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Clinical Research Article

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A Serum Resistin and Multicytokine Inflammatory Pathway Is Linked With and Helps Predict All-Cause Death in Diabetes

Maria Giovanna Scarale,[1] Alessandra Antonucci,[1] Marina Cardellini,[2,3] Massimiliano Copetti,[4] Lucia Salvemini,[1] Rossella Menghini,[2] Tommaso Mazza,[5] Viviana Casagrande,[2,1] Gianluigi Ferrazza,[3] Olga Lamacchia,[6] Salvatore De Cosmo,[7] Rosa Di Paola,[1] Massimo Federici,[2,3] Vincenzo Trischitta,[1,8,*] and Claudia Menzaghi[1,*]

[1]Research Unit of Diabetes and Endocrine Diseases, Fondazione IRCCS "Casa Sollievo della Sofferenza," 71013 San Giovanni Rotondo, Italy; [2]Department of Systems Medicine, University of Rome Tor Vergata, Rome 00133, Italy; [3]Center for Atherosclerosis, Department of Medical Sciences, Policlinico Tor Vergata University, Rome 00133, Italy; [4]Unit of Biostatistics, Fondazione IRCCS "Casa Sollievo della Sofferenza," San Giovanni Rotondo 71013, Italy; [5]Bioinformatics Unit, IRCCS "Casa Sollievo della Sofferenza," San Giovanni Rotondo 71013, Italy; [6]Unit of Endocrinology and Diabetology, Department of Medical and Surgical Sciences, University of Foggia, Foggia 71100, Italy; [7]Department of Clinical Sciences, Fondazione IRCCS "Casa Sollievo Della Sofferenza," San Giovanni Rotondo 71013, Italy; and [8]Department of Experimental Medicine, "Sapienza" University, Rome 00185, Italy.

ORCiD number: 0000-0002-7438-8955 (C. Menzaghi), 0000-0003-1174-127X (V. Trischitta).

*These authors oversaw the entire study.

Abbreviations: BMI, body mass index; CEA, carotid endarterectomy; cNRI, category-free net reclassification improvement; ENFORCE, Estimation of Mortality Risk in Type 2 Diabetic Patients; FMS, Foggia Mortality Study; GHS-prospective, Gargano Heart Study–prospective design; GMS, Gargano Mortality Study; HbA_{1c}, glycated hemoglobin A_{1c}; HR, hazard ratio; hs-CRP, high-sensitivity C reactive protein; IL, interleukin; MACE, major adverse cardiovascular event; OR, odds ratio; RECODe, Risk Equations for Complications of Type 2 Diabetes; REMAP, resistin and multicytokine inflammatory pathway; rIDI, relative integrated discrimination improvement; TNF, tumor necrosis factor.

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Abstract

Context: Type 2 diabetes (T2D) shows a high mortality rate, partly mediated by atherosclerotic plaque instability. Discovering novel biomarkers may help identify high-risk patients who would benefit from more aggressive and specific managements. We recently described a serum resistin and multicytokine inflammatory pathway (REMAP), including resistin, interleukin (IL)-1β, IL-6, IL-8, and TNF-α, that is associated with cardiovascular disease.

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Objective: We investigated whether REMAP is associated with and improves the prediction of mortality in T2D.

Methods: A REMAP score was investigated in 3 cohorts comprising 1528 patients with T2D (409 incident deaths) and in 59 patients who underwent carotid endarterectomy (CEA; 24 deaths). Plaques were classified as unstable/stable according to the modified American Heart Association atherosclerosis classification.

Results: REMAP was associated with all-cause mortality in each cohort and in all 1528 individuals (fully adjusted hazard ratio [HR] for 1 SD increase = 1.34, $P < .001$). In CEA patients, REMAP was associated with mortality (HR = 1.64, $P = .04$) and a modest change was observed when plaque stability was taken into account (HR = 1.58; $P = .07$). REMAP improved discrimination and reclassification measures of both Estimation of Mortality Risk in Type 2 Diabetic Patients and Risk Equations for Complications of Type 2 Diabetes, well-established prediction models of mortality in T2D ($P < .05$-$< .001$).

Conclusion: REMAP is independently associated with and improves predict all-cause mortality in T2D; it can therefore be used to identify high-risk individuals to be targeted with more aggressive management. Whether REMAP can also identify patients who are more responsive to IL-6 and IL-1β monoclonal antibodies that reduce cardiovascular burden and total mortality is an intriguing possibility to be tested.

Key Words: resistin, cytokines, mortality, prediction models, type 2 diabetes, plaque instability

Type 2 diabetes is a leading cause of death, accounting for 11% of global all-cause mortality among adults (1).

