**Serum oxidative-anti-oxidative stress balance is dysregulated in pulmonary hypertensive patients with liver cirrhosis: a case control study**

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**Abstract**

**Objective:** Hepatopulmonary syndrome (HPS) is characterized by vascular dilatation and hyperdynamic circulation, while portopulmonary hypertension (POPH) is characterized by vasoconstriction with fibrous obliteration of the vascular bed. Vasoactive molecules such as nitric oxide (NO) are candidate factors for cirrhotic complications. However, oxidative stress balance is not well characterized in HPS and POPH. The present objective is to investigate the oxidative stress and anti-oxidative stress balance and NO pathway balance in patients with potential HPS and POPH.

**Methods:** We recruited decompensated cirrhosis patients (69 potential HPS and 61 potential POPH) admitted to our hospital as liver transplantation candidates. Patients exhibiting $pO_2$ lower than 80 mmHg and $AaDO_2 \geq 15$ mmHg were categorized as potentially having HPS (23/69). Patients exhibiting a tricuspid regurgitation pressure gradient $\geq 25$ mmHg were categorized as potentially having POPH (29/61). Serum reactive oxygen metabolites and anti-oxidative OXY-adsorbent tests (OXY) were performed and the balance of these tests was defined as the oxidative index. The correlation between these and clinical characteristics were assessed as a cross-sectional study.

**Results:** Potential HPS patients exhibited no correlation with oxidative stress markers. Potential POPH patients exhibited lower OXY (p=0.037) and higher oxidative index (p=0.001). Additionally, the vascular NO synthase enzyme inhibiting protein asymmetric dimethylarginine

was higher in potential POPH patients (p=0.049). The potential POPH patients exhibited elevated $AaDO_2$, suggesting the presence of pulmonary shunting.

**Conclusions:** Potential POPH patients exhibited elevated oxidative stress with decreased anti-oxidative function accompanied by inhibited NO production. Anti-oxidants represent a candidate treatment for potential POPH patients.

**Background**

Portopulmonary hypertension (POPH) and hepatopulmonary syndrome (HPS) are liver

cirrhosis complications that exhibit opposite features in the pulmonary vasculature(1). POPH is

defined as pulmonary arterial hypertension associated with portal hypertension. The current

criteria defining POPH are as follows: 1) the presence of portal hypertension, with or without

complicating cirrhosis; 2) hemodynamic measurements from right heart catheterization

including mean pulmonary artery pressure > 25 mmHg at rest, mean pulmonary capillary

wedge pressure < 15 mmHg, and pulmonary vascular resistance > 240 dyn-s-cm-5 or >3 Wood

units. These criteria indicate that POPH is a form of pulmonary hypertension not due to left

ventricular heart failure in portal hypertensive patients. In comparison, current criteria for HPS

are as follows: 1) arterial deoxygenation (a widened alveolar-arterial oxygen gradient ($AaDO_2$)

with or without hypoxemia); 2) intrapulmonary vascular dilation (diagnostic procedures are not

defined); 3) complicating liver diseases. These criteria explain why the hallmark of HPS is

intrapulmonary vasodilatation with pulmonary arterial shunting resulting in arterial

deoxygenation. There is no explanation why the pathophysiologically opposite phenomenon

occurs in liver diseases.

HPS and POPH exhibit poor outcomes that require treatment. One year after diagnosis, the

natural course of POPH results in mortality rates of up to 54%(2). Similarly, 2.5 years after

HPS diagnosis mortality has been reported to be 40 to 60%(3-6). Importantly, orthotopic liver

transplantation (OLT) is not beneficial for severe POPH and is regarded as a contra-indication, while > 85% of HPS patients experience a successful outcome with OLT within one year(7). The treatment strategies for POPH are the same as primary pulmonary hypertension specific treatments, such as prostacyclin analogues, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors. While these drugs promise to improve pulmonary arterial hypertension, cardiologists restrict their use to severe forms of POPH because of their numerous side effects and very high cost. For HPS, OLT is the only treatment that holds promise. Some reports found that Allium sativum L. (garlic) exhibits nitric oxide (NO) inhibitory effects and improves hypooxygenation in HPS(8, 9).

