Osteoprotegerin: A Novel Independent Marker for Silent Myocardial Ischemia in Asymptomatic Diabetic Patients

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OBJECTIVE — We sought to evaluate osteoprotegerin, an inhibitor of osteoclastogenesis involved in atherosclerosis, and other novel risk factors as predictive markers of silent myocardial ischemia (SMI).

RESEARCH DESIGN AND METHODS — A total of 465 consecutive diabetic patients with more than one additional risk factor were evaluated for SMI using stress myocardial perfusion imaging (MPI). We studied the association of SMI (positive stress electrocardiogram and/or abnormal MPI) with osteoprotegerin, other novel risk factors (lipoprotein[a], homocysteine, adiponectin, C-reactive protein, and fibrinogen), and conventional risk factors (total, LDL, and HDL cholesterol and triglycerides).

RESULTS — A total of 92 patients were diagnosed with SMI. Of the six novel markers, osteoprotegerin was the only one associated with SMI; the relative risk (RR) of SMI in patients with osteoprotegerin values above the 75th percentile was 3.19 (95% CI 1.99–5.18; $P < 0.001$) in comparison with those with osteoprotegerin below the 75th percentile. In univariate analyses, the other plasma markers significantly associated with SMI were higher triglycerides ($P = 0.04$) and lower HDL cholesterol ($P = 0.02$). The association of osteoprotegerin with SMI remained significant after correcting for other variables associated with SMI at $P < 0.15$ in univariate analysis (RR 3.95 [95% CI 2.21–7.06]; $P < 0.0001$). The association of osteoprotegerin with SMI was observed in male ($P < 0.0001$) and female ($P = 0.03$) patients, in type 1 ($P = 0.002$) and type 2 ($P = 0.0004$) diabetic patients, in patients with ($P = 0.0004$) or without ($P = 0.03$) nephropathy, and in patients without ($P < 0.0001$) but not with ($P = 0.2$) peripheral arterial disease.

CONCLUSIONS — Osteoprotegerin measurement, together with other conventional factors, can help to better define the diabetic population with an increased likelihood for SMI.

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The risk of coronary artery disease (CAD) is markedly increased in diabetic patients, in whom CAD is the leading cause of death (1). Silent myocardial ischemia (SMI) occurs frequently in numerous individuals and may result in more severe CAD upon initial presentation and worse outcomes in diabetic patients (2,3). Once CAD is symptomatic, morbidity and mortality are higher and significantly worse in patients with than without diabetes.

In patients with diabetes, risk factors should be managed aggressively to prevent the development and progression of CAD (4–7). The American Diabetes Association consensus guidelines recommend screening for SMI when two or more additional risk factors are present (8) as is already done by many physicians. However, the outcome of such screening in asymptomatic patients has been inconsistent (9–12).

Several novel risk factors have been proposed as potential criteria to refine the detection of subclinical atherosclerosis in patients, including diabetic patients. In particular, clinical interest has focused on promising lipid parameters, such as lipoprotein(a), and inflammatory biomarkers like C-reactive protein (CRP) and fibrinogen and on nutritional markers associated with premature atherothrombosis, such as total plasma homocysteine.

Osteoprotegerin, a key factor in bone remodeling (13), is a member of the tumor necrosis factor receptor family and a decoy receptor for the receptor activator of nuclear factor-κB ligand and tumor necrosis factor–related apoptosis-inducing ligand (14). Recently, we reported that osteoprotegerin is an independent predictor of angiographically diagnosed but asymptomatic CAD in type 2 diabetic patients (15). In the present study, we sought to determine whether the measurement of osteoprotegerin in addition to the recommended factors may provide a clinically useful method for selecting patients who should benefit from SMI screening. In addition, to compare osteoprotegerin with other novel markers, we measured the plasma levels of CRP, fibrinogen, lipoprotein(a), homocysteine, and adiponectin.