Because of the epidemic proportion type 2 diabetes is assuming (1), this dramatic load will surge further over the next decades, thus greatly contributing to increased economic, social, and human costs worldwide (2).

To tackle such a burden, highly performing models able to predict the most high-risk patients who would benefit from the most aggressive, expensive, and burdensome prevention strategies are mandatory.

Unraveling novel biomarkers that improve stratification of all-cause mortality risk is a way to address this need (3). Coincidentally, such efforts can also highlight pathogenic pathways that are still unknown or poorly understood and that eventually become the target of new treatments (4).

It has long been known that low-grade inflammation is pathogenic for atherosclerotic cardiovascular disease (5, 6) and a key player in atherosclerotic plaque formation, progression, instability, and healing (7), all major causes of total death, especially in individuals with diabetes (8). Among the proinflammatory cytokines is resistin, which has been associated with cardiovascular disease and mortality rate in patients with type 2 diabetes (9-11). Resistin itself controls the expression of several other proinflammatory cytokines (12-16), so the existence of a resistin and multicytokine inflammatory pathway, now named resistin and multicytokine inflammatory pathway (REMAP), operating in cells and tissues, has been hypothesized (17) Our recent findings also speak in favor of the existence in vivo of such a pathway, including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, whose serum concentrations are strictly correlated with those of resistin (17). Notably, a REMAP score, created by summing up circulating levels of resistin and the aforementioned cytokines, is associated with cardiometabolic traits in the general population and with major adverse cardiovascular events (MACE) in type 2 diabetes (17). Based on this encouraging background, we investigated whether it is associated with and helps improve the prediction of all-cause mortality as assessed by established prediction models (18, 19), which, though well performing, need to be further improved with the aim of reaching outstanding discriminatory abilities (20, 21). In a small subgroup of high-risk individuals enriched by type 2 diabetes, we also investigated whether the association between REMAP and all-cause mortality was dependent on atherosclerotic plaque stability.

Materials and Methods

Participants

Three cohorts of patients with type 2 diabetes (diagnosed according to the American Diabetes Association 2018 criteria), the Gargano Mortality Study (GMS), Foggia Mortality Study (FMS), and the Gargano Heart Study (GHS)-prospective design from Apulia, central-southern Italy, were analyzed.

The GMS and GHS-prospective design were recruited at IRCCS "Casa Sollievo della Sofferenza," San Giovanni Rotondo, Italy (3). The GMS comprises 1028 patients recruited from 2000 to 2005, with all-cause mortality as the end point and followed until December 2014 (3). The FMS comprises 1115 patients consecutively recruited at

the University of Foggia from 2002 to 2008, with all-cause mortality as the end point and followed until March 2015 (3). The GHS-prospective design consists of 368 patients with coronary artery disease consecutively recruited from 2001 to 2008, and followed yearly until 2011 for MACE, cardiovascular, and all-cause mortality (9, 17).

For all patients, follow-up duration, exclusion criteria, and vital status ascertainment were assessed as previously described (3). For this specific study, serum resistin, IL-1β, IL-6, IL-8, and TNF-α concentrations were assessed in 643 individuals from the GMS (62.5% of the total sample, randomly extracted), 532 from the FMS (48%, randomly extracted), and 353 (96%) from the GHS-prospective design.

In addition, to address the role of plaque instability in mediating the association between REMAP and all-cause mortality, a selected cohort of 59 patients (16 with major stroke or transient ischemic attack and 43 with no symptoms) was recruited. All patients underwent carotid endarterectomy (CEA) by patch reconstruction with the use of shunt when indicated by electroencephalogram monitoring, performed at Tor Vergata University Hospital, Rome, Italy (22). Further details on this sample have been previously reported (22). Both serum levels and carotid plaque gene expression of the 5 cytokines mentioned earlier were measured in these patients.

Measurement of Circulating Cytokines

Serum resistin concentrations were measured in duplicate by a commercial enzyme-linked immunosorbent assay (Bio Vendor) as previously described (17). Interassay and intra-assay coefficients of variation were 7.0% to 8.1% and 5.2% to 6.6%, respectively. Serum IL-1β, IL-6, IL-8, and TNF-α circulating levels were measured in duplicate, using a multiplex detection 4-plex kit from Bio-Rad. The median coefficient of variation was less than 25% for all analyzed cytokines. Data were analyzed as previously described (17).

