First evaluation of real-time nitric oxide changes in the coronary circulation in patients with non-ischaemic dilated cardiomyopathy using a catheter-type sensor

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Aims
No direct method has yet been developed to measure real-time plasma nitric oxide (NO) concentration in humans. In this study, we evaluated a new method for measuring plasma NO concentration in patients with dilated cardiomyopathy (DCM) and in normal controls using a catheter-type sensor.

Methods and results
We simultaneously measured average peak velocity (APV) of the coronary artery flow and change in plasma NO concentration using the NO sensor placed in the great cardiac vein of 10 DCM patients and 10 control subjects. These evaluations were performed in response to sequential intracoronary infusions of acetylcholine (ACh, 10^{-8}–10^{-6} M), N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA, 200 \textmu mol) and co-infusion of ACh and L-NMMA. The change in plasma NO concentration in DCM patients was significantly impaired compared with the control group (P < 0.01). Pretreatment with L-NMMA completely suppressed the ACh-induced NO concentration, whereas APV in the left anterior descending coronary artery was partially suppressed in both groups. Plasma NO concentration reached its peak value later than the maximum APV following the injection of ACh (10^{-6} M) in both groups.

Conclusion
The catheter-type NO sensor could be applied to clinically evaluate the endothelial function (i.e. reduced endothelium-derived NO bioavailability) in patients with cardiovascular diseases.

Keywords
Acetylcholine • Microcirculation • Nitric oxide

Introduction
The endothelium has a crucial role in regulating vasomotor tone,1 platelet activity,2 leucocyte adhesion,3 and vascular smooth muscle proliferation through the release of several paracrine factors, and particularly nitric oxide (NO). In the clinical setting, endothelial dysfunction can be evaluated in several ways, resulting in the increased understanding of the mechanisms underlying the development of atherosclerosis. However, it has been very difficult to measure real-time NO levels, because NO is oxidized or inactivated by dissolved oxygen, oxyhaemoglobin, and/or reactive oxygen species (ROS) immediately after release by vascular endothelial cells into the blood stream.5,6

Berkels et al.7 reported a method for measuring NO using an NO-selective electrochemical sensor. They demonstrated for the first time that basal and agonist-induced NO release could be...
measured with an electrochemical electrode by converting nitrite back to NO. This sensor has enabled us to evaluate dynamic changes in NO concentration in solutions and in tissues in response to agonists. From this, we have developed a catheter-type NO sensor which can directly measure intra-arterial NO concentration in vivo.8–10 The NO sensor showed high sensitivity and high selectivity to NO compared with various NO-related reagents.9,10 We also showed more recently that the chronic application of angiotensin II reduces the plasma NO concentration induced by acetylcholine (ACh).11 Furthermore, the NO sensor enabled us to record accurate changes in plasma NO levels as a result of release from the endothelium in response to ACh infusion. Using this sensor, we previously demonstrated the dynamic state of NO in a canine model.8

After carefully examining the safety of this method for human use, we have carried out the first real-time measurements of dynamic changes in NO concentration in the coronary circulation in patients with non-ischaemic dilated cardiomyopathy (DCM) and in normal controls.

Methods

Study population

The study design was approved by the ethical committee of Wakayama Medical University. Written informed consent was obtained from each subject after a full explanation of the possible complications (e.g. coronary sinus rupture, arrhythmia, and hypotension). From April 2007 to January 2009, 12 DCM patients were admitted to our hospital with congestive heart failure. DCM was defined as the presence of left ventricular ejection fraction (LVEF) dysfunction (ejection fraction <40%), but without coronary artery disease (determined by coronary angiography), valvular disease, myocarditis, or secondary cardiomyopathy (determined by echocardiography and laboratory findings). After medical treatment, 10 DCM patients were enrolled in the study. As the control group, we selected 10 sex-matched patients with normal left ventricular function and no evidence of coronary artery disease, who underwent cardiac catheterization for chest pain or palpitations. The control group was selected from June 2007 to November 2008, taking the age range (40–70 years) into consideration. Both groups consisted of three women and seven men. Age was not significantly different between patients and controls; the average age of the patients was 56±10 years (43–70 years) and that of controls was 58±7 years (45–68 years). Eight of the 10 patients in the control group had been admitted due to incessant palpitations and underwent electrophysiologic catheterization. For the procedure, a 6-French (Fr) guiding catheter was inserted via the coronary sinus to record the mitral annulus potential using an electrical catheter. Following the electrophysiologic study, coronary angiography was performed to rule out coronary artery disease in symptomatic patients, and then this study protocol was started.

