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Safety of the oral methionine load test: effects on the clinical performance and laboratory tests

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ABSTRACT

Introduction. Hyperhomocysteinemia is a prothrombotic risk factor. Homocysteine is evaluated during fasting and after an oral methionine load (OML). **Aim.** To determine the safety of the OML test according to the general performance status and clinical laboratory tests. We studied healthy nonsmoking volunteers and patients with several thrombotic conditions. Before and after receiving an OML, blood samples were obtained to perform several laboratory tests. We also evaluated acute and subacute adverse effects and 30-day associated morbidity and mortality. Of 353 individuals, three were eliminated because they did not tolerate the OML. We studied 175 healthy individuals and 175 patients without age differences. After OML, mild to moderate clinical abnormalities were recorded in 78 subjects (22.1%): nausea (n = 69; 88.5%), dizziness (n = 13; 16.7%) and decreased or increased blood pressure (n = 8; 10.2%). Nausea always disappeared after breakfast in affected individuals. Prevalence of complications was similar in patients and controls. No patient required hospitalization and there was no mortality during the 30-day study period. In conclusion, OML test had no significant undesirable effects on the clinical status or the general laboratory tests of patients and healthy controls. Some mild and moderate symptoms associated with OML tests were observed, and OML test did not negatively affect general laboratory tests. OML test is a safe diagnostic procedure in patients with previous thrombotic events (and with the consequent associated risk factors such as diabetes mellitus or dyslipidemia) and in healthy subjects.

Key words. Hyperhomocysteinemia. Methionine load. Laboratory tests. Thrombosis. Post-oral methionine load test.

Seguridad de la prueba de carga oral de metionina: efectos sobre el estado clínico y pruebas de laboratiorio

RESUMEN

Introducción. *La hiperhomocisteinemia es un factor de riesgo trombótico. La homocisteína se evalúa en ayuno y luego de una carga oral de metionina (COM).* ***Objetivo.*** *Establecer la seguridad de la prueba de COM en términos del estado clínico general y las pruebas generales de laboratorio.* ***Material y métodos.*** *Se estudiaron adultos de ambos sexos, sanos y no fumadores, y pacientes con diversas condiciones trombóticas. Antes y después de recibir la COM se obtuvieron muestras de sangre para realizar las pruebas de laboratorio. También se evaluaron los efectos adversos agudos y sub-agudos y la morbilidad y mortalidad asociadas a los 30 días. De 353 individuos, tres fueron eliminados ya que no toleraron la COM. Se estudiaron 175 individuos sanos y 175 pacientes sin diferencias significativas en términos de edad. Luego de la COM se encontraron algunas alteraciones clínicas leves y moderadas en 78 individuos (22.1%): náusea (n = 69; 88.5%), mareo (n = 13; 16.7%) y aumento o disminución de la presión arterial (n = 8; 10.2%). La náusea siempre desapareció luego del desayuno. La prevalencia de las complicaciones fue similar en los pacientes y en los controles. Ningún paciente requirió hospitalización y la mortalidad en los 30 días del periodo de estudio fue cero.* ***Conclusión.*** *La COM no induce efectos indeseables significativos sobre el estado clínico o las pruebas de laboratorio en pacientes y en voluntarios sanos. La COM se asocia con algunos efectos clínicos leves a moderados, pero no afecta ninguna prueba de laboratorio significativamente. Por lo tanto, la COM es una prueba diagnóstica segura en pacientes con eventos trombóticos previos que son portadores de factores de riesgo asociados como diabetes mellitus o dislipidemia, así como en controles sanos.*

Palabras clave. *Hiperhomocisteinemia. Carga de metionina. Estudios generales de laboratorio. Trombosis. Prueba de carga oral de metionina.*

INTRODUCTION

Hyperhomocysteinemia (HHC) is a prothrombotic risk factor that has gained importance during recent years. It has been demonstrated that HHC is an independent risk factor for venous and arterial thromboembolic diseases.[1,2] Depending on the plasma levels of homocysteine (Hcy), HHC may be classified as mild (16-30 μmol/L), moderate (31-100 μmol/L), and severe (> 100 μmol/L).[3,4]

