD (2, 9, 12, 19, 20), we first investigated whether the MPXI values in IHD patients may also change. To compare values of both MPXI and plasma MPO between peripheral blood and catheter-drawn coronary arterial blood, only patients that underwent coronary catheterization were enrolled in the study.

The MPXI values of patients with various IHDs, including SAP (–1.2 ± 0.8, n = 8), UAP (–1.0 ± 1.5, n = 9), and AMI (0.9 ± 0.8, n = 12), were not significantly different from those of healthy volunteers (0.4 ± 0.5, n = 10) (Figure 1A). In contrast, the serum MPO concentrations in SAP (154.9 ± 25.3 ng/mL, n = 8), UAP (106.9 ± 21.7 ng/mL, n = 9), and AMI (157.8 ± 34.2 ng/mL, n = 12) patients were significantly higher than those in normal volunteers (29.5 ± 1.0 ng/mL, n = 10). Contrary to the previous studies that have reported higher plasma MPO in UAP and AMI compared to SAP (12), we observed prominently and similarly elevated MPO in all categories of IHD (Figure 1B).

While MPXI did not change significantly among different patient groups, individual cases did show changes in MPXI levels after acute IHD events. A typical course of AMI is shown in Figure 1C. MPXI was stable with a value of approximately 1 to 2 before the onset of AMI. At the onset of AMI, when the peak serum CK was 1988 IU/L and the WBC count was 12.88 × 109/L, the MPXI value changed inversely with the change in CK and WBC, decreasing rapidly to -3.3. The attenuation of MPXI continued for up to 1 week while serum CK was elevated, followed by a gradual increase in the MPXI values to near baseline levels over the next week. This indicates that MPXI appears to decrease during acute IHD events, at least in a few cases.

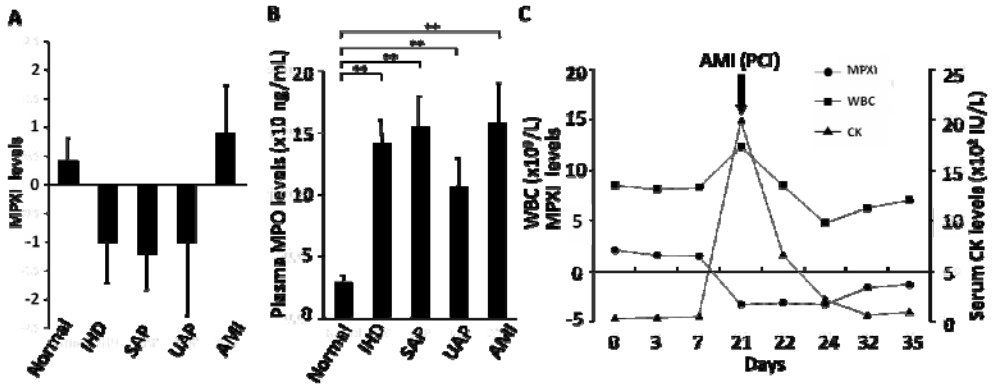

Figure 1. Peripheral blood MPXI and plasma MPO in various IHD patients. **A**: MPXI for various IHD categories is shown. **B**: Plasma MPO for various IHD categories is shown. The values represent the mean ± SE, and the significance of differences between independent means was assessed by the Kruskal-Wallis test (*$p < .05$, **$p < .01$) (**A**, **B**). **C**: Typical clinical course in a patient with AMI is shown. Upon the onset of AMI, MPXI was attenuated, and the attenuation continued for a few weeks.

The effect of co-morbidity of IHD and diabetes mellitus was then assessed. When the MPXI and serum MPO values in cases with co-morbidity were compared with those with IHD without diabetic complication, there was no significant difference in these values in each subgroup of IHDs (data not shown).

