Clinical Relevance of Nitric Oxide Metabolites and Nitrative Stress in Thrombotic Primary Antiphospholipid Syndrome

PAUL R.J. AMES, JOANA R. BATUCA, ANTONIO CIAMPA, LUIGI IANNACCONE, and JOSE DELGADO ALVES

ABSTRACT. Objective. To assess the role of nitrite (NO2–), nitrate (NO3–), and nitrative stress in thrombotic primary antiphospholipid syndrome (PAPS).

Methods. We investigated 46 patients with PAPS: 21 asymptomatic but persistent carriers of antiphospholipid antibodies (PCaPL), 38 patients with inherited thrombophilia (IT), 33 patients with systemic lupus erythematosus (SLE), and 29 healthy controls (CTR). IgG anticardiolipin (aCL), IgG anti-beta2-glycoprotein I (anti-β2-GPI), IgG anti-high density lipoprotein (aHDL), IgG anti-apolipoprotein A-I (aApoA-I), crude nitrotyrosine (NT) (an indicator of nitrative stress), and high sensitivity C-reactive protein (CRP) were measured by immunoassays. Plasma nitrite (NO2–), nitrate (NO3–), and total antioxidant capacity (TAC) were measured by colorimetric spectroscopic assays.

Results. Average plasma NO2– was lower in PAPS, PCaPL, and IT (p < 0.0001); average NO3– was highest in SLE (p < 0.0001), whereas average NT was higher in PAPS and SLE (p = 0.01). In thrombotic PAPS, IgG aCL titer and number of vascular occlusions negatively predicted NO2– (p = 0.03 and p = 0.001, respectively), whereas arterial occlusions and smoking positively predicted NO3– (p = 0.05 and p = 0.005), and CRP positively predicted NT (p = 0.004). In the PCaPL group IgG aCL negatively predicted NO2– (p = 0.03). In the SLE group IgG aCL negatively predicted NO2– (p = 0.03) and NO3– (p = 0.02).

Conclusion. PAPS is characterized by decreased NO2– in relation to type and number of vascular occlusions and to aPL titers. Nitrative stress and low grade inflammation are linked phenomena in PAPS and may have implications for thrombosis and atherosclerosis. (First Release Oct 1 2010; J Rheumatol 2010;37:2523–30; doi:10.3899/jrheum.100494)

Key Indexing Terms:
ANTIPHOSPHOLIPID SYNDROME
NITRIC OXIDE
THROMBOSIS
NITRITE
NITRATE

The primary antiphospholipid syndrome (PAPS) is characterized by venous and arterial thromboses, recurrent miscarriages, and premature atherosclerosis in persistent carriers of antibodies against β2-glycoprotein I (anti-β2-GPI) and other coagulation proteins in the absence of any other underlying immune disorder1,2. From a biochemical standpoint PAPS is also characterized by an antioxidant/oxidant balance tilted towards the latter, partly due to decreased paraoxonase activity and enhanced oxidative stress3,4. Indeed, IgG anticardiolipin (aCL) antibody titers positively correlated to plasma levels of F2-isoprostanes, a marker of increased oxidative stress, and to decreased urinary excretion of nitric oxide (NO•) metabolites in PAPS3. NO• is the main endothelial vasodilator agent, and interference with NO• biology induces vascular dysfunction, particularly in the early phases of atherosclerosis5. After physiological stimulation of constitutive endothelial nitric oxide synthase (eNOS)6 or inflammatory activation of inducible (iNOS) enzyme7, NO• is released at higher rates and behaves as a pathogenic mediator or a cytotoxic molecule.