Mediators of vasodilation, such as NO and prostaglandin $I_2$, may be decreased in POPH. NO is generated by the NO synthase (NOS) enzyme family, including inducible NO synthase (iNOS) and endothelial NO synthase (eNOS), and is expressed in a variety of cells, such as macrophages, hepatocytes, and endothelial cells. NOS inhibitors, such as asymmetric dimethylarginine (ADMA), regulate NO production. ADMA is inactivated by hydrolysis to citrulline and dimethylamines by the action of dimethylarginine dimethylaminohydrolases (DDAH)-1 and 2(10). The DDAH-ADMA-NOS pathway controls NO production and regulates endothelial motility. The interaction between NO and reactive oxygen species (ROS) is an important factor in determining their net effects. While NO can inhibit the generation of oxygen radicals, NO-related cytotoxicity can occur in the presence of high ROS levels because of its

interaction with either $O_2$ or $O_2^-$ to generate $N_2O_3$ or peroxynitrite. The imbalance between vasodilators and vasoconstrictors may ultimately affect the outcome of POPH or HPS. Oxidative stress is recognized as one of the causes of chronic liver disease progression. The vascular endothelium is susceptible to oxidative stress and can be targeted with anti-oxidative treatment. Since liver mitochondria can serve as a source of ROS, chronic liver disease could induce oxidative stresses.

The objective of the present study was to investigate the balance between oxidative stress and anti-oxidative mechanisms, as well as the role of NO-related enzymes in patients with decompensated liver diseases complicated by potential POPH and potential HPS. Although patients with confirmed POPH and HPS are rare, patients with pulmonary hypertension (potential POPH) and pulmonary shunting (potential HPS) could be targeted with preventive therapies. Preventing progression to HPS and POPH is important for reducing mortalities in liver cirrhosis patients. Revealing the precise mechanisms of these diseases is necessary to identify novel treatment options. For these reasons, we evaluated oxidative stress conditions and the NO related pathway in decompensated liver cirrhosis patients with potential HPS or POPH.

Serum levels of reactive oxygen metabolites (ROM) were determined as a marker of circulating ROS(11, 12). The OXY-adsorbent test (OXY) was also performed to evaluate the corresponding anti-oxidative status(13). Clinical parameters related to liver cirrhosis and

cardio-pulmonary function were confirmed in relation to ROM, OXY, oxidative index, and NO pathway-related enzymes.

**Methods**

*Subjects*

The study group consisted of 69 liver cirrhosis patients who were admitted to our hospital according to a pre-transplantation general assessment (Table 1A). All patients were recruited at the Clinic of Gastroenterology and Hepatology, Okayama University Hospital, from December 2009 to December 2013. The baseline liver diseases were 33 hepatitis C virus (HCV)-related cirrhosis, four hepatitis B virus (HBV)-related cirrhosis, 12 non-alcoholic steatohepatitis (NASH) cirrhosis, and 20 other etiologies. Twenty-one patients (30.4%) had complications associated with hepatocellular carcinoma (HCC). The median total bilirubin level was 4.0 mg/dL and the Mayo end stage liver disease (MELD) score was 16, reflecting decompensated liver cirrhosis. The patients were classified according to severity of pulmonary shunting, presenting with elevated $AaDO_2$ levels and pulmonary arterial pressure. The patients with higher $AaDO_2$ were defined as potentially having HPS (23 cases) and others as non-HPS (46 cases). The $AaDO_2$ threshold was 15 mmHg as defined by a European Respiratory Society task force(14). As POPH is defined using right coronary catheterization, it was impossible to conclusively diagnose our patients. Therefore, we categorized patients according to the median

range of transthoracic echocardiographic measurement of tricuspid regurgitation peak gradient (TR-PG) (mmHg). As with the noninvasive estimation of pulmonary vascular resistance by Doppler echocardiography in patients with pulmonary arterial hypertension, TR-PG has been shown to have the highest positive correlation coefficient with pulmonary vascular resistance(15). Other candidate causes of pulmonary hypertension, such as left-heart disease, valvular heart disease, interstitial or obstructive pulmonary disease, sleep-related breathing disorders, and chronic thromboembolism(16), were excluded by general examination performed during pre-transplantation assessment. The TR-PG data was obtained in 61 patients. The patients with TR-PG greater than or equal to 25 mmHg (29 cases) were defined as potential POPH and others were as potential non-POPH (32 cases). In addition, patients exhibiting both potential HPS and POPH (15 cases) were compared with patients exhibiting neither HPS nor POPH (26 cases) to define their characteristics (Table 1B).