Hcy can be quantified in serum and plasma using several methods including high-performance liquid chromatography (HPLC), gas chromatography with mass spectrophotometry, and amino acid analysis, among others. Hcy may be evaluated in fasting state and after administering the patient an oral methionine load (OML) using a single dose of 100 mg/kg of methionine, namely, the OML test, a widely used diagnostic tool to detect abnormal metabolism of Hcy in patients with various conditions. An oral load with L-methionine was first reported in homocysteinuric patients[5] and was subsequently adapted as a test to identify heterozygous deficiency of the enzyme cystathionine b synthase (CBS).[6-10] However, it may also detect the homozygous state for the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) enzyme, which induces a selective defect in the remethylation pathway of the Hcy metabolism.[11] When Hcy metabolism is normal, following the OML test the level of this amino acid increases an average 20 μmol/L higher than basal levels but returns to normal levels during the next few hours. Hcy is quantified 2 to 8 h after OML and is considered abnormal when its concentration is > 2 standard deviations above basal fasting Hcy level.[10-14] The OML test also identifies patients with HHC who have normal fasting Hcy levels, a fact that allows the identification of > 50% of patients with HHC. Therefore, the OML test is considered the most sensitive test for diagnosing moderate HHC.[15]

It is clear that the OML test is necessary in order to obtain an appropriate diagnosis of HHC; however, concerns remain in regard to its safety. Among the adverse effects reported, dyspnea is the most frequent complaint but somnolence, nausea, polyuria, dizziness, and arterial hypo- and hypertension have been described.[16,17] Moreover, some disturbances in lipid profile have also been described.[16,18] To the best of our knowledge, only one fatal case was reported after an OML test; however, most reports are associated with mild or moderate adverse effects.

It is likely that an excess of methionine administered to an individual and the subsequent increase in Hcy plasma levels may acutely affect individual clinical status, overall metabolism or endothelial function. Therefore, our objective was to determine the safety of the OML test in terms of the general performance status but more importantly in regard to the general clinical laboratory tests because this analysis has not been previously reported in the literature.

MATERIAL AND METHODS

General characteristics of the study

This was a prospective, longitudinal, nonrandomly assigned study of adult Mexicans of both genders who required an OML test. Between January 2010 and January 2012 we included two groups of subjects: healthy nonsmoking volunteers with no history of thrombotic diseases or with several conditions predisposing to thrombosis. Volunteers were blood donors, patient caregivers at our hospital, and health services personnel working at our hospital. The second group was comprised of patients with several thrombotic conditions who required the quantification of Hcy. All participants were instructed about the nature of the study and received precise instructions in regard to the OML test. We followed one of the most commonly accepted protocols.[19] Briefly, we take a blood sample in the morning in order to obtain a basal sample to measure fasting Hcy. Subjects must carry out their normal daily activities and, after dinner, were asked to take methionine (100 mg/kg of body weight) (L-methinone, Sigma Aldrich, St. Louis, MO, USA), which was diluted in 250-500 mL orange juice. Patients were asked to drink the methionine during a 20 min period and 8 h later a blood sample was obtained. Patients were instructed to continue with any required medications.

Blood sample collection

Before and after the OML test we obtained 3 mL of blood from each patient in vacuum plastic tubes added with sodium citrate 0.109 M (9:1, vol:vol) (Na Citrate, BD Vacutainer, Franklin Lakes, NJ, USA); 5 mL was drawn in a vacuum glass tube with EDTA (K3 EDTA, BD Vacutainer) and 10 mL in two glass tubes without anticoagulant (SST, BD Vacutainer). All samples were centrifuged at 2,000 g for 15 min in order to obtain platelet poor plasma and serum.

Acute and subacute adverse clinical effects and 30-day morbidity and mortality associated with the OML test

To ascertain any possible subjective complications, patients were asked how they felt prior to the test and 30 min and 8 h after the test began. The interval could be proportionally shortened for subjects reporting any problems. Blood pressure and heart rate were measured and recorded before the OML test, 30 min after the test, and 8 h after the OML test. In case of any complaints, the participants were evaluated more frequently. To determine whether the OML test could result in a possible subacute or lethal complication, we gathered information from direct interviews and from the clinical chart of the patients during the 30 day period after performance of the OML test. Our objective was to determine any clinical worsening of pre-existing conditions or novel occurrence of abnormal clinical data. Mortality within 30 days after the OML test was analyzed in all participants in this study using interviews and searching in the death registry of our hospital.