In the latter case, most NO• mediated pathogenicity depends on formation of secondary intermediates such as peroxynitrite anion (ONOO–) and nitrogen dioxide (∙NO2), which are typically more reactive and toxic than NO• per se8. In the presence of oxidants such as superoxide radical (O2●) NO• gives rise to ONOO–, a strong 1-electron and
2-electron oxidant with such a short biological half-life (10–20 ms) that it cannot be measured directly but must be inferred by indirect methods. In fact ONOO\(^{-}\) interacts with CO\(_2\) to give nitrosoperoxycarbonate (ONOO\(\mathrm{CO}_2\)) that will nitrate tyrosine residues in proteins: measurement of nitrated proteins therefore represents a fingerprint of the interaction of O\(_2^\bullet^+\) with NO\(_3\)^\(-\). Possible involvement of NO\(^\bullet\) inAPS has been explored in animal studies and in a few patient series whose numbers were too limited to provide a full understanding of its significance. We therefore hypothesized that NO\(^\bullet\) might play a role in the vascular pathogenesis of PAPS, and compared the behavior of NO\(^\bullet\) metabolites nitrite (NO\(_2^\bullet\)) and nitrate (NO\(_3^\bullet\)), total antioxidant capacity (TAC) (expressed as ONOO\(^{-}\) quenching), and nitrotyrosine (NT) in patients with thrombotic PAPS, in asymptomatic but persistent carriers of antiphospholipid antibodies (PcAPL), in patients with inherited thrombophilia (IT) with vascular occlusions, in patients with systemic lupus erythematosus (SLE), and in healthy subjects. Possible relationships between NO\(_2^\bullet\), NO\(_3^\bullet\), C-reactive protein (CRP), and several aPL were also investigated.

**MATERIALS AND METHODS**

**Patients.** Our study was devised as a cross-sectional case-quadruple control: PAPS patients with vascular occlusions represented cases; IT patients with vascular occlusions represented thrombotic controls PcAPL without vascular occlusions represented nonthrombotic aPL-positive controls; patients with SLE represented inflammatory controls and healthy subjects represented normal controls. All participants were age- and sex-matched (where possible), except for SLE patients, who were all female. Consecutive patients with thrombotic PAPS, according to recent criteria, with IT and persistent aPL attending the Coagulation Unit of the Cardarelli Hospital (Naples, Italy) were invited to participate between January 2008 and July 2008. Our study was carried out according to the revised Declaration of Helsinki, with approval of the Ethics Board of the hospital and written consent of all participants. Exclusion criteria were acute or chronic hepatic, renal, and lung disease; diabetes; acute infection (within 6 weeks); post-thrombotic syndrome with or without venous ulcerations; positive urinary dipstick for nitrates on the day of sampling and treatment with statins or fibrates. PAPS and IT patients are seen on average every 3 to 4 weeks for oral anticoagulation monitoring and are instructed to self-report any illness during the intervening periods; their lipid profiles and kidney and liver function tests are checked annually. PAPS PAPS subjects were diagnosed as such either because of the presence of prolonged clotting tests in routine assays, subsequently confirmed as lupus anticoagulant (LAC), or because of thrombocytopenia or other symptoms that prompted a search for aPL. Of the PAPS attendees (n = 50), 2 were excluded because they had gradually developed ankylosing spondylitis and SLE, one had developed kidney cancer, 2 were pregnant, one had suffered a recent recurrent event, one had post-thrombotic syndrome, and 2 were evasive regarding their smoking and contraceptive status. Of the IT (n = 46) attendees, 2 were excluded for post-thrombotic syndrome and venous ulcerations in lower limbs. Of the PAPS attendees (n = 27) one was excluded for development of non-insulin-dependent diabetes; one for the development of SLE, hemolytic anemia, nephrotic syndrome, and pulmonary embolism after ovarian hyperstimulation; one for the development of chronic lymphoid leukemia; one for spontaneous onset of ischemic stroke; and 2 had moved to a different town. Of the remaining aPL subjects 4 had moderate thrombocytopenia (platelets < 100 × 10\(^9\)/l) not requiring treatment.

Consecutive patients with SLE fulfilling the American Rheumatism Association (ACR) criteria were enrolled among those attending the Autoimmune Outpatient Clinic of the Curry Cabral Hospital, Lisbon (Portugal) between January 2008 and August 2008. Exclusion criteria included acute or chronic renal impairment that would significantly alter NO metabolites, liver cirrhosis, diabetes, acute infection (within 6 weeks), post-thrombotic syndrome with or without venous ulcerations, positive urinary culture following positive dipstick for nitrite (urinary excretion in SLE may be increased in the absence of infection), and treatment with statins or fibrates.