Plaque Histology and Measurements of Cytokine Expression

All plaques had histology classified according to previously reported methods (22) and were separated into 2 categories according to the modified American Heart Association atherosclerosis classification: unstable and stable plaques (23). Unstable plaques comprise thrombotic and vulnerable plaques. Thrombotic plaques included: (a) plaque rupture with the luminal thrombus, (b) ulceration, (c) erosion, and (d) a calcified nodule. Vulnerable plaque or a thin-cap fibroatheroma was characterized by a fibrous cap less than 165 µm thick heavily infiltrated by macrophages, CD68 positive (> 25 per high-magnification field), without plaque rupture. Stable plaques were divided in fibrocalcific and healed plaques. Fibrocalcific plaques showed a thick fibrous cap (> 165 µm) associated with the presence of calcification and a variable necrotic core. Healed plaques were defined as those showing multilayers of fibrous tissue and a lipid-rich necrotic core.

Gene expression quantification of RETN, IL-1, IL-6, CXCL8, and TNF were derived from a whole-transcriptomic analysis conducted in 59 carotid plaques from patients with CEA, by means of high coverage (30 million pairs of 100 bp reads/sample) paired-end RNA-sequencing on Illumina NexSeq 500, following Illumina-based strand specific protocol (Illumina Inc). Data from the whole-transcriptomic analysis remain under deeper, ongoing investigation and will be the subject of another manuscript.

Statistical Analysis

Patients' baseline characteristics were reported as mean ± SD or median and interquartile range and frequency and percentage for continuous and categorical variables, respectively. Since the rate of missing values was low (< 5%) in all samples, data were imputed using the random forest framework (24). Because of skewed distribution, values of all molecules belonging to the REMAP were log-transformed, standardized, and then summed up in a REMAP score. In the 3 cohorts of individuals with type 2 diabetes, to be conservative enough, our analyses were kept linked to the background derived from similar, unselected diabetic patients (17) on whom our present study is based. Each standardized cytokine serum value was therefore summed in the REMAP score after being weighted by the regression coefficient previously obtained in the association with MACE (17). The weights used to estimate the REMAP score and the overall rationale behind their use are described in the supplementary data (25). In all prospective studies, the time variable was defined as the time between the baseline examination and date of the event (ie, all-cause mortality) or, for individuals who did not experience the event, the date of the last available clinical follow-up. The incidence rate for all-cause mortality was expressed as the number of events per 100 person-years.

To investigate the relationship between REMAP score and the all-cause mortality rate, REMAP score was included in the multivariable analyses as: 1) a linear term only, 2) a quadratic term only, 3) both linear and quadratic terms. Goodness of fit for each Cox model was evaluated by Akaike information criterion. The linear term was the one obtaining the minimum Akaike information criterion value among all the Cox models tested. To assess the association between REMAP score and

all-cause mortality, unadjusted and adjusted Cox proportional hazard models were estimated. In the GMS, FMS, and GHS-prospective and in the pooled sample, age at recruitment, sex, smoking habit, body mass index (BMI), HbA_{1c}, diabetes duration, and antihypertensive, insulin, and statin treatments were considered as possible confounders in the fully adjusted model. Moreover, high-sensitivity C reactive protein (hs-CRP), a marker of acute-phase inflammation, was considered as an additional covariate after log transformation and standardization. Analyses in the pooled sample were also adjusted for study cohort. Our pooled sample of 1528 patients, with an event rate of 26.8%, achieves 90% power at a 5% significance level to detect, for a 1-SD increment of the REMAP score, a hazard ratio (HR) equal to at least 1.18.

In the sample with CEA, comprised also of individuals with no diabetes, HbA_{1c} and diabetes duration were excluded and diabetes status (yes/no) and plaque status (unstable/stable) were included in the model.

Risks were reported as odds ratios (ORs) or HRs along with their 95% CIs per 1-SD increase in standardized log REMAP score values.

To deeply study the association between REMAP and all-cause mortality, the pooled sample was stratified according to the study-specific tertiles of REMAP score values and incidence rates were computed within each stratum. Moreover, study-adjusted Cox survival curves were estimated (26) in the whole sample according to REMAP tertiles.

To examine whether the REMAP score increases the prediction accuracy of all-cause mortality in type 2 diabetes, 2 different, well-established models were used, including Estimation of Mortality Risk in Type 2 Diabetic Patients (ENFORCE) (18) and Risk Equations for Complications of Type 2 Diabetes (RECODe) (19). Predictors included in the 2 models are reported in the supplementary data (25).