Serum levels of ROM and OXY were determined and the balance between ROM and OXY was defined as the oxidative index. Correlation analyses between ROM, OXY, oxidative index, NO pathway-related enzymes and clinical characteristics were performed.

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the ethical guidelines for analytical research on the human genome/genes in 2001 Japan as reflected in approval by the ethics committee at Okayama University Hospital. After obtaining written

informed consent, a detailed medical questionnaire was completed by doctors.

*Blood sample collection and preparation*

Fasting blood samples were collected from patients. Serum samples were collected at the time of admission or at the outpatient clinic, with no intervention performed prior to collection. The collected blood samples were centrifuged at 3000 rpm for 5 minutes to isolate serum. Serum aliquots were stored at -80°C until subsequent analysis. The obtained samples were used to determine serum levels of biochemical markers (ROM and OXY, and ADMA). For arterial blood gas analysis, femoral arterial blood or brachial arterial blood was drawn.

*Measurement of ROM serum levels*

Measurement of ROM serum levels (ROM) was performed using a spectrophotometer (Diacron International, Grosseto, Italy), as reported previously[11]. The Carratelli unit (CARR U), where one CARR U corresponds to 0.08 mg/dL hydrogen peroxide, was used as the measurement unit.

*Measurement of total serum anti-oxidant capacity*

In order to determine total serum anti-oxidant capacity, data from the OXY-adsorbent test was collected using a spectrophotometer (Diacron International)[13]. This test evaluates the capacity of serum to counteract the massive oxidative action of a hypochlorous acid (HClO) solution, with total anti-oxidant capacity expressed in terms of HClO (μmol) consumed by one mL of sample (μmol HClO/mL).

*Calculation of oxidative-anti-oxidative balance*

The balance between oxidative stress and anti-oxidative capacity was calculated as an oxidative index. To utilize parameters with different measurement units, the standardized values of ROM and OXY were assessed using the formula reported by Vassale et al(17). The formula is as follows: *sv-var = (v-var － m-var) / ds-var,* where *sv-var* represents the standard value of the oxidative index, *v-var* corresponds to the original value, and *m-var* and *ds-var* are the mean and standard deviation of the parameter.

*Measurement of ADMA*

The concentration of the NOS inhibiting enzyme ADMA was measured by HPLC, using pre-column derivatization with o-phthalaldehyde after isolation of samples with carboxylic acid solid-phase extraction cartridges (SRL Inc., Tokyo, Japan).

*Immunohistochemical staining*

Immunohistochemical staining of the oxidative stress marker 4-hydroxynonenal (4-HNE) and the NO-related enzymes DDAH-1 and eNOS was performed. Liver samples were fixed in 10% formaldehyde and sections were incubated with a 1:100 dilution of a primary antibody for 4-HNE (clone HNEJ-2; JaICA, Shizuoka, Japan) at 4°C overnight. Subsequently, the sections were incubated with a secondary antibody (LSAB + System-HRP; Dako, CA), universal biotinylated link antibody, and streptavidin-HRP. The slides were subsequently stained with fresh 3,3'-diaminobenzidine chromogen (Dako, Liquid diaminobenzidine + Substrate

Chromogen System). For eNOS staining, the primary antibody (Cell Signaling Technology, Danvers, MA) was used at a 1:200 dilution. Quantitative comparison of 4-HNE and eNOS staining was performed by computerized image analysis with Olympus cellSens imaging software (Olympus, Tokyo, Japan). For DDAH-1 staining, the primary antibody (Abcam, Tokyo, Japan) was used at a 1:100 dilution. As DDAH-1 staining patterns were classified into hepatocyte and ductular patterns, which was not suited for automated calculation, staining strength of hepatocyte cytoplasm was assessed in a blinded manner by two hepatologists (TY and FI). Negative control staining was evaluated with mouse IgG as the primary antibody for 4-HNE and rabbit IgG for eNOS and DDAH-1, according to the origin of the primary antibodies.