Of 52 patients with SLE, 16 were excluded on the basis of the above criteria. Of the remaining 36, one was found weeks later to have tuberculosis and 2 were pregnant; their samples were discarded. Therefore 33 SLE patients participated in the study: of these, 9% had visceral involvement without renal disease, 12% cardiac and lung involvement, 9% central nervous system involvement, 66% arthritis, 3% myositis, 9% alopecia, 3% hemolytic anemia, 21% thrombocytopenia, 9% neutropenia, 81% presence of anti-DNA antibodies, and 90% presence of antinuclear antibodies. The average SLE Disease Activity Index (SLEDAI) score was 4.85 ± 3.96 (median 3.5, range 0–16). Their medication intake was: prednisolone in 54%, (< 6 mg/day in 27%, 6–10 mg/day in 18%, > 10 mg/day in 9%) azathioprine in 24% (100 mg/day in 18%, 150 mg/day in 6%), hydroxychloroquine 200 mg/day in 60%, aspirin in 12%, warfarin in 9%. Twenty-nine healthy hospital staff served as normal controls: 15 from Cardarelli Hospital in Naples and 14 from the Curry Cabral Hospital in Lisbon. To minimize dietary influences on nitric oxide metabolite concentrations all participants were asked to refrain from foodstuffs containing high concentrations of nitrate/nitrite (such as lettuce, spinach, beetroot, radish, salami, and pickled items) for 3 days before blood sampling, which was drawn between 8:00 and 10:00 AM. Blood samples were drawn by neat venepuncture into 5 ml citrate vacutainers, spun immediately at room temperature at 4000 rpm for 6 min; supernatant plasma was spun again at room temperature at 12,000 rpm for 4 min to obtain platelet-poor plasma: aliquots were frozen at −80°C and thawed on the day of testing. The study was therefore carried out on 46 thrombotic PAPS patients, 21 PcAPL subjects, 38 IT patients, 33 SLE patients, and 29 control subjects. Their demographics are shown in Table 1.

**Determination of antiphospholipid antibodies.** All participants had their aPL determined according to established criteria; LAC screened by activated partial thromboplastin time (aPTT) and dilute Russell’s viper venom time (DRVVT). A clotting time ratio between sample and control plasma > 1.2 for aPTT and > 1.18 for DRVVT indicated an abnormal result. After demonstrating the presence of an inhibitor using mixing studies, the platelet neutralization procedure confirmed the presence of a lupus inhibitor in aPTT and DRVVT. IgG aCL (Cambridge Life Sciences, Ely, UK) and IgG anti-ß2-GPI (Corgenix, Broomfield, CO, USA) were measured by ELISA according to manufacturer’s instructions. Since the inception of the PAPS cohort (1994), after initial diagnosis with repeat testing of aPL after 6 weeks, IgG aCL was measured yearly, whereas IgG anti-ß2-GPI was measured yearly only since 2004.

**Measurement of IgG anti-high density lipoprotein (aHDL) antibodies, IgG anti-apolipoprotein A-I (aApo A-I) antibodies, plasma nitrotyrosine, and high sensitivity C-reactive protein (CRP).** HDL and aApo A-I were measured by ELISA as described; similarly ELISA was employed to measure nitrotyrosine (HyCult Biotechnology, Uden, The Netherlands) and high sensitivity CRP (Biosupply Ltd., Bradford, UK) according to the manufacturer’s instructions. “CRP” stands for the high-sensitivity test throughout this article.

**Measurement of plasma nitrate and nitrite.** Nitric oxide metabolites nitrate (NO\(_2^\bullet\)) and nitrite (NO\(_3^\bullet\)) were determined using a modified Griess reaction, following the reduction of nitrate to nitrite using nitrate reductase and nicotineamide adenine dinucleotide phosphate (NADPH). Briefly, the assay was performed in a standard flat-bottomed 96-well microtiter plate half divided for simultaneous measurement of nitrate and nitrite concentration. To each well was added 50 µl/well of standard or diluted sample (1 in 4

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The Journal of Rheumatology 2010; 37:12; doi:10.3899/jrheum.100494

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with phosphate buffer pH 7.4) in duplicate. The assay was blanked against phosphate buffer. In half plate, 4 µl of nitrate reductase (Sigma-Aldrich) and 10 µl of NADPH (Sigma-Aldrich) were added to each well, giving a final concentration of 6.3 U/l and 550 µmol/l, respectively. The plate was incubated at room temperature for 2 h. Griess reaction was initiated by addition to each well of equal volumes of 2% sulfanilamide (Sigma-Aldrich) in H₃PO₄ 5% and 0.2% N-(1-naphthyl)-ethylenediamine dihydrochloride (Sigma-Aldrich) in water, mixed just before use. After 10 min incubation at room temperature the absorbance of the reaction mixture was measured at 540 nm and the levels expressed as µM.