According to the models' setting, the time horizon prediction was 6 years for ENFORCE and 10 years for RECODe. Each model was tested without (reference model) and with the addition of REMAP score values. Discrimination was measured by survival *c* statistic (27), the improvement in discrimination by Δ *c* statistic (27), and the survival version of the relative integrated discrimination improvement (rIDI) (28). In addition, the survival version of the category-free net reclassification improvement (cNRI) (29), which examines whether the predicted probabilities of individuals with and without events move in the right directions (upward and downward, respectively) from the reference to the enriched model was evaluated.

The 95% CIs for discrimination and reclassification measures were computed by bootstrap. A *P* value of less than .05 was considered significant. All analyses were performed using SAS Release 9.4 (SAS Institute).

Results

The clinical features of patients from the GMS, FMS, and GHS-prospective design are summarized in Table 1. In the GMS, during follow-up (10.7 ± 3.5 years; 6897.5 person-years), 185 deaths occurred. In the FMS, during follow-up (7.1 ± 2.5 years; 3773.3 person-years), 143 deaths occurred. In the GHS-prospective design, during follow-up (5.4 ± 2.5 years; 1907.6 person-years), 81 deaths occurred. The association between any single cytokine comprised in the REMAP and all-cause mortality in each sample is shown in Supplementary Table 1 (25). In all 3 samples, the REMAP score was significantly associated with all-cause mortality (HR [95% CI] for 1-SD increase = 1.47 [1.28-1.68], 1.47 [1.28-1.69] and 1.56 [1.28-1.89] in GMS, FMS, and GHS-prospective, respectively; all $P < .001$]). The 3 associations remained significant in a fully adjusted model comprising age at recruitment, sex, smoking habit, BMI, HbA_{1c}, diabetes duration, and antihypertensive, insulin, and statin treatments (HR [95% CIs] = 1.36 [1.17-1.57], 1.27 [1.09-1.47], and 1.47 [1.20-1.81] in GMS, FMS and GHS-prospective, respectively; all $P < .01$). In the GHS-prospective design, where data on cardiovascular mortality were available, the association between the REMAP score and all-cause mortality was superimposable to that of cardiovascular origin (HR; 95% CI = 1.62; 1.29-2.04 and 1.48; 1.17-1.87 in the unadjusted and the fully adjusted model, respectively). In the pooled analysis comprising all 3 samples, the REMAP score was strongly associated with all-cause mortality with an adjusted HR = 1.34 (1.22-1.47; $P < .001$) (Table 2), with no difference between male and female participants (*P* of heterogeneity = .92). Of note, this association, even if weakened, remained strongly significant after also adjusting for hs-CRP (HR; 95% CI = 1.20; 1.08-1.34; $P < .001$). Interestingly, a negative REMAP-by-CRP interaction was observed (β value [SE]: –0.12 [0.04]; $P < .01$), with the association between REMAP and mortality being stronger in patients with lower CRP levels. Finally, in a fully adjusted model also comprising serum resistin levels, the REMAP score (HR; 95% CI = 1.37; 1.13-1.65; $P < .01$), but not resistin (HR; 95% CI = 0.98; 0.81-1.18; $P = .81$), was associated with all-cause mortality.

When the pooled sample was stratified according to study-specific tertiles of REMAP values (cutoffs being –0.49 and 0.35; –0.50 and 0.21; –0.40 and 0.23 for GMS, FMS, and GHS-prospective, respectively; distributions of

Table 1. Clinical characteristics of patients from the 3 study cohorts

	GMS	FMS	GHS-prospective
	(n = 643)	(n = 532)	(n = 353)
Male, n (%)	307 (47.7)	269 (50.6)	238 (67.4)
Age at recruitment, y	61.5 ± 9.7	62.7 ± 11.6	64.5 ± 8.1
Smoking habit, n (%)	87 (13.5)	82 (15.4)	64 (18.1)
Diabetes duration, y	10.4 ± 8.9	13.0 ± 9.8	13.9 ± 9.1
BMI	31.0 ± 5.7	30.0 ± 5.8	30.1 ± 4.7
HbA_{1c}, %, mmol/mol	8.6 ± 1.9 (70 ± 20.8)	9.0 ± 2.1 (75 ± 23.0)	8.6 ± 1.9 (70 ± 20.8)
Antihypertensive therapy, n (%)	340 (52.9)	386 (72.6)	306 (86.7)
Insulin therapy, n (%)	241 (37.5)	181 (34.0)	195 (55.2)
Statins therapy,(n (%)	167 (26.0)	166 (31.2)	220 (62.3)
hs-CRP, mg/L	2.6 (1.2-5.4)	2.4 (1.2-6.0)	1.4 (0.7-5.5)
Resistin, ng/mL	8.2 (5.9-11.5)	6.6 (4.9-9.8)	9.0 (6.8-12.5)
IL-1 β, pg/mL	6.5 (4.4-10.9)	6.8 (3.8-8.5)	4.5 (2.6-6.8)
IL-6, pg/mL	9.5 (6.8-15.3)	10.3 (8.2-13.4)	18.9 (10.7-36.9)
IL-8, pg/mL	52.1 (30.1-87.8)	40.0 (30.9-53.6)	166.1 (73-403)
TNF-α, pg/mL	21.4.5 (15.9-27.2)	37.9 (31.4-45.5)	69.5 (35.8-111.5)
Follow-up, y (py)	10.7 ± 3.5 (6897.5)	7.1 ± 2.5 (3773.3)	5.4 ± 2.5 (1907.6)
All-cause death, n (%)	185 (28.8)	143 (26.9)	81 (22.9)
IR, n events per 100 py[a] (95% CI)	2.3 (2.0-2.7)	2.6 (2.1-3.1)	2.8 (2.2-3.5)