RESEARCH DESIGN AND METHODS

— Since March 2001, all asymptomatic patients referred to our diabetes clinic (either new referrals or returning for routine follow-up) have been regularly considered for stress testing according to the French diabetic association

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Abbreviations: Apo, apolipoprotein; CAD, coronary artery disease; CRP, C-reactive protein; ECG, electrocardiogram; hs-CRP, high-sensitivity CRP; HPLC, high-performance liquid chromatography; MPI, myocardial perfusion imaging; PAD, peripheral arterial disease; SMI, silent myocardial ischemia.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

(ALFEDIAM) guidelines currently available at that time (16). The present report describes a cross-sectional analysis of 465 consecutive asymptomatic diabetic patients recruited between March 2001 and October 2006 with at least one of the following characteristics: age ≥60 years, active smoking, albuminuria, hypertension, dyslipidemia, family history of premature CAD, and peripheral arterial disease, with normal baseline electrocardiogram (ECG). These subjects were subjected to a cardiac stress test to ascertain the presence/absence of SMI. Exclusion criteria were age <35 or >85 years, history of coronary events, symptoms of coronary events as defined by the Rose Questionnaire, abnormal result at the ECG, chronic or acute diseases, pregnancy, neoplasia, and contraindications to dipyridamole infusion, such as asthma. Diabetes was diagnosed according to American Diabetes Association criteria (17). Participants were considered to have type 2 diabetes if they had no history of ketosis and did not start any insulin treatment either in the 2 years following the diagnosis or before the age of 40 years. Otherwise, they were considered to have type 1 diabetes. Hypertension was diagnosed if at least one of the following conditions was present: systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or presence of at least one antihypertensive medication. Dyslipidemia was considered when any of the following conditions were present: LDL cholesterol ≥3.35 mmol/l, HDL cholesterol <1.05 mmol/l, triglycerides ≥1.70 mmol/l, and/or lipid-lowering therapy. Patients with albumin excretion rate >30 mg/day and/or macroproteinuria were considered to have diabetic nephropathy. Peripheral arterial disease (PAD) was diagnosed when one or more peripheral arterial pulse was abolished at clinical examination and/or when intermittent claudication or a past history of revascularization of the lower limbs was present.

Stress test and myocardial perfusion imaging were performed and interpreted as previously described (15,18). Briefly, all subjects underwent a combined dipyridamol (0.75 mg/kg) exercise stress test followed by a same-day stress-rest imaging protocol using ^{99m}Tc-sestamibi (1 mCi/10 kg body wt with a minimum of 7 mCi; 259 MBq). Stress and rest images were acquired, in the prone position, 1 h after injection of ^{99m}Tc-sestamibi. Acquisition was performed using a double-headed gamma camera (GE-SMV DST-XL) with low-energy, high-resolution, parallel-hole collimators. Stress and rest acquisitions were gated (10% R-R interval acceptance window, eight gated intervals). Images were reconstructed (butterworth filter order 4, cutoff frequency 0.25/cm), and short-axis, horizontal long-axis, and vertical long-axis sections were obtained. MPI reconstructions were divided into 20 segmental regions. Inside a segment, a hypoperfusion was considered significant if the mean activity in the segment was <70% of the maximal myocardial activity.

When stress images showed a significant defect in at least three segments (i.e., ≥15% of the whole left ventricular myocardium), they were scored as abnormal, and a resting study was performed. For resting studies, patients were injected with three times the activity injected for the stress study (minimum 21 mCi; 777 MBq) of ^{99m}Tc-sestamibi 3 h after stress. Stress images showing smaller defects (<15%) were scored as normal, and no rest acquisition was performed. Defects were classified as reversible (normalization after injection), fixed (persistent defect after injection), or partially reversible. Subjects were considered to have SMI in the presence of a positive stress ECG (i.e., horizontal or descending ST segment depression ≥1 mm relative to the isometric line and measured at the J-junction) and/or an abnormal scintigraphic image.

Details of all patients were recorded at the time of their clinic attendance. Each patient enrolled in this study had given oral informed consent in accordance with the European directives as edited in 2001 (available at http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_121/l_12120010501en00340044.pdf), which require no approval from an ethical committee for a study design as described herein.

Measurement of risk factors

Peripheral blood samples were collected in evacuated tubes containing heparin as anticoagulant the morning of the stress test after a 12-h fast, and an aliquot was frozen at −80°C until the time of osteoprotegerin and adiponectin dosages. Then, the aliquots were thawed and plasma levels of these factors determined by enzyme-linked immunosorbent assay (Biovendor Laboratory Medicine, Brno, Czech Republic).