Measurement of total antioxidant capacity of plasma. TAC of plasma was measured by peroxynitrite (ONOO–) quenching: 100 µl of phosphate buffer (50 mM, pH 7.4) containing Pholasin® (1.7 µg/ml) was pipetted into a microcuvette. Plasma or buffer for control (5 µl) was added. The reaction was initiated by adding 3-morpholino-sydnonimine HCl (SIN-1; 2 µl of 2 mg/ml in water), and light emission was measured continuously at 5 min intervals until the maximum reading was obtained. Antioxidant capacity was expressed as the time at which maximum light was emitted. Lower values reflect decreased plasma TAC (peroxynitrite-related).

Statistical analysis. Variables were compared by ANOVA (post-hoc analysis) and ANCOVA with log transformation of variables that did not follow a normal distribution. The assumptions of univariate analysis within groups (not shown) were tested by multiple regression models. All statistical analyses were done using SPSS (SPSS, Chicago, IL, USA).

RESULTS

Comparison of variables in PAPS, PCaPL, IT, SLE, and healthy controls. Average plasma NO₂⁻ was lower in the PAPS, PCaPL, and IT groups (Figure 1A), whereas NO₃⁻ was higher in SLE (Figure 1B) and NT was higher in SLE and PAPS (Figure 1C). Mean plasma TAC was lowest in SLE (Figure 2A), where CRP was highest (Figure 2A and 2B). Average TAC was higher in males than in females in all non-SLE groups: in PAPS 11280 ± 3041 versus 9749 ± 2967 µmol/l (p = 0.02); in IT 6392 ± 1399 versus 5325 ± 1720 µmol/l (p = 0.04); and in healthy controls 7967 ± 991 vs 6194 ± 1265 µmol/l (p = 0.001).

Age, sex, and smoking correlated to NO₂⁻, NO₃⁻, and TAC but had no confounding effect on resulting significance findings by ANCOVA. Age and IgG aCL related to NT and their confounding effect by ANCOVA reduced the comparative significance (p < 0.02).

Relationship among variables in PAPS. The effect of antibodies and that of other clinical and laboratory variables on plasma concentrations of NO₂⁻, NO₃⁻, and NT was tested by
separate multiple regression models. In the model with NO$_2^-$ as the dependent variable and IgG aCL, IgG anti-ß2-GPI, IgG aHDL, and IgG aApoA-I antibodies as independent variables, IgG aCL resulted in the only negative predictor of NO$_2^-$ ($p = 0.03$; Table 2). Average NO$_2^-$ was lower in patients with a history of arterial thrombosis versus those with venous thrombosis (11.41 ± 7.6 vs 18.43 ± 11.06 µmol/l; $p = 0.03$) although in a separate model with NO$_2^-$ as the dependent variable and age at first thrombotic event and thrombosis number and type as the independent variables, thrombosis number negatively predicted NO$_2^-$ ($p = 0.001$; Table 2).

In the model with NO$_3^-$ as the dependent variable and IgG aCL, IgG anti-ß2-GPI, IgG aHDL, and IgG aApoA-I antibodies as independent variables, IgG aCL was the only negative predictor of NO$_3^-$ ($p = 0.03$) (Table 2). In a different model, with NO$_3^-$ as the dependent variable and age,
sex, thrombosis type, smoking, and TAC as the independent variables, arterial thrombosis and smoking independently predicted NO₃⁻ (p = 0.05 and p = 0.005, respectively).

In a further model with NT as the dependent variable and the antibodies as the independent variables none of the latter bore any relationship with NT; but in a similar model NT as the dependent variable and with NO₃⁻, NO₂⁻, TAC, smoking, and CRP as independent variables, CRP was the only independent predictor of NT (p = 0.004) (Table 2).

**Relationship among variables in PcaPL.** In the regression model with NO₂⁻ as the dependent variable and IgG aCL, IgG anti-ß2-GPI, IgG aHDL, IgG aApoA-I, aPTT, and DRVVT as independent variables, IgG anti-ß2-GPI showed only a negative trend with NO₂⁻ (p = 0.07) (Table 3).

In the model with NO₃⁻ as the dependent variable and IgG aCL, IgG anti-ß2-GPI, IgG aHDL, IgG aApoA-I, aPTT, and DRVVT as independent variables, negative predictors were IgG aCL (p = 0.03) and DRVVT (p = 0.03), and a trend was seen for IgG anti-ß2-GPI (p = 0.06; Table 3).