Peripheral and coronary arterial blood MPXI and MPO are comparable

It was hypothesized that neutrophilic MPXI or plasma MPO at the site of arterial lesions might differ from those of the peripheral blood. To investigate this possibility, catheter-derived arterial blood drawn from the sites of the coronary arterial lesions was compared with peripheral venous blood drawn at the same time for both MPXI and plasma MPO. The MPXI values were generally consistent between catheter-derived coronary blood (0.3 ± 0.6, n = 68) and peripheral blood (–0.2 ± 0.6, n = 68) (Figure 2A). Plasma MPO concentrations were also comparable between catheter-derived coronary blood (131.6 ± 30.4 ng/mL, n = 53) and peripheral blood (121.1 ± 34.1 ng/mL, n = 53) within each subject, but were variable between subjects (Figure 2B). Thus, peripheral blood MPXI and MPO appear to represent those of arterial lesions and suffices for analyses.

MPXI and prognosis of IHD

Since it has been reported that plasma MPO levels predict the prognoses of patients with IHD and that higher MPO levels are associated with less favorable outcomes (2, 9,12), we

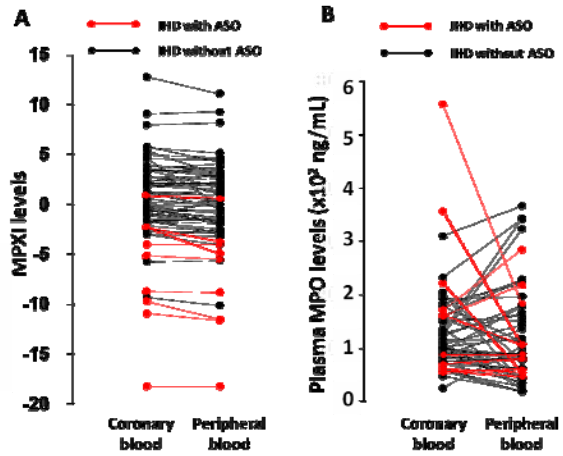

Figure 2. MPXI and serum MPO at coronary arterial lesions and in peripheral blood. Both MPXI (**A**) and serum MPO (**B**) values were approximately equivalent when comparing coronary arterial blood drawn at the sites of lesions by catheter, with peripheral venous blood, among patients with IHD. MPXI (**A**) and MPO (**B**) in IHD associated with ASO are denoted in red.

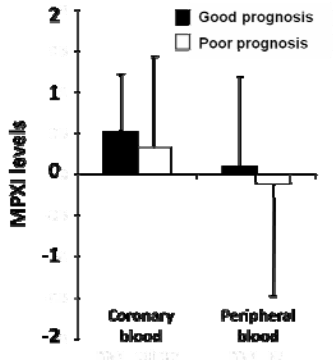

Figure 3. MPXI and prognosis. MPXI values of both coronary arterial and peripheral blood at initial presentation are indistinguishable between patients with good and poor prognoses, as defined by the absence and presence, respectively, of major cardiac events during the subsequent 6 months. The significance of differences between independent means was assessed by the Student's t-test.

next investigated whether MPXI might also predict the outcome of IHD. To this end, we prospectively followed the cohort patients for major adverse cardiac events (myocardial infarction, reinfarction, the need for revascularization, or death) after the initial coronary catheterization examinations. Poor prognosis was defined by morbidities of major adverse cardiac events within 6 months of catheterization-mediated coronary arterial intervention (2).

Among the cohorts that could be assessed, peripheral blood MPXI values did not differ significantly between the group with good prognoses and the group with poor prognoses (0.1 ± 0.8, $n = 13$, versus 0.1 ± 1.4, $n = 12$). Values of coronary arterial blood MPXI did not differ significantly between these groups either (0.5 ± 0.7, $n = 13$, versus 0.3 ± 1.1, $n = 12$) (Figure 3). These results suggest that MPXI levels do not predict the prognosis of IHD.