The other measurements were performed on the collection day. Total cholesterol, HDL cholesterol, and triglyceride levels were measured in serum by routine enzymatic methods (KonePro; Konelab, Epoo, Finland). Lipoprotein(a), apolipoprotein (apo)A, and apoB concentrations were determined by immunonephelometric assay using an Immage x (Beckman-Coulter) Behring Nephelemeter 100 (Behring Diagnostic, Marburg, Germany). High-sensitivity CRP (hs-CRP) (from 0.5 to 20 mg/l) was determined by latex-enhanced immunoturbidimetric method on an Olympus AU2700 biochemistry analyzer (Olympus, Rungis, France). A1C was measured by a routine high-performance liquid chromatography (HPLC)-based ion-exchange procedure (HA-8140; Menarini, Rungis Cedex, France). Total plasma homocysteine was measured by chromatography method. HPLC with fluorimetric detection was carried out on a Dionex HPLC device equipped with an RF 1000 Dionex spectrofluorimeter (Dionex, Sunnyvale, CA) using a commercial Recipe HPLC analytical kit including a calibrator and internal standard (Recipe Chemicals and Instruments, Munich, Germany). Fibrinogen measurements were carried out according to the Von Clauss method on an STA-R analyzer with commercial reagents STA-fibrinogen 5 (Diagnostica Stago, Taverny, France).

Statistical analysis

Means, medians, or proportions for cardiovascular risk factors were calculated for subjects with and without SMI. The significance of mean differences between the two groups was assessed by Student's *t* test or Mann-Whitney's *U* test as adequate, and the significance of differences in proportions was tested with the χ^2 statistic tool. After dividing subjects into quartiles on the basis of the distribution of osteoprotegerin values, we used logistic regression analyses to compute the relative risks (RRs) of SMI when associated with increasing levels of osteoprotegerin. Accordingly, the magnitude of the risk associated with levels in the highest versus lowest quartile could be directly compared. Multivariable estimates of risk were computed in similar models, which were adjusted for factors that were associated with SMI at $P < 0.15$ in a univariate analysis. Moreover, we addressed the potential additive value of osteoprotegerin as a risk factor with different variables—such as type 1 and type 2 diabetes, sex, and presence or absence of nephropathy or PAD—by using the likelihood ratio test to determine whether the addition of osteoprotegerin improved the risk predic-

Table 1—*Characteristics of patients with or without SMI*

Variable	Patients without SMI	Patients with SMI	*P*
n	373	92	—
Age (years)	59.1 ± 10.7	61.3 ± 9.0	0.07
Male sex (%)	60	76	0.003
Diabetes duration (years)	16.8 ± 11.9	12.9 ± 8.9	0.004
Type 2 diabetes (%)	73	90	0.001
BMI (kg/m²)	28.4 ± 5.1	30.0 ± 5.2	0.006
A1C (%)	8.35 ± 1.52	8.26 ± 1.84	0.6
Treatment (%)			0.0002
Diet only	2	4	—
Oral agent only	38	50	—
Insulin only	42	42	—
Insulin + oral agent	18	4	—
Hypertension (%)	70	83	0.02
Diabetic nephropathy (%)	38	51	0.02
Diabetic retinopathy (%)	39	35	0.5
PAD (%)	10	23	0.0004
SBP (mmHg)	128.5 ± 16.4	134.5 ± 16.8	0.002
DBP (mmHg)	71.9 ± 9.9	72.4 ± 9.6	0.7
Dyslipidemia (%)	74	83	0.09
Statin treatments (%)	46	57	0.07
Family history of CAD (%)	14	11	0.4
Smoking status (%)			0.03
Never	46	44	—
Past	30	43	—
Current	24	13	—

Data are means ± SD or percent unless otherwise indicated. DBP, diastolic blood pressure; SBP, systolic blood pressure.

tion of SMI in each of these conditions. Similar logistic regression analyses were then performed after dividing the study subjects into four groups based on presence or absence of the considered factor and on the level of osteoprotegerin (i.e., below or above the 75th percentile). Based on our previouly published work (15), we made the a priori hypothesis that there would be a relationship between SMI and serum osteoprotegerin. Thus, *P* values <0.05 were considered significant, and no adjustment was made for multiple testing.