Laboratory tests

Blood cell count was performed using Cell-Dyn 3700 equipment (Abbott Park, IL, USA). Erythrocyte sedimentation rate and blood group analysis were performed according to worldwide accepted techniques. Glucose, urea, creatinine, triglycerides, total cholesterol, HDL-cholesterol (HDL-c), alkaline phosphatase, lactate dehydrogenase, alanine transaminase, aspartate transaminase, total bilirubin, direct bilirubin, total serum protein, albumin, globulins, and gamma-glutamyl transferase levels were assayed using Synchron LX20 equipment (Beckman Coulter, Fullerton, CA, USA); high-sensitivity C-reactive protein (hsCRP) was evaluated with a AXSYM System analyzer (Abbott Park, IL, USA). Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen level (Clauss technique) were evaluated using commercially available tests (Neoplastin Plus, PTT Reagent, STA Thrombin, Fibrinogen Reagent, Stago, Asnieres, France) in STA Compaq equipment (Stago).

Ethics

Each patient received information in regard to the study characteristics. Informed written consent was obtained from all subjects before study enrollment. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was accepted by the Local Ethics Committee and the Investigation Research Board of our hospital.

Statistical analysis

For analysis of the general patient characteristics and clinical abnormalities, we used descriptive statistics. Continuous data are expressed as means and ranges, whereas categorical data are presented as percentages. The significance of the differences between continuous variables was determined by paired *t* test. Results of laboratory tests are expressed as medians and percentiles 2.5-97.5. To determine the presence of significant differences between basal and after OML test laboratory values, paired t test or Wilcoxon test was used according to the distribution of the results. To analyze the results of the PT and APTT tests, we used χ^2 test. For these two tests, normal values were obtained from pooled plasmas obtained from healthy individuals; p value < 0.05 was considered significant. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) software (version 16; SPSS Inc., Chicago, IL, USA).

RESULTS

Three hundred and fifty-three individuals were originally enrolled in the study and all of them were analyzed in terms of clinical performance changes: 176 healthy individuals (72 females and 104 males) and 177 patients (92 females and 85 males) with a mean age of 43.0 years (range: 18-93 years) and 46.5 years (range: 18-91 years), respectively. When analyzing both groups separately and as a single group, there were no significant differences between females and males in terms of age.

Clinical complications of the OML test

For the whole study group, within 8 h of OML ingestion, subjective and objective complaints were observed in 81 loaded subjects (22.9%). Except for 3 cases with severe vomiting (3.7%), most clinical abnormalities were mild to moderate and the most frequent transient complications were nausea (n = 69; 85.2%), dizziness (n = 13; 16.0%), and decreased or increased blood pressure (n = 8; 9.8%). All these clinical disturbances usually appeared within 2 h after methionine ingestion, ranging between 30 min and 4 h.

It is worth noting that nausea and vomiting always disappeared after breakfast in all affected individuals. There was no correlation between vomiting and nausea with dizziness and abnormalities in blood pressure according to age or gender. Symptoms and/or signs were isolated in 65 individuals, whereas in 16 persons a combination of symptoms/signs was present and all experienced nausea. The prevalence of complications was not significantly different between females and males: 42 (51.8%) *vs.* 39 (48.1%) (p = 0.07), respectively. The prevalence of complications was similar in patients and controls: 41 (50.6%) *vs.* 40 (49.4%) (p = 0.52), respectively. The OML test did not lead to significant changes in systolic or diastolic blood pressure in both groups. No cases were reported of serious worsening of the overall clinical condition requiring hospitalization.

In terms of subacute adverse effects and mortality within the 30-day period after the OML test, none of the subjects experienced clinical worsening of the pre-existing disease or novel clinical abnormalities. Finally, there was no mortality during the 30-day study period.

Effects on laboratory tests

As previously mentioned three patients experienced intense vomiting and were excluded from the analysis of laboratory changes. Therefore, the analysis about changes in the laboratory tests was performed in only 175 healthy individuals (71 females and 104 males) and 175 patients (91 females and 84 males) with a mean age of 42.5 years (range: 18-93 years) and 46.5 years (range: 18-91 years), respectively. Results of all metabolic tests performed before and after the OML test are shown in table 1. Except for basal total cholesterol levels, all results obtained before and after the OML test had an abnormal distribution. Descriptive analysis showed

Table 1. Changes in general laboratory tests before and after OML test.