Cardiac catheterization

Following diagnostic catheterization, heparin was administered (total dose 5000 IU), and a coronary-infusion catheter equipped with a 0.014-inch Doppler guide wire (Flowire, Volcano Inc., Mountainview, CA, USA) was positioned in the left anterior descending (LAD) coronary artery via a 6-Fr Judkins guiding catheter. A coronary-infusion catheter was positioned distal to the first major septal branch, and a Doppler guide wire was positioned distal to the infusion catheter to monitor coronary flow velocity. An NO sensor was positioned at the proximal end of the great cardiac vein (GCV) via a 7-Fr Amplatz guiding catheter to continuously monitor the plasma NO concentration (Figure 1). Blood in the GCV was obtained at 4 min after the administration of 30 μg ACh. Then, the plasma nitrite level was measured by the Griess reaction to compare the NO levels with those obtained using the NO sensor.

Quantitative coronary angiography and coronary velocity measurements

Coronary arteriograms were recorded and analysed using a Philips angiographic system (Philips Medical, The Netherlands). We measured the coronary artery diameter at a point 5 mm distal from the tip of the Doppler wire. An appropriate view that permitted clear visualization of the site was selected. The diameter was measured three times by two examiners who had no knowledge of the patients’ clinical characteristics, and the average value was used for analysis. Inter- and intra-observer reproducibilities were high (r = 0.96 and 0.98, respectively).

The average peak velocity (APV) of flow in the LAD was continuously monitored using a fast Fourier transform-based spectrum analyser (FloMap, Cardiometrics Inc.). Systemic arterial pressure and heart rate were continuously recorded. The steady-state signals of APV, blood pressure, and heart rate were used for analysis.

Real-time measurements of nitric oxide in the coronary circulation

The performance of the amperometric NO-selective sensor (amin-o-700XL, Innovative Instruments, Tampa, FL, USA) has been previously described.11 In brief, the NO sensor was mounted in a 4-Fr catheter (1200 mm long; Hirakawa Hewtech) and fixed with silicon adhesive. Polyurethane was attached to the detection tip to prevent physical damage of the vessel wall, and two metal wires were also attached along the detection tip to provide mechanical support to the electrodes. The oxidative current generated by NO was monitored using an NO monitor (model inNO-T, Innovative Instruments). The sensor could not measure the absolute level of circulating NO because of the differences between the calibration sites and the measuring site. Therefore, we evaluated ACh-induced plasma NO levels as the peak response in the current from the baseline over the entire period, which is expressed as ‘change in plasma NO concentration (nM)’ based on the calibration.

Study design

All cardiovascular drugs were withheld for 24 h before the start of the study. The same study protocol was used for all patients (Figure 2). Acetylcholine was infused into the LAD via the infusion catheter at 5 mL/min for 2 min in the following order: (i) three 2-min infusions of ACh at 0.15, 1.5, and 15 μg/min, yielding estimated intracoronary concentrations of 10−8, 10−7, and 10−6 M, respectively; (ii) a 5-min infusion of Nω-monomethyl-L-arginine (L-NMMA) (Calbiochem) at 40 μmol/min; and (iii) three ACh infusions at the above three concentrations immediately after the L-NMMA infusion.