*Statistical analysis*

JMP software (Version 9.0.0, SAS Institute Inc., NC) was used to conduct the statistical analysis. Continuous variables were expressed as median (interquartile range) and the Mann-Whitney *U*-test or chi-squared test was used to compare parameters between potential HPS and potential non-HPS or potential POPH and potential non-POPH. Correlations between oxidative stress-related markers and NO-related markers were analyzed using Spearman's rank correlation method. Statistical significance was set at $P < 0.05$. Univariate analysis was performed to identify the potential factors correlated with oxidative stress-related markers. Age and HCV positivity, which are widely accepted as oxidative stress factors, and any variables

yielding $P < 0.05$ in the univariate analysis were analyzed further by multivariate analysis to identify independent factors correlated with oxidative stress-related markers.

**Results**

*General characteristics of potential HPS and potential POPH*

The potential HPS patients exhibited higher prothrombin time international ratio (PT-INR) and moderately elevated model for end stage liver disease (MELD) scores, indicative of severe cirrhosis (Table 1). The potential POPH patients exhibited significantly lower diastolic blood pressure only, with no difference in liver function tests. The potential HPS with POPH patients exhibited significantly higher MELD scores.

*Blood gas analysis and transthoracic echocardiography (TTE)*

Blood gas analysis and TTE observations for the present cohorts are shown in Table 1B and C. The potential HPS and potential non-HPS patients showed no definite difference in TTE parameters (Table 1). The potential POPH patients exhibited elevated $AaDO_2$, reflecting HPS with complications. These patients exhibited diastolic left ventricular disorder as higher E / e', which is indicative of the presence of a diastolic disorder despite not reaching the diagnostic value of >15(18).

*Serum oxidative stress-related markers in all patients*

Differences in the serum oxidative stress marker ROM, OXY, and the oxidative index,

sub-divided according to clinical findings, are summarized in Table 2. In multivariate analysis,

higher OXY was correlated with higher systolic blood pressure, while higher oxidative index

was correlated with higher TR-PG.

*Oxidative stress-related markers and NOS-related markers in all patients*

Immunohistochemical staining for the oxidative stress marker 4-HNE was positively

correlated with oxidative index, although the correlation with ROM was not statistically

significant (Figure 1A, B). Staining for the ADMA inhibiting enzyme DDAH-1 was classified

into hepatocyte and ductular patterns, and the hepatocyte staining-pattern was further assessed

(Figure 2A). Consistent with expectations, patients with strong hepatocyte DDAH-1 staining

exhibited lower serum ADMA levels and lower oxidative index (Figure 2B). Hepatic eNOS

staining was negatively correlated with hepatic 4-HNE staining and serum ROM levels (Figure

2C). Serum ADMA levels were positively correlated with ROM and oxidative index. In

summary, strong oxidative stress or oxidative balance correlated with weak hepatocyte

DDAH-1 staining, weak hepatic eNOS staining, and increased NOS inhibiting ADMA levels.

*Oxidative stress-related markers and NOS-related markers in potential HPS and potential*

*POPH*

The above-mentioned oxidative stress-related markers were compared to define their impact

on potential HPS and potential POPH. Serum oxidative stress-related markers were not

different between potential HPS and potential non-HPS (Figure 3A). However, potential POPH

patients exhibited moderately elevated ROM and significantly lower OXY and higher oxidative index (Figure 3B). Staining for the hepatic oxidative stress marker 4-HNE was also increased in potential POPH patients. Hepatic DDAH-1 staining was higher in both potential HPS and POPH (Figure 3C, D). Hepatic eNOS staining was equal, while serum ADMA levels were higher in POPH.

*Comprehensive characteristics of potential POPH*

A multivariate analysis was utilized to assess the characteristics of potential POPH (Table 3). Of the clinical findings and markers tested, diastolic blood pressure, blood gas analysis-related $AaDO_2$, TTE-related E / e', oxidative stress-related oxidative index were selected as factors for analysis. Cardiac function-related E / e' and oxidative index were the factors selected as defining phenomena for potential POPH.

*Characteristics of potential HPS complicating with POPH*

Patients with potential HPS complicating with POPH exhibited lower OXY with higher oxidative index and higher hepatic 4-HNE staining (Figure 4). Multivariate analysis was utilized to assess the complicated pathology of potential HPS and POPH. The factors selected were diastolic blood pressure, E-wave, MELD score, oxidative index, and hepatic 4-HNE expression, which correlated with the clinical phenomena in the univariate analysis (Table 3B). Oxidative index was the only defining factor for potential HPS with POPH.