Variable	Basal* (n = 350)	OML* (n = 350)	p
Glucose (70-100 mg/dL)	93 (73-284)	97 (75-304)	< 0.001
Creatinine (0.6-1.30 mg/dL)	0.8 (0.5-1.3)	0.8 (0.5-1.2)	0.456
Urea (15-39 mg/dL)	28 (14-64)	30 (15-71)	< 0.001
Triglycerides (35-160 mg/dL)	149 (56-461)	153 (55-499)	0.186
Total cholesterol (140-220 mg/dL)†	197 (110-284)	192 (109-276)	< 0.001
HDL-c (45-60 mg/dL)	39 (20-72)	38 (19-69)	< 0.001
Hemoglobin (13.8-16.7 g/dL)	15 (9-18)	15 (9-19)	0.196
Hematocrit (40.5-52%)	43 (29-55)	42 (28-52)	0.140
Leukocytes (5.3-9.7 x $10^3/\mu L$)	5,320 (2,837-9,431)	5,710 (2,685-9,724)	< 0.001
Platelets (150-450 x $10^3/\mu L$)	237 (91-412)	240 (93-404)	0.416
ESR (0-20 mm/h)	20 (4-34)	19 (3-35)	0.499
hsCRP (0-0.8 mg/dL)	0.28 (0.03-0.88)	0.31 (0.03-1.04)	0.463
Fibrinogen (150-450 mg/dL)	373 (230-667)	373 (233-667)	0.276
PT (s)‡	11.7 (11.9)	11.7 (11.9)	0.188
APTT (s)‡	28.5 (28.0)	28.5 (28.0)	0.696
Total bilirubin (0.2-1.0 mg/dL)	1.4 (0.4-2.7)	1.4 (0.2-2.7)	0.416
Direct bilirubin (< 0.2 mg/dL)	0.8 (0.1-2.1)	0.8 (0.1-2.2)	0.875
Indirect bilirubin (0-0.8 mg/dL)	0.4 (0-1.8)	0.4 (0-1.8)	0.368
Total protein (6.7-8.2 g/dL)	6.7 (4.9-8.1)	6.5 (4.9-8.0)	0.059
Albumin (3.8-5.1 g/dL)	4 (2.9-5.1)	4 (3.1-5.0)	0.905
Globulin (g/dL)	2.6 (1.2-4.0)	2.5 (1.2-3.7)	0.024
A/G ratio	1.6 (0.9-3.6)	1.6 (1.0-3.2)	0.078
ALP (37-110 U/L)	63 (42-204)	68 (42-168)	0.324
LDH (106-274 U/L)	223 (98-395)	228 (113-388)	0.696
AST (15-37 U/L)	29 (13-54)	30 (13-54)	0.668
ALT (8-35 U/L)	31 (12-76)	29 (12-75)	0.557
GGT (5-24 U/L)	28 (12-65)	29 (12-60)	0.603

* Medians and percentiles 2.5-97.5. † Mean (95% CI = median ± 1.96 SD). ‡ As compared against the values obtained from a pool of normal plasmas. Reference values for each laboratory test are shown in parentheses. ESR: erythrocyte sedimentation rate. hsCRP: high-sensitivity C-reactive protein. A/G ratio: albumin/globulin ratio. PT: prothrombin time. APTT: activated partial thromboplastin time. ALP: alkaline phosphatase. LDH: lactate dehydrogenase. ALT: alanine transaminase. AST: aspartate transaminase. GGT: gamma-glutamyl transferase.

Table 2. Changes in laboratory tests before and after OML test in patients with diabetes, insulin resistance, and chronic renal failure.