Systemic arterial pressure, heart rate, 12-lead electrocardiogram, APV in the LAD, and changes in plasma NO concentration in the GCV were continuously measured throughout the study. In addition, coronary angiography to measure the diameter of the LAD at the target site was performed at the time points indicated in Figure 2.
Assay of nitric oxide concentration

Twenty blood samples from 10 DCM patients and 10 control subjects were obtained through the catheters with the use of disposable syringes and centrifuged immediately at 300 rpm for 5 min at 4°C. Plasma was stored at −80°C until use. Plasma samples were diluted 1:2 with deionized water and filtered through a 0.45-μm microfilter. Plasma nitrite and nitrate concentrations were measured with an NOx analyzer (Eno10, Eikon, Kyoto, Japan) with high-performance liquid chromatography and the Griess reaction. In brief, the samples were first passed through a column to separate nitrate from nitrite. Then, the samples were passed through a second column containing copper-coated cadmium to reduce the nitrate from nitrite. Next, the nitrite was detected by reaction with the Griess reagent (0.03 mol/L sulfanilamide plus 0.15 mol/L HCl solution containing 1.0 × 10⁻³ mol/L N-[1-naphthyl] ethylenediamine). Absorption was measured at 546 nm with use of a spectrophotometer. Thus, the separate nitrite and nitrate levels could be measured simultaneously. The detection limit of the assay was 1.0 × 10⁻⁸ mol/L.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The non-parametric Mann–Whitney U test was used to compare the numerical clinical data between the DCM group and the control group. Categorical variables were compared by the χ² test or Fisher exact test, as appropriate. Statistical analyses for comparison of changes in plasma NO concentration and coronary flow velocity were conducted by
In addition, as previously reported, no significant changes in the plasma NO concentration of saline did not cause any change in the plasma NO concentration. The incidence of hypertension was significantly higher in the DCM group compared with the control group (Cohen’s d = 1.5 and 1.7, respectively; P < 0.01 by Mann–Whitney U test). As shown in Table 3, the ACh (0.3, 3, and 30 μg)-induced increase in NO concentration was almost completely inhibited by pretreatment with L-NMMA in both the DCM and the control groups (Cohen’s d = 0.3, 0.1, and 0.3, respectively; Table 3).

### Results

#### Study population

A comparison of demographic data between the studied DCM group and the control subjects is shown in Table 1. No significant differences were observed with respect to age, smoking habit, or the incidence of diabetes mellitus and/or hyperlipidaemia between groups. The incidence of hypertension was significantly higher in the DCM group than in the control group (P < 0.05). On the other hand, the incidence of LVEF was significantly lower in the DCM group than in the control group (P < 0.01).

#### Validation of no sensor

To evaluate the NO level using another methodology, we measured NO change by the Griess reaction, which detects nitrite based on the reaction with sulphonic acid to form the diazonium ion. There was a weak but significant correlation between the NO levels measured by the catheter-type NO sensor and the nitrite level measured by the Griess reaction (r = 0.41, P = 0.048) (data not shown).

We used saline as a control marker of the NO sensor. Infusion of saline did not cause any change in the plasma NO concentration. In addition, as previously reported, no significant changes in the baseline current were observed with and without mixing, suggesting no direct (primary) effect of fluid (blood) motion on measurement of the NO sensor.

#### Changes in coronary nitric oxide production induced by acetylcholine and N^G^-monomethyl-L-arginine

Figure 3 shows representative tracings of the change in plasma NO concentration in the GCV after intracoronary infusion of ACh at different concentrations (0.3, 3, and 30 μg) and infusion of ACh immediately after L-NMMA in the DCM and control groups. The ACh induced change in plasma NO concentration in the DCM and the control groups (Table 2). Acetylcholine increased the plasma NO concentration in a concentration-dependent manner only in the control group. The changes in NO concentration after 3 and 30 μg ACh infusion were significantly blunted in the DCM group compared with the control group (Cohen’s d = 1.5 and 1.7, respectively; P < 0.01 by Mann–Whitney U test). As shown in Table 3, the ACh (0.3, 3, and 30 μg)-induced increase in NO concentration was almost completely inhibited by pretreatment with L-NMMA in both the DCM and the control groups (Cohen’s d = 0.3, 0.1, and 0.3, respectively; Table 3).