**Discussion**

In the present study, serum ROM was higher and OXY was lower in potential POPH. In contrast, these parameters were not significantly different between potential HPS and non-HPS. ADMA, a molecule upstream of the NO and NOS system, was significantly higher in potential POPH, suggesting that this pathway is activated by oxidative stress and might represent a target for antioxidant treatment. These results indicate that controlling oxidative stress could possibly protect cirrhotic liver from progressing to POPH, thereby resulting in longer survival.

ROM is considered to be a reliable parameter for determining circulating ROS levels(11, 12). It has been reported that ROS induces the progression of HCC(19), involving the transcription and activation of a large series of cytokines and growth factors, which ultimately lead to malignant transformation(20). However, the correlation between oxidative stress and the severity of conditions involving liver failure, such as HPS or POPH, has not been well characterized.

Oxidative stress is also involved in pulmonary hypertension. A recent study of 347 patients who received TTE revealed that pulmonary artery systolic pressure was correlated with oxidative stress markers cysteine, mitral regurgitation, statin use, left ventricular ejection fraction, and age(21). ROS promote smooth muscle contraction by triggering elevated cytosolic free $Ca^{2+}$ concentrations. In addition, under hypoxic conditions, which induce pulmonary hypertension, mitochondria-derived ROS was shown to be elevated with concomitant increases

in $Ca^{2+}$ influx, pulmonary arterial smooth muscle contraction, and proliferation. Hypoxia induces the expression of growth factors such as platelet derived growth factor and transforming growth factor-β, which promotes distal arterial muscularization, while antioxidants such as rosiglitazone reverse these effects(22).

The NO and NOS system is recognized as the main pathway for maintaining endothelial motility. Elevated levels of exhaled NO derived from the HPS lung have been reported to be normalized after orthotopic liver transplantation(23). Moreover, NO is hypothesized to be a potential regulatory effector for prolidase and may regulate matrix metalloproteinase activity. An iNOS knockout study revealed decreased collagen accumulation in chemically induced liver fibrosis, as well as increased apoptotic signals(24). ADMA promotes the dissociation of the ferrous-dioxy species from the heme group of NOS, resulting in the production of superoxide rather than NO. In pulmonary artery endothelial cells, ADMA has been shown to induce mitochondrial dysfunction by increasing uncoupling protein 2 levels and mitochondrial ROS in a dose-dependent manner(25). ADMA has been found to be increased by 4-HNE via miR-21 dependent DDAH-1 down-regulation(26). In the present study, DDAH-1 expression was found to be increased in liver expressing high levels of 4-HNE. During oxidative stress, reduced anti-oxidant protection of the cysteine residue in the reactive site of DDAH can result in decreased DDAH activity and elevated ADMA levels(27). DDAH-1 is highly expressed in brain and liver(28). ADMA is reported to be correlated with oxidative stress markers, such as

oxidized LDL(29). Oxidative stress and the NO-NOS system are correlated, which is consistent with the present observation that hepatic oxidative stress, reflected by higher 4-HNE staining, is evident in potential POPH.

The question of what factors lead to the occurrence of patients with elevated hepatic and general oxidative stress remains unanswered. Our present results are insufficient to answer this question, as we found only that oxidative stress balance and the cardiac diastolic failure correlated marker E / e' are predictive of potential POPH. Eighty seven percent of chronic hepatitis C patients are reported to have myocardial perfusion defects, as assessed with thallium-201 myocardial scintigraphy(30). This cardiac complication can be improved after viral eradication, suggesting that HCV or chronic hepatitis affects heart function, even after mild liver injury. Hepatopulmonary syndrome is reported to occur relatively frequently in cirrhosis, with a 5% incidence in cirrhosis outpatients and 25-65% in liver transplantation candidates(1). In the present study, potential POPH patients exhibited higher $AaDO_2$, suggesting that shunting affects potential POPH pulmonary vessels. The etiological factors of pulmonary hypertension are reported to be inflammation, shear stress, and hypoxia-induced oxidative stress(31). Pulmonary shunting in HPS is accompanied by shear stress and hypoxia in pulmonary vessels, while baseline liver cirrhosis produces inflammatory cytokines. As HPS occurs relatively frequently in cirrhosis, such conditions might induce pulmonary hypertension. Patients exhibiting both potential HPS and POPH showed a strong correlation with oxidative

index, indicating the importance of oxidative stress balance in more severe conditions.