Variable	Basal*	OML*	p
Diabetic patients(n = 59)			
Glucose(70-100 mg/dL)	163 (126-355)	177 (112-373)	0.227
Creatinine(0.6-1.30 mg/dL)	0.8 (0.6-2.3)	0.8 (0.5-2.4)	0.622
Urea(15-39 mg/dL)	32 (17-126)	32 (19-151)	0.445
Triglycerides(35-160 mg/dL)	170 (77-525)	186 (74-609)	0.398
Total cholesterol(140-220 mg/dL)	210 (129-336)	206 (124-303)	0.071
HDL-c(45-60 mg/dL)	41 (23-64)	39 (22-56)	0.094
Insulin resistant patients(n = 77)			
Glucose(70-100 mg/dL)	106 (101-124)	108 (88-147)	0.157
Creatinine(0.6-1.30 mg/dL)	0.8 (0.6-1.1)	0.8 (0.5-1.2)	0.094
Urea(15-39 mg/dL)	28 (16-49)	32 (19-52)	0.005
Triglycerides(35-160 mg/dL)	181 (77-454)	209 (85-609)	0.014
Total cholesterol(140-220 mg/dL)	198 (131-280)	193 (133-282)	0.266
HDL-c(45-60 mg/dL)	39 (23-71)	36 (17-59)	0.007
Renal failure patients(n = 8)			
Glucose(70-100 mg/dL)	133 (80-344)	107 (91-229)	0.742
Creatinine(0.6-1.30 mg/dL)	2.3 (2.1-4.0)	2.3 (0.8-2.4)	0.875
Urea(15-39 mg/dL)	77 (29-171)	85 (29-175)	0.089
Triglycerides(35-160 mg/dL)	153 (74-295)	114 (57-296)	0.360
Total cholesterol(140-220 mg/dL)	180 (95-257)	160 (99-244)	0.080
HDL-c(45-60 mg/dL)	44 (17-58)	45 (19-56)	0.761

*Medians and percentiles 2.5-97.5. Reference values for each laboratory test are shown in parentheses.

that means for four basal variables were not between the reference values: gamma-glutamyl transferase, HDL-c, total bilirubin, and direct bilirubin. We found that six variables were significantly modified after the OML test as compared with basal levels. Glucose and urea levels as well as leukocyte count showed higher levels than the corresponding basal levels. Moreover, levels of total cholesterol, HDL-c, and serum globulins decreased after the OML (Table 1). Except for HDL-c, mean values observed for all the variables analyzed were always between the normal reference ranges.

Because the study population included several clinical disorders, laboratory abnormalities could be completely different and may have different degrees of impact on the patients. Therefore, we performed a specific analysis considering only diabetic, insulin resistant, and chronic renal failure patients (Table 2). Except for basal total cholesterol levels, all results obtained before and after the OML test had an abnormal distribution. Descriptive analysis showed that there were not significant changes in all variables analyzed in diabetic and renal patients. In insulin resistant patients, three variables significantly changed as compared with basal levels: urea, triglycerides, and HDL-c. Except for triglycerides, mean values observed for all the variables analyzed were always between the normal reference ranges.

DISCUSSION

Thrombotic disease represents the most frequent cause of morbidity and mortality worldwide. During the last decades, advances in regard to the traditional atherothrombotic risk factors (diabetes, high blood pressure, dyslipidemia, obesity, and smoking) have grown exponentially. However, we are aware that these risk factors cannot explain all cases with atherothrombotic diseases because in up to 25% of patients with premature vascular disease there is no well-established risk factor.[20] In 1996, the 27th Bethesda Conference described the so-called new cardiovascular risk factors including HHC.[20,21] It is currently well known that HHC is a risk factor associated with venous thromboembolic disease and atherothombosis. As a consequence, a body of information has been published during recent years about the importance of HHC, mainly in human thrombotic disease.

Following reports on abnormal Hcy metabolism in patients with atherosclerosis.[22] the OML test was used to determine heterozygosity for cystathionine

beta-synthase (CBS) deficiency in patients with premature atherosclerosis.[7,23,24] Subsequently, this test was accepted as a regular procedure in patients with venous and arterial thrombosis, especially at a young age because elevated HHC was found in 60% of patients in fasting plasma samples, whereas in the remaining 40% of patients HHC was apparent only in the post-load specimens.[12] It is currently suggested that the OML test should be performed in persons at high risk of atherosclerosis or venous thrombotic disease or with a history of thrombosis and normal fasting Hcy concentration.[25]

Methionine, a normal component of alimentary proteins, is a harmless compound. The dose of methionine used in the OML test is almost 2.5-4 times the amount of a regular Western diet containing 1.6-2.8 g/day.[26] Although higher doses of methinone (70-300 mg/kg) have been safely used in patients with several conditions requiring an OML test, incidence of adverse events increases with these high doses.[27-29] Therefore, an appropriate methionine dose should always be indicated in a patient requiring an OML test.