#### Estimation of coronary flow velocity

The ratio of APV increase by ACh ((ACh infusion−baseline) × 100/ACh infusion) in both the DCM and the control groups was changed in a concentration-dependent manner. The changes in APV (%) after 0.3, 3, and 30 μg ACh infusion were significantly blunted in the DCM group compared with the control group (Cohen’s d = 1.9, 1.8, and 1.9, respectively; P < 0.01 by Mann–Whitney U test; Table 2). The changes in APV after 0.3 and 3 μg ACh infusion were not significantly different between the DCM group and the control group (Cohen’s d = 0.6 and 0.7, respectively; Table 3). Interestingly, the change in APV in response to 30 μg ACh infusion after L-NMMA was significantly blunted in the DCM group compared with the control group (Cohen’s d = 1.1; P < 0.01 by Mann–Whitney U test; Table 3), which suggests that the ACh-induced increase in the APV was partially suppressed by pretreatment with L-NMMA in contrast to the response plasma NO concentration.

#### Real-time changes in coronary diameter, coronary blood flow, and nitric oxide production induced by acetylcholine stimulation

During ACh infusion, APV and NO concentration were measured simultaneously using a Doppler guide wire and an NO sensor, respectively. Figure 4 shows APV and NO concentration plotted every 5 s in a representative control subject. A comparison of dynamic changes in NO in response to ACh between the DCM and the control groups is shown in Figure 5. The APV and plasma NO concentration response starting times following the ACh injection (30 μg/min) were 20 ± 2 and 72 ± 11 s in the control group and 20 ± 2 and 82 ± 13 s in the DCM group.

### Table 1 Characteristics of the study population

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<th>DCM</th>
<th>Control</th>
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<td>No. of patients</td>
<td>10</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>56 ± 10 (43/70)</td>
<td>58 ± 7 (45/68)</td>
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<tr>
<td>Sex (male/female)</td>
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<td>Smoking habit</td>
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<td>Hypertension</td>
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<td>0.043</td>
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<td>Hyperlipidaemia</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
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<td>LVEF (min/max)</td>
<td>41 ± 6 (32/49)</td>
<td>67 ± 5 (61/78)</td>
<td>&lt;0.01</td>
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</table>

Categorical data were compared by the χ² test. The non-parametric Mann–Whitney U test was applied to evaluate differences in LVEF between two groups. Data are expressed as mean ± SD or number: DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction.
respectively. In the control group, APV reached its peak value 77 ± 12 s after intracoronary ACh infusion, whereas the NO concentration started to increase 1 min after Ach infusion and reached its peak value after 230 ± 45 s. In the DCM group, APV reached its peak value after 79 ± 12 s and NO concentration started to increase at approximately the same time as in the control group.

**Discussion**

No direct method has been developed so far to measure real-time plasma NO concentration in humans. In this study, we evaluated a new method to measure real-time plasma NO concentration in the coronary circulation in patients with DCM and normal control using a catheter-type sensor. The results of the present
study showed for the first time that the NO sensor could be applied to clinically evaluate endothelial function (i.e. reduced endothelium-derived NO bioavailability) in patients with cardiovascular diseases.