Co-morbidity of IHD and ASO is associated with lower MPXI

While investigating the MPXI values among various subpopulations with certain diseases, we noticed that patients with IHD complicated by ASO were among those with very low MPXI levels (Figure 2A). In contrast, however, plasma MPO concentrations did not appear to be related to the presence of ASO (Figure 2B).

Prompted by this observation, we reanalyzed the MPXI and plasma MPO values in terms of the absence or presence of ASO. Indeed, the peripheral blood MPXI values in patients with both IHD and ASO (–6.1 ± 1.8) were profoundly lower than those of patients with IHD but without ASO (0.8 ± 0.5) and of normal subjects (0.42 ± 0.45). The MPXI values of catheter-drawn coronary arterial blood in patients with both IHD and ASO (–5.4 ± 1.9) were also significantly lower than those of patients with IHD but without ASO (–1.2 ± 0.4) (Figure 4A). In contrast, the MPO values of both peripheral blood and coronary arterial blood plasma both in patients with IHD and ASO and in patients with IHD but without ASO

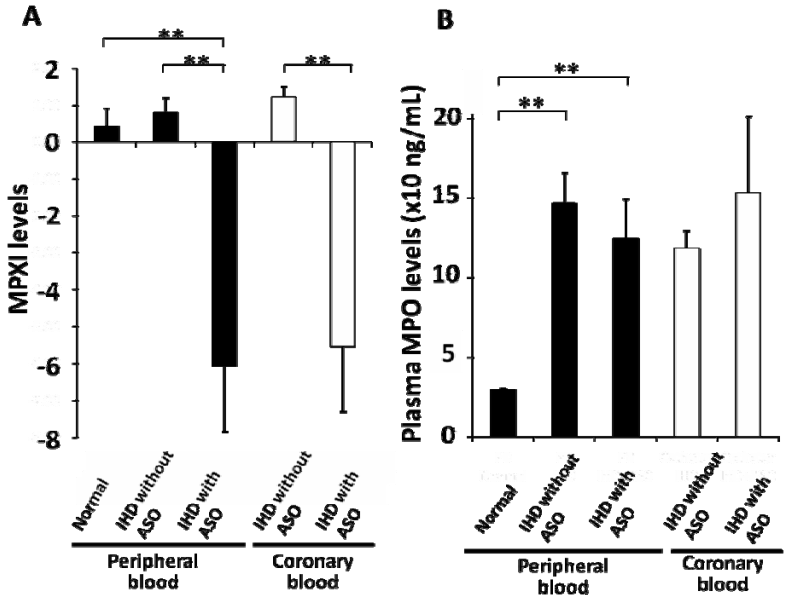

Figure 4. MPXI and plasma MPO in IHD with or without ASO. **A**: MPXI of coronary or peripheral blood in IHD patients with co-morbidity of ASO is lower than MPXI in IHD patients without ASO. **B**: Plasma MPO of coronary or peripheral blood is comparable among IHD patients with and without ASO. The values represent the mean ± SE, and the significance of differences between independent means was assessed by the Kruskal-Wallis test (peripheral blood) or the Student's *t*-test (coronary blood) (*$p < .05$, **$p < .01$) (**A**, **B**).