The endothelium is perhaps the main target for the HHC-induced vascular damage.[30-32] Some endothelial abnormalities have been reported following acute administration of methionine as happens in the OML test: endothelial dysfunction,[31,33,34] impaired endothelium dependent relaxation of larger arteries,[30,35-37] impaired nitric oxide activity without change of oxidative status,[32] and endothelial cell desquamation.[38] Other abnormalities associated with acute HHC include renal metabolic dysfunction,[39] reduction of cerebral blood flow,[40] changes in pulmonary vascular function,[41] increased lipid peroxidation,[42] elevated oxidative stress status,[43] arterial smooth muscle dysfunction, disturbances in plasma lipid profile and in blood coagulation tests,[44,45] elevated acute endogenous fibrinolytic capacity,[46] increased plasma oxidation markers,[44] and transient abnormalities in perception and vigilance without effects on the vasculature.[17] Of course, all these functional disturbances may be especially important in individuals with pre-existing endothelial dysfunction[40,47] as occurs in patients with a history of venous or arterial thrombotic events. Because the possibility of impairment of vascular function secondary to an acute rise in Hcy plasma levels may occur after an OML test, especially in individuals with a prothrombotic status, reports in regard to the clinical safety of the test are always useful.

In our 30-day study interval, only three individuals had an immediate severe complication (vomiting in all of them), and there was no mortality related to the OML test. In fact, all symptoms appearing after the OML disappeared immediately after breakfast (none of the adverse clinical effects persisted > 4 h after the meal), a fact that suggests that these symptoms may be attributed to a local effect of the OML. Indeed, because nausea was the most common complication of the OML test, we feel that this reaction may be caused by a direct reaction of the stomach secondary to the methionine itself, as strongly suggested by the immediate improvement after breakfast. Finally, vomiting and nausea may also be related to the intake of large amounts of fruit juice.

Laboratory abnormalities induced by the OML test have been poorly described in the literature and some of these data were derived from studies in which the intention was not directly to evaluate the presence or absence of these side effects. For example, there are reports indicating an increase in serum triglyceride levels and in the total cholesterol/HDL-c ratio.[16,18] However, to our knowledge, our report is the first attempt to prospectively evaluate the changes in the general laboratory tests in individuals requiring an OML test. We attempted to analyze the general laboratory tests that are most widely used in medical practice because they provide information about an individual's hematological, metabolic, hepatic, and inflammatory status. Our study included patients with several clinical disorders such as diabetes mellitus, dyslipidemia, high blood pressure, and mild to moderate liver and renal failure. Therefore, our main objective was to establish whether the OML may induce negative changes in these variables that may have a deleterious effect on the primary disease of the patient. Our results are quite clear. Serum and plasma levels of most of the laboratory tests studied were similar before and after the OML test and although, from the statistical point of view, we found some significant differences these changes were clinically irrelevant. For example, statistical analysis showed that the mean glucose level rose significantly after the OML test; however, the real increase was only 4 mg/dL, which may be considered as clinically irrelevant from the clinical perspective. This conclusion may apply to other tests showing significant differences before and after the OML testing. Of course, in some cases the results of these tests showed improvement, but they were also clinically irrelevant. On the other hand, because our sample included patients with several diagnoses we performed a specific analysis of the three most representative subgroups namely diabetic, insulin resistant, and chronic renal failure patients.

Only insulin resistant patients showed statistical differences before and after the OML test and, as previously described for the whole group of individuals studied, most of these changes appear clinically irrelevant. Finally, we feel that the sample size of our study provides us with a realistic view of the safety of the OML both in patients and controls.

CONCLUSION

We found that the OML test had no significant undesirable effects on clinical status (as previously demonstrated) or general laboratory tests of patients and healthy controls. Although some symptoms associated with the OML test were observed, none of the subjects experienced a worsening of the primary disease during the 30-day study period. Moreover, the OML test did not negatively affect general laboratory tests. Therefore, the OML test can be considered a safe diagnostic procedure in patients with a previous thrombotic event (and with the consequent associated risk factors such as diabetes mellitus or dyslipidemia), as well as in healthy subjects.

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