In a further model none of the antibodies bore any relationship with NT as the dependent variable but with NO₃⁻, NO₂⁻, TAC, and CRP set as independent variables, NO₂⁻ negatively predicted NT (p = 0.05; Table 3).

**Relationship among variables in IT.** In the regression model with NT as the dependent variable and NO₃⁻, NO₂⁻, TAC, smoking, and CRP as independent variables, only CRP independently predicted NT (p = 0.0006; Table 3).

**Relationship among variables in SLE.** In the regression model with NO₂⁻ as the dependent variable and IgG anti-ß2-GPI, IgG aHDL, IgG aApoA-I, and IgG aCL as the independent variable, IgG aCL negatively predicted NO₃⁻ (p = 0.03); a similar result was obtained when NO₂⁻ was substituted for NO₃⁻ (p = 0.02; Table 3). In the model with NT as the dependent variable and NO₃⁻, NO₂⁻, TAC, smoking, and CRP as independent variables, NO₂⁻ negatively predicted NT (p = 0.002) and NO₃⁻ positively predicted NT (p = 0.001; Table 3). Finally, in the model with SLEDAI as the dependent variable and NO₃⁻, NO₂⁻, TAC, CRP, smoking,

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**Table 2. Regression model predictors of nitric oxide metabolites and nitrotyrosine in primary antiphospholipid syndrome.**

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Dependent Variables</th>
<th>Predictors</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG aCL, IgG anti-ß₂-GPI, IgG aApoA-I</td>
<td>NO₂⁻</td>
<td>IgG aCL</td>
<td>-1.87</td>
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<td>Age at first thrombosis, thrombosis number, thrombosis type</td>
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<td>Thrombosis number</td>
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<td>0.001</td>
</tr>
<tr>
<td>IgG aCL, IgG anti-ß₂-GPI, IgG aHDL, IgG aApoA-I</td>
<td>NO₃⁻</td>
<td>IgG aCL</td>
<td>-1.93</td>
<td>0.03</td>
</tr>
<tr>
<td>Age, sex, thrombosis type, smoking, TAC</td>
<td>NO₃⁻</td>
<td>Arterial thrombosis</td>
<td>1.67</td>
<td>0.05</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoking</td>
<td>2.66</td>
<td>0.005</td>
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</tbody>
</table>

NO₃⁻, NO₂⁻, TAC, smoking, CRP

**Table 3. Regression model predictors of nitric oxide metabolites and nitrotyrosine in non-primary antiphospholipid antibody syndrome groups.**

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Dependent Variables</th>
<th>Predictors</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent carriers of antiphospholipid antibodies</td>
<td>NO₃⁻</td>
<td>IgG aCL</td>
<td>-2.06</td>
<td>0.03</td>
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<tr>
<td>IgG aCL, IgG anti-ß-GPI, IgG aHDL, IgG aApoA-I, aPTT, DRVVT</td>
<td>NO₂⁻</td>
<td>DRVVT</td>
<td>-1.93</td>
<td>0.03</td>
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<tr>
<td>NO₃⁻, NO₂⁻, TAC, smoking, CRP</td>
<td>NT</td>
<td>NO₃⁻</td>
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<td>0.05</td>
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<td>Inherited thrombophilia</td>
<td>NT</td>
<td>CRP</td>
<td>3.75</td>
<td>0.0006</td>
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<tr>
<td>NO₃⁻, NO₂⁻, TAC, smoking, CRP</td>
<td>NT</td>
<td>SLEDAI</td>
<td>2.55</td>
<td>0.009</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>NT</td>
<td>CRP</td>
<td>1.44</td>
<td>0.08</td>
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<tr>
<td>Age, sex, TAC, CRP, smoking, NT</td>
<td>NO₃⁻</td>
<td>Smoking</td>
<td>2.90</td>
<td>0.003</td>
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<tr>
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<td>Smoking</td>
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<td>0.002</td>
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NO₃⁻, NO₂⁻, TAC, CRP, smoking, NT

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IgG aCL: anticardiolipin; IgG anti-ß₂-GPI: beta-2-glycoprotein-I; IgG aHDL, anti-high-density lipoprotein; IgG aApoA-I, anti apolipoprotein A-I; NO₂⁻: nitrite; NO₃⁻: nitrate; TAC: total antioxidant capacity; NT: nitrotyrosine; CRP, C-reactive protein.
and NT as the independent variables, NT predicted SLEDAI (p = 0.009) and a trend was seen for CRP (p = 0.08; Table 3).