RESULTS — Of the 465 patients of the present study, 92 (20%) had SMI. Sixty-five patients (14%) had abnormal MPI (defect in at least 3 of 20 segments, i.e., ≥15% of the whole myocardium), including 15 patients who also had a positive exercise ECG; of these, 44, 7, and 14 had reversible, fixed, and partially reversible defects, respectively. Despite normal MPI, 27 additional patients (6%) had a positive exercise ECG.

The clinical characteristics significantly associated with SMI were male sex, type 2 diabetes, BMI, treatment type, hypertension, nephropathy, PAD, and smoking status (Table 1). Patients with SMI tended to be older but had shorter diabetes duration.

As shown in Table 2, the biological markers significantly associated with SMI were osteoprotegerin, HDL cholesterol, and plasma triglycerides. LDL cholesterol tended to be higher in patients with SMI in comparison with patients without SMI. No significant associations were found among SMI and plasma levels of total cholesterol, apoA-1 and apoB-100, lipoprotein(a), CRP and fibrinogen, homocysteine, and adiponectin.

The RRs of SMI for increasing quartiles of osteoprotegerin are presented in Fig. 1. No difference in RRs of SMI was noted for the first three quartiles, whereas the 4th quartile was associated with a significant increase in RR in comparison with the previous three quartiles. Hence, a plasma value of osteoprotegerin ≥8.19 pmol/l (i.e., above the 75th percentile) was associated with an RR of SMI of 3.19 (95% CI 1.99–5.18; $P < 0.001$); the positive and negative predictive values of osteoprotegerin plasma measurements above this level were 38 and 85%, respectively. This association was not altered by further analyses, which were controlled for factors associated with SMI at $P < 0.15$ in univariate analysis, i.e., age, sex, BMI, PAD, diabetes type, diabetes treatment, statin treatment, smoking status, blood pressure, dyslipidemia, diabetes duration, and adiponectin (RR 3.95 [95% CI 2.21–7.06]; $P < 0.0001$) .

We then explored whether an osteoprotegerin value above the 75th percentile had a predictive value for SMI in different conditions, such as type 1 and type 2 diabetes, male or female sex, and presence or absence of nephropathy or PAD, by undertaking a series of additional analyses. We computed the RR of SMI in analyses that stratified the study participants into four groups based on having an osteoprotegerin value below or above the

Table 2—*Comparison of plasma levels of risk factors in patients with or without SMI*

Variable	Patients without SMI	Patients with SMI	*P*
n	373	92	—
hs-CRP (mg/dl)	0.17 (0.09–0.36)	0.19 (0.09–0.42)	0.4
Fibrinogen (g/dl)	0.33 ± 0.08	0.32 ± 0.08	0.3
Homocysteine (mg/l)	1.43 ± 0.74	1.42 ± 0.50	0.7
Osteoprotegerin (pmol/l)	6.57 ± 3.12	8.92 ± 5.5	<0.0001
Adiponectin (μg/ml)	9.67 ± 6.71	8.44 ± 6.35	0.1
Total cholesterol (mg/dl)	195 ± 43	203 ± 41	0.1
LDL cholesterol (mg/dl)	108 ± 35	115 ± 35	0.06
HDL cholesterol (mg/dl)	60 ± 19	55 ± 17	0.02
Triglycerides (mg/dl)	115 (79–162)	134 (96–194)	0.04
ApoA-1 (mg/dl)	153 ± 31	150 ± 27	0.3
ApoB-100 (mg/dl)	100 ± 27	104 ± 24	0.2
Lipoprotein(a) (mg/dl)	13 (11–33)	11 (11–37)	0.4

Data are means ± SD or median (interquartile range) unless otherwise indicated.