Continuous variables are reported as mean ± SD, and categorical variables as total frequencies and percentages. Skewed variables are presented as median (interquartile range).
Abbreviations: BMI, body mass index; FMS, Foggia Mortality Study; GHS-prospective, Gargano Heart Study–prospective design; GMS, Gargano Mortality Study; HbA_{1c}, glycated hemoglobin A_{1c}; hs-CRP: high-sensitivity C-reactive protein; IL, interleukin; IR, incidence rate of all-cause death events; py, person-years; TNF, tumor necrosis factor.
[a]Adjusted for age and sex.

Table 2. Multivariable associations with all-cause mortality in the pooled sample (n = 1528)

	HR	*P*
REMAP score, per 1 SD	1.34 (1.22-1.47)	< .001
Male vs female	1.45 (1.17-1.80)	< .001
Age at recruitment, per 5 y	1.60 (1.50-1.70)	< .001
Smoking habit, yes vs no	1.41 (1.04-1.92)	.03
BMI, per 1 unit	0.99 (0.97-1.01)	.23
HbA_{1c}, per 1 unit	1.09 (1.04-1.15)	.001
Disease duration, per 10 y	1.02 (0.92-1.16)	.74
Antihypertensive therapy, yes vs no	1.74 (1.34-2.25)	< .001
Insulin treatment, yes vs no	1.68 (1.35-2.09)	< .001
Statins treatment, yes vs no	0.77 (0.62-0.95)	.02

REMAP obtained as described in "Materials and Methods" (SD are of log-transformed values). Analyses are adjusted for study cohort.
Abbreviations: BMI, body mass index; HbA_{1c}, glycated hemoglobin A_{1c}; HR, hazard ratio; REMAP, weighted serum resistin and inflammatory multicytokines pathway.

single inflammatory molecules in each tertile are reported in Supplementary Table 3) (25), a clear trend of mortality incidence rate was observed from 2.1% (96 events/505; 4522.9 person-years) to 2.5% (107 events/503; 4220.8 person-years), and 5.3% (206 events/520; 3851.1 person-years) in tertile 1, 2, and 3, respectively (*P* for trend < .001), with tertile 3 being by far the most at-risk subgroup (Fig. 1).

REMAP, All-Cause Mortality, and Plaque Instability

The clinical features of patients who underwent CEA are summarized Table 3. During follow-up (6.2 ± 2.2; 365.3 person-years), 24 deaths occurred. Also in this cohort, serum IL-1β, IL-6, IL-8, and TNF-α were significantly correlated with serum resistin (all *P* < .001). The REMAP score was significantly associated with all-cause