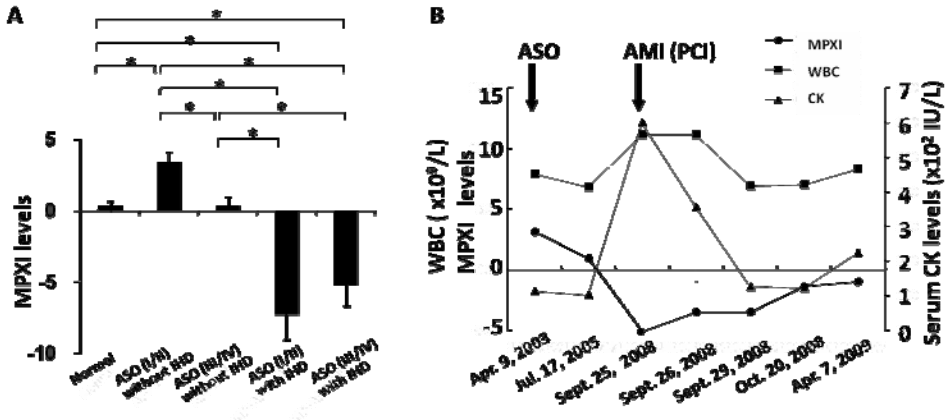

Figure 5. Distinct MPXI among patients with ASO, with or without IHD. **A**: MPXI values are elevated in milder ASO patients (Fontaine's stages I/II), but normalized in patients with more severe ASO (stages III/IV), when they do not suffer from IHD. However, when complicated by IHD, MPXI values decrease prominently in all ASO cases. The values are the mean ± SE, and the significance of differences between independent means was assessed by the one-way ANOVA (*p < .05, **p < .01). **B**: The typical clinical course of a patient with ASO complicated by AMI is shown. Upon the onset of AMI, MPXI is attenuated, and the attenuation continues over months.

were prominently and similarly elevated compared to normal levels (Figure 4B). These data suggest that co-morbidity of large atherosclerotic changes in peripheral arteries might be associated with decreases in MPXI, but not be related to plasma MPO.

We next investigated whether MPXI specifically changes in ASO, and retrospectively analyzed the charts of patients with ASO. In those without IHD, the MPXI values in milder cases (Fontaine's stages I and II, 3.4 ± 0.8; n = 21) were significantly higher than those of normal subjects (0.4 ± 0.4, n = 20). In contrast, the values in patients with more severe cases of ASO (Fontaine's stages III and IV, 0.3 ± 0.8; n = 22) were significantly lower than those with milder cases. Interestingly, when ASO was complicated by IHD, the MPXI values of the patients dramatically decreased (stages I and II, –7.3 ± 1.9; n = 10; and stages III and IV, –5.2 ± 1.6, n = 10) (Figure 5A).

The clinical course of a typical case is presented in Figure 5B. The patient presented with Fontaine's stage II ASO (co-morbidities were non-insulin dependent diabetes mellitus and hypertension) in 2003, which was followed by the gradual augmentation of ASO status to stage III over the next 5 years. MPXI gradually decreased from 3.1 (April 2003) to 0.9 (July 2005). In 2008, AMI developed and percutaneous coronary intervention (PCI) was performed. The peak serum CK was 599 IU/L and WBC count was 11.1 × 10^9/L. At this point, MPXI promptly and prominently decreased to –5.1. The MPXI then gradually increased over months but did not reach the baseline level previously observed in the patient.

The effect of co-morbidity of ASO and diabetes mellitus was then assessed. When the MPXI and serum MPO values in cases with co-morbidity were compared with those with ASO without diabetic complication, there was no significant difference in these values in each Fontaine's stages (I/II, and III/IV) (data not shown).

These data suggest that MPXI is elevated in mild ASO cases but not in more severe cases. It also suggests that MPXI decreases dramatically when IHD develops in ASO patients.

DISCUSSION

Using the MPXI values calculated routinely during automated CBC counting, we have shown that neutrophilic MPO activity is variable or unchanged in IHD per se, specifically upregulated in mild (stages I/II) ASO, but not in severe (stages III/IV) ASO cases, and attenuated when IHD and ASO are co-morbid. These findings may have important pathophysiological and diagnostic implications.

Pathophysiological implication of MPXI in atherosclerosis

MPO is mainly synthesized in promyelocytes and stored in azulophilic granules. When myelopoiesis is activated in response to inflammation, it is conceivable that MPO production is highly upregulated in these cells. In contrast, mature neutrophils do not synthesize, but store MPO. Meanwhile, activated neutrophils degranulate and release large amounts of MPO at the site of inflammation. These neutrophils possess reduced amounts of MPO as a result of consumption. Thus, MPXI is determined by both the levels of production in the bone marrow, and consumption in response to inflammation.