**Relationship among variables in the control group.** No effect on NO$_3^-$ was seen in a multiple regression model with NO$_2^-$ as the dependent variable and age, sex, smoking, IgG aHDL, and IgG aApo-I as explanatory variables. In a similar model where NO$_3^-$ was set as the dependent variable, smoking independently predicted NO$_2^-$ (p = 0.003; Table 3). Similarly, when NT was set as the dependent variable with age, sex, smoking, TAC, NO$_2^-$, and NO$_3^-$ as explanatory variables, smoking independently predicted NT (p = 0.02) alongside NO$_3^-$ (p = 0.04; Table 3).

**DISCUSSION**

NO• is synthesized in the vasculature by 2 related nitric oxide synthases (NOS), constitutive eNOS and inducible NOS; both convert L-arginine to NO• and citrulline at different concentrations according to substrate availability. The role of NO• in PAPS is unknown: one study found lower urinary NO$_2^-$ in a small number of patients with PAPS in negative correlation with IgG aCL titer. Of the NOS metabolites, it is widely accepted that in humans, only NO$_2^-$ reflects changes in eNOS activity and endothelial dysfunction known to be impaired in APS.

To evaluate the clinical significance of NO$_2^-$ and NO$_3^-$ in PAPS we employed as comparator patients with IT who had vascular occlusions, patients with PCaPL who had no vascular occlusions, patients with SLE as an inflammatory disease control group, and healthy subjects. The low average concentration of NO$_3^-$ found in PAPS and IT suggests that reduced NO$_2^-$ may be involved in the vascular events of these patients, although causality cannot be established since vessel occlusion might have led to reduced NO$_3^-$; in fact, the number of vascular occlusions was a negative independent predictor of NO$_2^-$ in PAPS. Nevertheless NO$_3^-$ was also low in the PCaPL group who never had vessel occlusions, suggesting that impaired NO$_2^-$ generation may precede and hence represent a predisposing factor for thrombosis.

Reduced NO• has a wider importance in the vascular biology of PAPS. NO• maintains vascular homeostasis against the vasopressor effects of endothelin-1, of isoprostanates derived from lipid peroxidation, and of thromboxane generated after platelet activation (as reviewed). Indeed, elevated plasma and/or urinary levels of the aforementioned molecules have all been described in PAPS and NT related to disease activity and was predicted by NO$_3^-$, in keeping with findings from other inflammatory rheumatic disorders. On the other hand, having demonstrated that low grade inflammation characterizes PAPS, we found that CRP was an independent predictor of NT in the PAPS group, suggesting that nitrative stress and low grade inflammation may be related phenomena in these thrombotic patients. Interestingly, smoking predicted NO$_3^-$ in PAPS, and it is known that active and passive smoking may induce oxidative stress.

Our study has several limitations: (1) its retrospective design prevented a full appreciation of the role of NO• in thrombosis as most PAPS patients were diagnosed after vascular occlusion; (2) our SLE group comprised female patients of whom only 5 had a history of thrombosis; however, we had opted for inclusion of the SLE group mostly to show the inflammatory behavior of NO• rather than to control for thrombosis, which was provided for by the IT group; (3) the method we employed for the measurement of NO• metabolites is not sensitive enough to detect nanomolar concentrations of NO$_2^-$ and NO$_3^-$, and we did not evaluate eNOS and/or iNOS gene polymorphisms that may have accounted for differences in measured metabolites, although our groups would have been too small to yield significant data.
In conclusion, our study, alongside our previous animal data, indicates a possible impairment of the vascular biology of NO• in PAPS, the consequences of which may be thrombosis and atherosclerosis. With regard to the former, we cannot define whether decreased NO2− is a cause or an effect of previous thromboses, but the low NO2− in PCaPL without vessel occlusions and the relationship between NO• metabolites and aPL in the PAPS, PCaPL, and SLE groups indicate that aPL may negatively influence some physiological activities of NO•. With regard to the latter, patients with PAPS exhibit a certain degree of nitrative stress that relates to low grade inflammation, also noted in other settings.

From a practical point of view, our study provides evidence that smoking should be avoided in patients with PAPS.

ACKNOWLEDGMENT

We are grateful to L. Lopez (Corgenix, Broomfield, CO, USA) for his help with the IgG anti-ß2-GPI assay.

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doi:10.3899/jrheum.100494C1