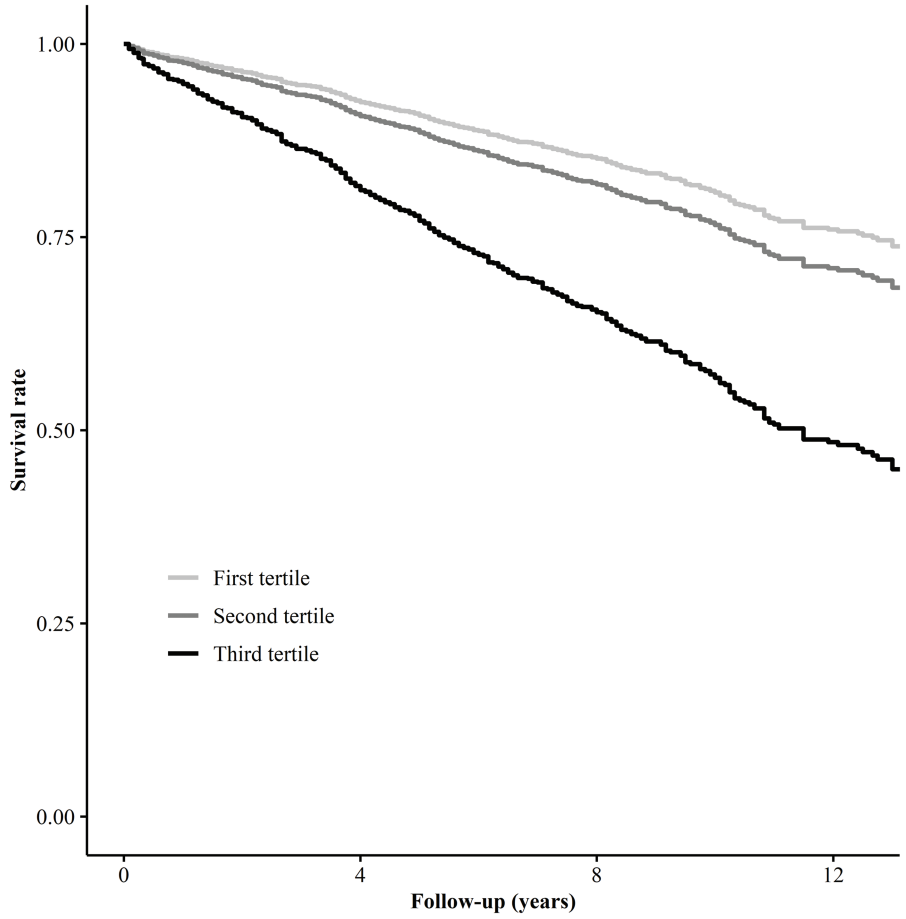

Figure 1. Survival curves for all-cause mortality in the pooled sample according to baseline tertiles of the resistin and multicytokine inflammatory pathway (REMAP) score. Ranges of tertiles (expressed for 1 SD of REMAP score): –3.1 to –0.5, –0.5 to 0.3, and 0.3 to 4.0 for the Gargano Mortality Study; –2.7 to –0.5, –0.5 to 0.2, and 0.2 to 4.3 for the Foggia Mortality Study; and –2.3 to –0.4, –0.4 to 0.2, and 0.2 to 3.2 for the Gargano Heart Study. Curves are estimated by Cox regression after adjusting for study sample.

mortality (HR [95% CI] for 1-SD increase = 1.51 [1.11-2.07]; $P < .01$). This association remained significant in a fully adjusted model comprising age at recruitment, sex, smoking habit, BMI, diabetes status, and antihypertensive and statin treatments (HR, 95% CI, for 1-SD increase = 1.64 [1.01-1.64]; $P = .04$). In the same fully adjusted model the REMAP score was also significantly associated with plaque instability (OR, 95% CI, for 1-SD increase = 3.04 [1.02-9.01]). Finally, only a modest weakening of the association between REMAP and all-cause mortality was observed in the fully adjusted model including also plaque status (HR, 95% CI, for 1-SD increase = 1.58 [0.96-2.56]; $P = .07$).

Though not reaching statistical significance, a tendency toward the expected positive association was observed when the correlation between *RETN* gene expression and all other cytokine expression levels was tested ($P = .08$-$.35$). The REMAP expression score was associated neither with all-cause mortality ($P = .43$) nor with plaque status ($P = .76$) and REMAP serum score ($P = .54$).

REMAP and Improved Mortality Risk Prediction

To examine whether the REMAP score is useful for improving the prediction of all-cause mortality in type 2 diabetes, 2 different validated prediction models were used, ENFORCE and RECODe. To this purpose the GMS and FMS, comprising 1175 patients and 187 (at 6 years for ENFORCE) and 277 (at 10 years for RECODe) deaths were used. Based on the results obtained when considering tertiles of REMAP score values (ie, tertile 3 being by far the most at risk), the REMAP score was used as a dichotomous variable (eg, tertile 3 = yes/no).

The addition of the REMAP score significantly ameliorated discrimination measures in both models, as indicated by the *c* statistic values and percentage rIDI (Table 4). Risk classification was also clearly ameliorated in both models, with a highly statistically significant and clinically meaningful proportion of nonevents being correctly reclassified (see Table 4).

Very similar results in terms of both rIDI and cNRI were obtained when the hs-CRP–enriched version of ENFORCE

(3) was tested in the 1175 individuals whose hs-CRP levels were available (Supplemental Table 3; (25).