These considerations lead to the hypotheses that in the acute phase of inflammation MPXI is attenuated promptly after neutrophilic degranulation, and that attenuated MPXI gradually recovers as a result of stimulated production of MPO within premature granulocytes in the bone marrow. MPXI may even become elevated when the production of MPO is highly stimulated while the consumption at the site of inflammation is relatively small.

In IHD, plasma MPO increases prominently while MPXI remains largely unchanged both at atherosclerotic coronary lesions and in peripheral circulation. These facts suggest a balanced net effect resulting from an increase in the release of MPO from activated neutrophils, and stimulated production of MPO in the bone marrow. The prompt decrease in MPXI at the onset of acute coronary events and the subsequent gradual recovery of MPXI observed in selected cases may reflect the increased consumption of MPO in response to these events, and subsequent upregulation of MPO synthesis.

Atherosclerotic lesions of peripheral arteries such as those observed in ASO may similarly constitute strong stimuli triggering an increase in the production of MPO in the bone marrow. When ASO is at milder stages, the rate of production of MPO presumably exceeds the rate of consumption, resulting in elevated MPXI. When ASO is at more severe stages, it is conceivable that consumption increases, and thus MPXI is no longer elevated. The later stages of ASO, as well as IHD without ASO, may be comparable to this situation.

When ASO is complicated by IHD, the consumption of MPO presumably predominates and MPXI is attenuated. The attenuation should be prompt and profound at the time of IHD events, with a subsequent gradual recovery of the MPXI in response to enhanced stimulation of production of MPO, but the level of production may not be sufficient to recover the MPXI to the previous baseline level.

Finally, our study showed similarly and highly upregulated plasma MPO in all categories of IHD, in contrast to previous studies that have reported greater upregulation of MPO in UAP and AMI than in SAP (12). This discrepancy may reflect the fact that only patients that underwent coronary catheterization were enrolled in this study. Therefore, the disease status of the SAP group in our study may differ from that of previous studies.

MPXI as a supplementary diagnostic modality

This study indicates that MPXI levels show specific changes in patients with atherosclerotic diseases, particularly ASO and IHD. Since the MPXI level in each individual is relatively stable, even a small change observed in an individual may be informative, even if the new value remains within the normal range. Additionally, a sudden decrease in MPXI

in patients with atherosclerosis may indicate serious acute events in the coronary artery. Such decreases, together with other laboratory and physical tests, could prompt health staff to conduct appropriate additional investigations and where appropriate, emergency intervention.

Since the MPXI is a byproduct of the routine CBC conducted in everyday clinical practice, it can be utilized without incurring any additional costs. MPXI values change independently of other laboratory tests including WBC, serum CK, CRP, and MPO. MPXI values reflect the severity of ASO and could serve as a biomarker that indicates the efficacy of ASO treatment. In combination with other laboratory tests and physical examination, MPXI may serve as an ideal supplementary modality for correct diagnosis, and appropriate treatment and follow-up of ASO.

The MPXI may change in peripheral arterial diseases other than ASO and in few forms of vasculitis. If the MPO levels change in any of these diseases, MPXI may also change in response to the induced consumption of it. Given this possibility, it would be intriguing to examine the levels of MPXI in MPO-ANCA positive vasculitis.

ACKNOWLEDGEMENTS

We thank Mitsuhiro Yokoyama, Tetsuji Kado, Ryuichiro Nishimura, Taku Shirakawa, Tohru Murayama, Miyako Matsuo, Yuriko Yamauchi, and members in our Laboratory and in Labs of Hematology at Hyogo Prefectural Awaji Hospital and Hyogo Prefectural Cancer Center for helpful discussion. This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

CONFLICT OF INTEREST

Hisataka Ikeda is an employee of Siemens Healthcare Diagnostics, Tokyo, Japan.

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