Rather than directly measuring NO concentrations, most previous studies have assessed the vasomotion after intracoronary injection of ACh or by flow-mediated dilation (FMD) to evaluate coronary endothelial function. However, evaluation of vascular endothelial function based on these two methods depends on the normal function of vascular smooth muscle.\(^{13}\) If both the basal tone and the response of vascular smooth muscle during the procedure are impaired, the utility of this measure is poor. Furthermore, the data are influenced by multiple endothelium-derived vasodilators other than NO and by vasoconstrictors and neural control of the vascular smooth muscle. Thus, FMD and vasomotion after ACh injection may not be adequate parameters of NO bioavailability and endothelial function. NO is immediately oxidized or inactivated in the blood stream after its release from vascular endothelial cells. However, Rassaf et al.\(^{14}\) suggest that NO can be transported in its bioactive form for significant distances along the vascular bed and may therefore exert remote effects. If this supposition proves true, then direct measurement of plasma NO concentration is necessary to characterize the kinetics and physiological role of NO and to evaluate endothelial function. In this study, we demonstrated for the first time that the catheter-type NO sensor are now allowing direct measurement of human plasma NO concentrations in coronary circulation, which will provide a powerful tool for the characterization of NO physiology and pathophysiology and may identify greater opportunities for therapeutic modulations in humans.

From an analytical point of view, detection of NO in biologic fluids is challenging, and several methods for determining NO in different cells and fluids have been reported, such as the Griess reaction, a bioassay,\(^{15}\) an oxyhaemoglobin assay,\(^{16}\) electron paramagnetic resonance,\(^{17}\) and chemiluminescence-based high-performance liquid chromatography. Compared with these reported methods, the advantage of the NO sensor is that it can measure accurately real-time NO concentration in the GCV in humans. The concentration of endothelium-derived NO measured in plasma using the NO sensor may be affected by various factors including: (i) oxidation or inactivation by dissolved oxygen, oxyhaemoglobin, and/or ROS, if present, and (ii) trapping and transport by haemoglobin and other thiol compounds including albumin and glutathione. In this study, we measured remaining plasma NO, which reflects in vivo NO bioavailability, in the coronary sinus.

**Table 3** Effect of intracoronary infusion of N\(^{G}\)-monomethyl-L-arginine on acetylcholine-induced coronary epicardial diameter, average peak velocity, and plasma NO concentration in patients with dilated cardiomyopathy and control subjects after infusion of N\(^{G}\)-monomethyl-L-arginine

<table>
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<th>DCM</th>
<th>Control</th>
<th>d&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in coronary epicardial diameter (%)</td>
<td>0.3</td>
<td>−0.6 ± 0.2</td>
<td>−0.7 ± 0.3</td>
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<tr>
<td>Acetylcholine (µg)</td>
<td>3</td>
<td>−3.4 ± 0.8</td>
<td>−0.6 ± 0.3</td>
<td>1.8</td>
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<tr>
<td></td>
<td>30</td>
<td>−8.0 ± 0.1</td>
<td>−7.3 ± 1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Change in average peak velocity (%)</td>
<td>0.3</td>
<td>0.6 ± 0.6</td>
<td>0.2 ± 0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Acetylcholine (µg)</td>
<td>3</td>
<td>5.1 ± 0.8</td>
<td>5.8 ± 0.9</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>24 ± 3.9</td>
<td>30 ± 5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Change in NO concentration (nM)</td>
<td>0.3</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Acetylcholine (µg)</td>
<td>3</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. The Mann–Whitney U test was used to compare between the DCM group and the control group. \(^{a}\)Values for effect size were expressed as Cohen’s d (the difference between two means divided by an SD for the total data.).

**Figure 4** Real-time profiles of nitric oxide (NO) concentration and averaged peak velocity. Representative real-time plot of changes in NO concentration and averaged peak velocity in the distal left anterior descending coronary artery (LAD).
Therefore, it is less likely that the levels of free NO measured would give an overall picture of the function of the endothelium. However, in the present study, pretreatment with L-NMMA completely suppressed ACh-induced NO concentration even in the control group. Thus, the measured NO level may reflect NO bioavailability in whole blood even if the NO sensor is located in flowing blood.