Conclusions

This study shows for the first time that the serum REMAP score, signaling a pathway comprising several inflammatory cytokines all under the control of resistin, is independently associated with all-cause mortality in patients with type 2 diabetes and in selected high-risk patients with carotid atherosclerosis. Notably, in a model comprising both resistin as singly considered and the REMAP, the latter but not the former remained significantly associated, thus suggesting that REMAP provides more information on the risk of death than resistin alone. The association between mortality rate and REMAP was replicable in all study samples, a consistency that increases the credibility of our finding. In addition and of utmost importance for possible clinical implications, the REMAP score improves both discrimination and reclassification of 2 well-established prediction models of all-cause death in type 2 diabetes (18, 19). These findings extend our previous evidence on the association between REMAP and cardiovascular disease in diabetes (17) to total mortality and are in line with the well-known role proinflammatory cytokines play on atherosclerotic processes (30, 31), cardiovascular events (32, 33) and all-cause mortality (10, 11, 34, 35) in several clinical sets. Our data on CEA patients suggest that atherosclerotic plaque instability, though strongly associated with REMAP, seems to play a marginal role in mediating the association between the latter and the risk of death. Also, no role seems to be attributable to the expression levels within the atherosclerotic plaque of the genes encoding the 5 inflammatory molecules, analyzed in a combined fashion as a REMAP expression score. Since CEA treatment has likely reduced the risk of mortality exerted by plaque instability, all findings in CEA patients have to be interpreted with caution. Taken altogether, our data suggest that plaque-independent mechanisms are likely to mediate most of the association between serum REMAP and all-cause mortality. On the other hand, it cannot be excluded that a greater percentage of this association would be influenced if, in an ideal experimental scenario, the whole burden exerted by all the atherosclerotic plaques affecting each individual could be considered.

Table 3. Clinical characteristics of patients who underwent carotid endarterectomy (n = 59)

Male, n (%)	45 (76.3)
Age at recruitment, y	72.6 ± 8.5
Current smoking habit, n (%)	12 (20.3)
Diabetes, n (%)	26 (44.1)
BMI	26.9 ± 4.3
Antihypertensive therapy, n (%)	52 (88.1)
Statins therapy, n (%)	37 (62.7)
Unstable plaque, n (%)	37 (62.7)
Resistin, ng/mL	5.7 (5.6-8.9)
IL-1 β, pg/mL	3.2 (2.8-4.0)
IL-6, pg/mL	7.8 (5.9-13.0)
IL-8, pg/mL	20.5 (18.0-28.2)
TNF-α, pg/mL	16.7 (15.1-18.2)
Follow-up, y (py)	6.2 ± 2.2 (365.3)
All-cause death, n (%)	24 (40.7)
IR, n events per 100 py[a] (95% CI)	4.4 (2.7-7.0)

Continuous variables were reported as mean ± SD, and categorical variables as total frequencies and percentages. Skewed variables are presented as median (interquartile range).
Abbreviations: BMI, body mass index; IL, interleukin; IR, incidence rate of all-cause death events; py, person-years; TNF, tumor necrosis factor.
[a]Adjusted for age and sex.

Table 4. Survival discrimination and reclassification measures of the ENFORCE and RECODe models

	Discrimination			Reclassification		
Prediction models	*c* Statistic (95% CI)	Δ*c* Statistic (*P*)	% Relative IDI (*P*)	1/2% cNRI (*P*)	% Events (*P*)	% Nonevents (*P*)
ENFORCE	0.793 (0.764-0.823)					
ENFORCE + REMAP score (tertile 3 = yes/no)	0.797 (0.766-0.829)	0.04 (.015)	5.8 (.011)	21 (< .001)	3 (.337)	39 (< .001)
RECODe	0.735 (0.705-0.764)					
RECODe + REMAP score (tertile 3 = yes/no)	0.741 (0.712-0.770)	0.06 (.031)	6.4 (.002)	21 (< .001)	–0.2 (.934)	43 (< .001)

Analyses were performed in Gargano Mortality Study and Foggia Mortality Study (n = 1175).
Abbreviations: cNRI, category-free net reclassification improvement; ENFORCE, Estimation of Mortality Risk in Type 2 Diabetic Patients; IDI, integrated discrimination improvement; RECODe, Risk Equations for Complications of Type 2 Diabetes; REMAP, weighted serum resistin and inflammatory multicytokines pathway.