In our previous study, HCC patients with periodontitis exhibiting elevated ROM levels and advanced HCC had severe periodontitis(32), while HCV positive HCC patients exhibited elevated ROM and reduced OXY(33). Our present results showed that there was no relation between ROM, OXY, and HCV positivity or HCC positivity. The reason for this is probably due to the present cohort consisting of decompensated liver cirrhosis patients preparing for liver transplantation. HCV positive patients registered for liver transplantation frequently suffer from HCC, and their conditions are relatively good, with non-HCC baring patients able to wait for transplantation until severe decompensation occurs. Therefore, HCV positive and/or HCC positive patients often have preserved liver function that may weaken oxidative stress dysregulation.

In conclusion, oxidative stress was higher in potential POPH and the anti-oxidant system was dysregulated, as the oxidative-anti-oxidative balance was observed to be severely distressed. The changes in oxidative stress balance correlated with hepatic and general NO-NOS system attenuation, as indicated by the reduced hepatic DDAH-1 expression and elevated serum ADMA. As the NO-NOS system is known to be correlated with pulmonary vessel contraction, unbalanced oxidative stress might be a treatment target for preventing the progression of potential POPH to definite POPH.

**Conflict of Interest**

No potential conflicts of interest are disclosed for all authors.

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Figure Legends

Figure 1: Correlations between hepatic 4-HNE staining and serum ROM, OXY, and oxidative

index in all patients. (A) The staining pattern of hepatic 4-HNE (arrow). Negative control

staining is shown in the right panel. (B) Correlation between 4-HNE positive area and

oxidative stress-related parameters. Oxidative index was significantly correlated with 4-HNE

staining area.

4-HNE; 4-hydroxynonenal, ROM; reactive oxygen metabolite, OXY; OXY-adsorbent test

Figure 2: Correlation analysis between NO-NOS-related pathway markers and oxidative

stress-related markers. (A) The staining pattern of hepatic DDAH-1 and eNOS are shown. (B)

The degree of DDAH-1 hepatocyte staining (weak or strong) was correlated with ADMA and

oxidative index. (C) eNOS staining was negatively correlated with hepatic 4-HNE staining

and serum ROM. (D) Serum ADMA levels were correlated with serum ROM and oxidative

index.

ADMA; asymmetric dimethylarginine, DDAH-1; dimethylarginine dimethylaminohydolases,

eNOS; endothelial nitric oxide synthase, 4-HNE; 4-hydroxynonenal, ROM; reactive oxygen

metabolite, OXY; OXY-adsorbent test

Figure 3: Assessment of NO-NOS-related pathway markers and oxidative stress-related markers

in potential HPS and POPH. (A) The oxidative stress-related markers were not different between potential HPS and potential non-HPS. (B) The anti-oxidative OXY was lower in potential POPH and the oxidative index was higher in potential POPH. (C) Hepatocyte DDAH-1 staining was weaker in potential HPS, although other markers were not different. (D) In potential POPH, hepatocyte 4-HNE staining was stronger, DDAH-1 staining was weaker, and serum ADMA was higher.

ADMA; asymmetric dimethylarginine, DDAH-1; dimethylarginine dimethylaminohydolases, eNOS; endothelial nitric oxide synthase, 4-HNE; 4-hydroxynonenal, HPS; hepatopulmonary syndrome, POPH; portopulmonary hypertension, ROM; reactive oxygen metabolite, OXY; OXY-adsorbent test

Figure 4: Assessment of an oxidative stress marker and NO-NOS-related pathway markers in potential HPS complicated with POPH. (A) Anti-oxidative OXY was lower in potential HPS with POPH, while the oxidative index showed the opposite pattern. (B) Hepatocyte 4-HNE staining was stronger in potential HPS with POPH.

ADMA; asymmetric dimethylarginine, DDAH-1; dimethylarginine dimethylaminohydolases, eNOS; endothelial nitric oxide synthase, 4-HNE; 4-hydroxynonenal, HPS; hepatopulmonary syndrome, POPH; portopulmonary hypertension, ROM; reactive oxygen metabolite, OXY; OXY-adsorbent test