Measurement of coronary diameter, coronary blood flow, and real-time changes in plasma NO concentration led to three major findings concerning coronary artery performance. (i) ACh-induced epicardial reactivity evaluated by quantitative coronary angiography as well as plasma NO concentrations in the coronary circulation were almost completely inhibited by pretreatment with L-NMMA, whereas microcirculatory coronary reactivity evaluated using a Doppler guide wire was partially inhibited. (ii) The NO concentration started to increase ≈50 s after the APV increase; this was similar in the DCM and the control groups. (iii) Both the peak change in plasma NO concentration and the total period of NO increase were reduced in the DCM group compared with the control group.

ACh is clinically used as a stimulus to assess endothelial function. However, we cannot exclude the possibility that the increase in ‘NO levels’ is due, at least in part, to an increase in flow because ACh increases both plasma NO concentration and blood flow. The associated elevation of shear stress may then further increase the plasma NO concentration by a positive feedback mechanism. In contrast, L-NMMA is a specific inhibitor of NO synthase and intracoronary infusion of L-NMMA reduces the basal and ACh-induced increase in coronary blood flow in humans. In this study, L-NMMA almost completely inhibited ACh-induced NO increase in the GCV and coronary dilatation in both the DCM and control groups, whereas it partially suppressed coronary blood flow in the LAD in both groups. Microvascular endothelial function has been evaluated based on the change in coronary blood flow, which is mostly regulated by the resistance of arterioles 200 μm or less in diameter. Therefore, our results strongly support the long-standing hypothesis that ACh-induced conduit endothelial vasodilation is largely mediated by NO, whereas microcirculatory vasodilation appears to be mediated only partly by NO, and mainly by other factors including endothelium-derived hyperpolarizing factor (EDHF).

The peak plasma NO concentration was reached later than the maximum APV after the injection of ACh in the DCM and control groups. The delayed effect of NO in vasorelaxation may be attributable to three mechanisms. First, ACh-induced vasodilation is caused not only by NO but also by other factors, particularly in the early phase. Indeed, there are many factors that may influence coronary vasodilation in addition to NO, including EDHF, ATP-dependent K+ channels, and prostaglandins. Thus, the coronary vascular response to ACh may not be primarily determined by NO. Second, the discrepancy between NO and APV timing may be partly due to differences in measuring sites. In this study, the NO sensor was placed in the coronary sinus, and a Doppler guide wire was located in the LAD. Most NO, once released from vascular endothelial cells into the blood stream, is immediately degraded by superoxide, if present. We measured the remaining plasma NO, which reflects in vivo NO bioavailability, in the coronary sinus. The relatively long distance between the vessel and the point of NO assessment might lead to the delayed effect of NO on vasorelaxation. Third, the delayed increase in NO following the increase in APV may reflect the fact that the increase in NO may be secondary to increased flow and shear stress rather than the direct effect of ACh on the endothelium. That is, the associated elevation of shear stress induced by the increased blood flow following ACh infusion may further increase the plasma NO concentration via a positive feedback mechanism. Furthermore, any flow-induced release of endogenous ACh from the endothelial cells may, at least in part, contribute to the sustained elevation of plasma NO concentration.

Figure 5 Comparative profiles of nitric oxide (NO) concentration and averaged peak velocity. Comparative plot showing changes in NO concentration and averaged peak velocity in 10 dilated cardiomyopathy (DCM) patients and 10 control subjects. Statistical analyses for comparison of changes in plasma NO concentration and averaged peak velocity were conducted by paired t-test. *P < 0.01 compared with time 0.
We also found that the plasma NO concentration in the human coronary circulation during ACh infusion (30 μg, 2 min) was approximately 12.0 nM in normal subjects and 2.3 nM in DCM patients with severe left ventricular dysfunction. With regard to normal subjects, our data are consistent with the results from a study in which a different type NO metre was used to measure NO concentration in the internal mammary artery and the saphenous vein taken from patients undergoing coronary surgery.23