Interestingly, the effect of REMAP on mortality is independent of and interacts negatively with hs-CRP, an acute-phase protein and a well-known marker of cardiovascular disease and all-cause death (36, 37). In detail, the association between a high REMAP score and high mortality rate decreases with increasing levels of hs-CRP, thus suggesting that the deleterious effect on mortality rate of low-grade inflammation and that of acute-phase inflammation cannot be mutually added. This is a typical negative synergy scenario that occurs when a certain biological effect (increasing the risk of death, in our case) approaches the plateau.

Prediction analyses highlighted that the REMAP score improves the discrimination ability both of ENFORCE (18) and RECODe (19), 2 established prediction models of all-cause mortality in patients with type 2 diabetes.

Though statistically significant, the Δc statistic is rather small, but it is worth noticing that in already well-performing models, as are those we used here, the *c* statistic lacks sensitivity in detecting further discrimination improvements (38). On the contrary, this is not the case of percentage rIDI, which, in fact, approximated 6%, the threshold requested by international guidelines for adding new biomarkers on top of established prediction models (39). A more important improvement, both statistically and clinically, was observed on reclassification measures. In fact, adding the REMAP score to ENFORCE and RECODe made it possible to correctly reclassify a high proportion (approximately 40%) of nonevent individuals, thus reducing risk overestimation. Conversely, adding the REMAP score did not help to correctly reclassify event individuals, thus showing no ability in addressing risk underestimation. Of note, a similar improvement in discrimination and reclassification measures was observed in the hs-CRP–enriched version of ENFORCE (3). The association between REMAP score and mortality rate has some potential clinical implication in the context of a precision medicine approach. In fact, 2 neutralizing monoclonal antibodies against IL-6 (ie, tocilizumab) (40) and IL-1β (ie, canakinumab) (41) have been proved to reduce the cardiovascular burden in high-risk patients with the latter also being able to reduce total mortality (42). One could therefore envision a future scenario in which individuals with a relatively high serum REMAP score (eg, those belonging to the highest tertile, by far those with the highest risk of mortality) become the most eligible to be targeted with novel anti-inflammatory therapies.

Strengths of our study are as follows. We used well-established, prospectively analyzed cohorts with complete information, including standardized clinical evaluations and mortality validated by death certificates. Measurements of cytokines were centralized. In addition, our data were confirmed in a well-characterized cohort of selected high-risk patients with carotid atherosclerosis. Moreover, the usefulness of the REMAP score for risk stratification purposes was tested on top of 2 well-established and validated models (18, 19), thus ensuring the feasibility of implementation in the real-life clinical set. A major limitation of our finding is due to the intrinsic nature of correlative studies like ours, which, by definition, cannot address the biology underlying the reported associations. At this stage of knowledge, we can say only that while the well-known deleterious effects of all REMAP components on low-grade inflammation represent a credible and strong biological background, the data obtained in individuals undergoing CEA suggest that most of the REMAP's effect on the risk of death is not mediated by an atherosclerotic plaque–dependent mechanism. Second, although in the GHS-prospective design data on cardiovascular death were superimposable to those of all-cause mortality, the lack of information on cardiovascular mortality in the other 2 larger studies makes it impossible to robustly extend our observation to death of cardiovascular origin. Finally, it is not known whether our present findings can be generalized to patients with different ethnic, environmental, and/or genetic backgrounds as well as to different clinical settings.

In conclusion, in patients with type 2 diabetes a serum resistin and multicytokine inflammatory pathway is not only independently associated with total death but also helps improve established all-cause mortality prediction models, thus paving the way to a precision medicine approach that allows us to focus our efforts on patients at the highest risk. Our finding also makes it possible to envision a further precision medicine approach in which REMAP is used to identify individuals who may be more responsive to treatments with neutralizing monoclonal antibodies against IL-6 and IL-1β that have been recently proven to reduce cardiovascular burden and total mortality.

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Additional Information

Present Affiliation: Maria Giovanna Scarale, University Centre for Statistics in the Biomedical Sciences (CUSSB), Vita-Salute San Raffaele University, Milan, Italy.

Correspondence: Claudia Menzaghi, PhD, Research Unit of Diabetes and Endocrine Diseases, IRCCS "Casa Sollievo della Sofferenza," Viale Padre Pio, 71013 San Giovanni Rotondo, Italy. Email: c.menzaghi@operapadrepio.it; or Vincenzo Trischitta, MD, Research Unit of Diabetes and Endocrine Diseases, IRCCS "Casa Sollievo della Sofferenza," Viale Padre Pio, 71013 San Giovanni Rotondo, Italy. Email: vincenzo.trischitta@uniroma1.it.

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