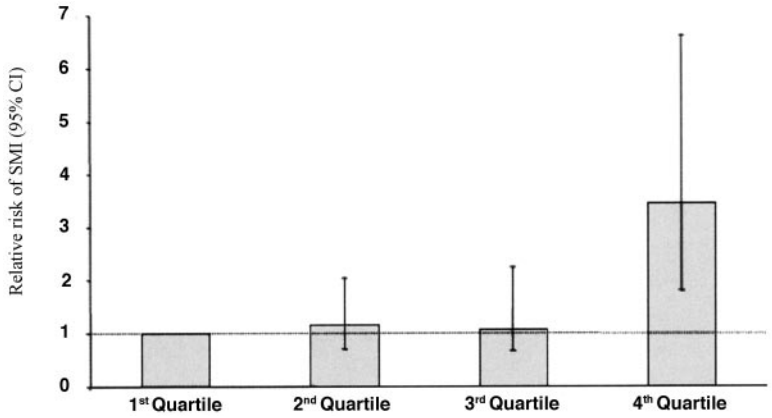

Figure 1— *RR of SMI according to osteoprotegerin quartile.*

75th percentile and then on sex, diabetes type, nephropathy, and PAD. Osteoprotegerin significantly improved the predictive value for SMI in all tested conditions except PAD (Fig. 2), where the predictive value of osteoprotegerin was enhanced only in patients without PAD.

CONCLUSIONS — In this study, we evaluated the strength of osteoprotegerin and five other novel risk factors (i.e., hs-CRP, lipoprotein[a], fibrinogen, homocysteine, and adiponectin) for the prediction of SMI in asymptomatic diabetic patients. Among these factors, osteoprotegerin was the only one significantly associated with SMI. In particular, when the values of this marker were above the 75th percentile (≥8.19 pmol/l), diabetic patients presented a 35% prevalence of SMI in comparison with a 14% prevalence when values were below that percentile. The predictive value of osteoprotegerin was observed in both sexes, in type 1 and type 2 diabetic patients, and regardless of the presence or absence of diabetic nephropathy. However, in patients with PAD, the increased RR of SMI at higher levels of osteoprotegerin was not significant. This might be due to the small size of this group (56 patients) and/or to the increased RR of SMI for patients with PAD at any osteoprotegerin level.

Besides osteoprotegerin, male sex was an independent predictor of SMI, whereas nephropathy, homocysteine, and hs-CRP were not. This finding is in agreement with the recently published Detection of Ischemia in Asymptomatic Diabetics Study (19). We also did not find any association with adiponectin, an adipocytokine reported to be negatively associated with CAD (20). This confirmed our previous results about the absence of any link between adiponectin and angiographically diagnosed coronary stenosis (15).

Surprisingly, shorter diabetes duration was significantly associated with SMI, which could be explained by the fact that type 1 diabetic patients, who had a much longer disease duration than type 2 diabetic patients, were predominantly in the group without SMI versus the one with SMI (26 vs. 10%, respectively, $P < 0.001$). Another unanticipated result was the higher proportion of current smokers in the group without SMI. However, since the proportion of past smokers was higher in the SMI group, it could be argued that the higher risk profile of these patients could have driven a higher proportion of them to quit smoking.

Our findings demonstrate the association between higher values of osteoprotegerin and higher risk of SMI and are consistent with previous reports that showed a close correlation between osteoprotegerin levels and surrogate measures of cardiovascular risk, including the following: *1*) carotid intima-media thickness