Limitations
First, although direct NO measurement in the coronary artery might provide valuable information to assess endothelial function of the epicardial conduit artery, it is currently impossible to insert the catheter-type NO sensor in the coronary artery due to its rigidity and size. Second, we used 200 μmol L-NMMA cumulatively in the present study in accordance with previous reports.24,25 This dose would be ideal for evaluating plasma NO concentration based on dose-dependent changes induced by L-NMMA infusion because it might provide more detailed information about basal NO in the coronary circulation. We did not use a higher L-NMMA dose in order to avoid secondary changes in coronary blood flow caused by elevated arterial pressure and hence increased myocardial oxygen demand. Third, magnetic resonance imaging examination should be performed in all patients to rule out secondary cardiomyopathy including myocarditis because the diagnosis of DCM is an exclusive one. However, we did not do so in this study. Finally, although we evaluated NO change by the Griess reaction to examine the NO levels with another methodology, there was a weak, but significant, correlation between the NO levels measured by the catheter-type NO sensor and nitrite levels measured by the Griess reaction ($n = 10$, $p = 0.048$, $r = 0.41$). The weak correlation may be due to the difference in the detection limit. That is, the detection limit of the sensor was reported to be 0.08 nM,26 whereas that of the Griess assay was 10 nM.12 Irrespective of underlying reasons, the validation study could not provide us with complete confidence.

Conclusions
In this study, we demonstrated for the first time that the catheter-type NO sensor allows the accurate measurement of real-time plasma NO concentration in the coronary circulation of non-ischaemic DCM patients and control human subjects. We confirmed the impairment of NO in DCM patients. More importantly, the NO sensor may be applied to clinically evaluate endothelial function and may open new diagnostic and therapeutic possibilities in patients with cardiovascular diseases. Further research on real-time plasma NO measurement in patients with endothelial dysfunction would clarify the interaction between the dynamic state of NO and coronary artery disease.

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Conflict of interest: none declared.

References
A 28-year-old man was admitted in the intensive care unit (ICU) for severe hypoxaemia with venous jugular distension 48 h after acute onset of asthenia without chest pain. Cardiovascular risk factors comprised only tobacco use. Anterior myocardial infarction (MI) was suspected because of elevated troponin level associated with a severe apical, septal, and anterior wall akinesia (see figure). ECG showed normal sinus rhythm with persistent ST-segment elevation and Q wave in the anterior precordial leads. Interestingly, apical akinesia extended to the right ventricular (RV) apex with a thrombus located along the right septal wall (Panels A and C). Anterior wall transmural necrosis extended to RV with a thrombus located in the RV apex, which was confirmed by magnetic resonance imaging (MRI) (Panel B); and coronary angiogram showed a monotoncular proximal left anterior descending coronary artery occlusion with smooth coronary arteries, suggestive of acute plaque rupture in a severe tobacco smoker. Revascularization was not attempted because of the delay between symptoms onset and presentation, and lack of viability on MRI. In addition, computed tomography scan showed a right segmental pulmonary embolism (Panel D). During hospitalization, the follow-up was marked by a pericardial effusion that required emergency pericardio-centesis at day 6 and a rapid ventricular tachycardia with cardiac arrest at day 8 successfully treated by external cardioversion. Implantable cardioverter defibrillator was implanted at day 16 and the patient was discharged from ICU. During follow-up no other cause (thrombophilia or embolic) was found other than heavy tobacco use. In patients with MI, RV extension is usually considered as a complication of inferior wall ST-elevation myocardial infarction (STEMI). However, MRI study reported that apical RV infarction during anterior MI is common. Importantly, the prevalence of RV infarction was similar in patients with anterior or inferior (65 vs. 47%) STEMI. In addition, a recent experimental study (Bodi et al. Cardiovasc Res 2010) demonstrated that prolonged left anterior descending occlusion may result in a large area of RV necrosis (30 ± 5%).

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