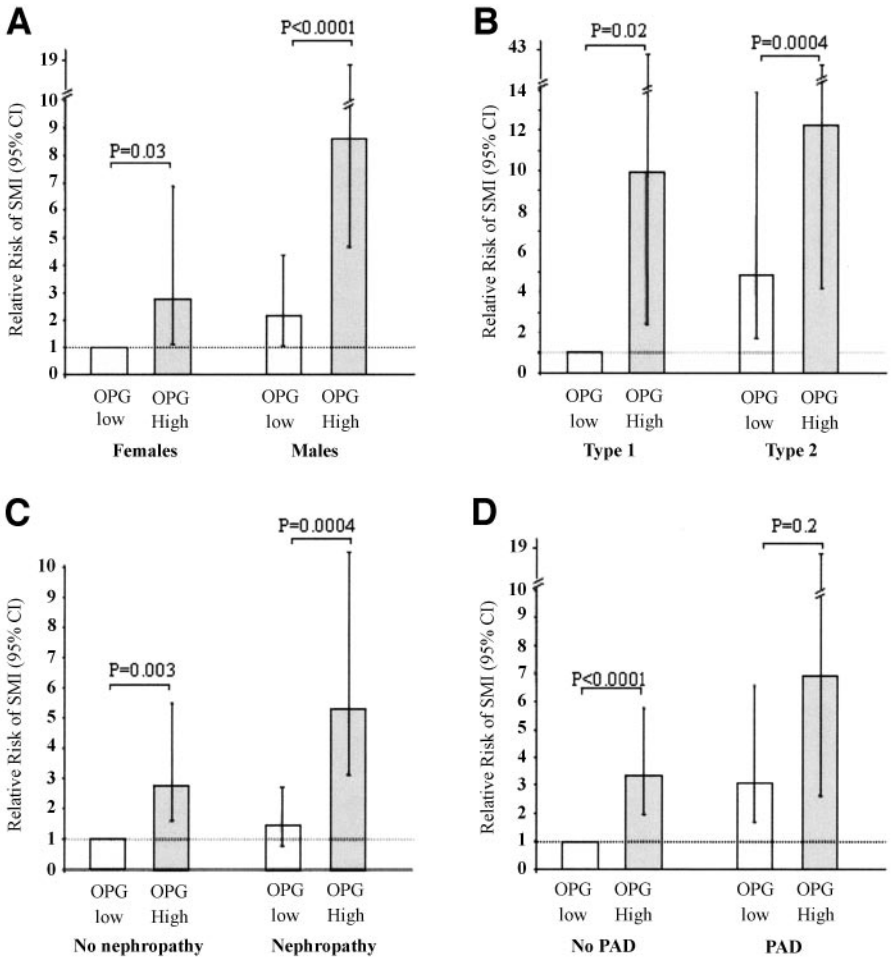

Figure 2— *Stratification by sex* (A)*, type of diabetes* (B)*, nephropathy* (C)*, and PAD* (D) *in combination with osteoprotegerin. Referents are female sex* (A)*, type 1 diabetes* (B)*, no nephropathy* (C)*, and no PAD* (D) *in combination with a plasma value of osteoprotegerin below the 75th percentile.*

in healthy men and women (21), 2) left ventricular hypertrophy (22), 3) microvascular disease in diabetic subjects (23), and 4) coronary artery calcification in type 2 diabetic subjects (24). Three cross-sectional studies have revealed a relationship between osteoprotegerin levels and the severity of coronary atherosclerosis in symptomatic CAD patients undergoing coronary angiography (22–24). More recently, we confirmed in a case-controlled study that osteoprotegerin is an independent predictor of angiographically diagnosed silent CAD in asymptomatic diabetic subjects (15). Recent reports have also established the prognostic value of osteoprotegerin measurements in healthy men and women (21), in hemodialysis patients (25), in patients with heart failure after acute myocardial infarction (26), and in type 2 diabetic patients (24).

Since in diabetic patients osteoprotegerin is associated with surrogate markers of cardiovascular disease, coronary stenosis at coronary angiography, and cardiovascular events, we believe that the results of the present report, which demonstrate a strong link between high levels of osteoprotegerin and SMI, have important clinical implications for the screening strategy of asymptomatic CAD in diabetic patients. From a practical perspective, the current data support the hypothesis that an osteoprotegerin value >8 pmol/l, in addition to other recommended factors (8), might represent a useful and cost-effective means to select the subset of diabetic patients who will require stress testing.

Our study has several limitations. It is a cross-sectional study, and inclusion criteria asked for the presence of at least another significant risk factor for CAD; therefore, the chosen patients could be considered at high risk for CAD. This choice limits the applicability of these data only to the highest-risk type 2 diabetic patients and not the general type 2 asymptomatic diabetic population, for whom they need to be confirmed.

Considering the high rates of cardiovascular events and mortality in diabetic patients, besides an aggressive management of cardiac risk factors in all patients (27), risk stratification should be optimized in this exponentially expanding population (28). Noninvasive imaging might be of particular value in this regard (29); however, the cost of performing this test among all asymptomatic diabetic patients is too high. Hence, we need better methods of assessing risk and to define a risk threshold above which noninvasive and invasive investigations are justified (30). Our current results demonstrate that the evaluation of osteoprotegerin can help better define the diabetic population with an increased likelihood for SMI.

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