UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

TESE DE DOUTORADO

CURSO EVOLUTIVO E FATORES DE PROGRESSÃO DA NEFROPATIA DIABÉTICA EM PACIENTES COM DIABETE MELITO TIPO 2

MARCIA MURUSSI

Porto Alegre, 2005

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Orientadora: Profª. Drª. Sandra Pinho Silveiro

Co-orientador: Prof. Dr. Jorge Luiz Gross

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DEDICATÓRIA

A todas as mulheres, que de uma maneira singular, têm trabalhado arduamente nas múltiplas funções, às vezes monumentais, que elas mesmas escolheram ao conduzir suas vidas, tais como filhas, esposas, mães, profissionais, amigas, madrinhas, administradoras no lar e no trabalho, voluntárias, professoras, síndicas, comerciantes, escritoras, artistas, atletas, etc, não muito diferente do que as mulheres sempre foram...

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LISTA DE ABREVIATURAS

CAPÍTULO 1

ADA: *American Diabetes Association*

BRA-II: bloqueador do receptor da angiotensina-II

DCCT: *Diabetes Control and Complications Trial*

DM: diabete melito

DRC: doença renal crônica

ECA: enzima conversora da angiotensina

EUA: excreção urinária de albumina

HAS: hipertensão arterial sistêmica

HPS: *Heart Protection Study*

IRT: insuficiência renal terminal

ND: nefropatia diabética

NKF: *National Kidney Foundation*

TFG: taxa de filtração glomerular

UKPDS: *United Kingdom Prospective Diabetes Study*

CAPÍTULO 2 e CAPÍTULO 3

ACE: angiotensin-converting enzyme

ADA: *American Diabetes Association*

BMI: body mass index

CI: confidence interval

CKD: chronic kidney disease

DBP: diastolic blood pressure

DM 2: type 2 diabetes mellitus

DN: diabetic nephropathy

DR: diabetic retinopathy

ESRD: end stage renal disease

FPG: fasting plasma glucose

GFR: glomerular filtration rate

HPLC: high-performance liquid chromatography

HR: hazard ratio

IT: immunoturbidimetry

KF: kidney failure

MA: macroalbuminuric

MDRD: *Modification of Diet in Renal Disease equation*

MI: microalbuminuric

NKF: *National Kidney Foundation*

NO: normoalbuminuric

RIA: radioimmunoassay

RRT: renal replacement therapy

SBP: systolic blood pressure

UAE: urinary albumin excretion

UKPDS: *United Kingdom Prospective Diabetes Study*

WHO: World Health Organization

DIAGNÓSTICO E CURSO EVOLUTIVO DA NEFROPATIA DIABÉTICA EM PACIENTES COM DIABETE MELITO TIPO 2

DIAGNOSIS AND CLINICAL COURSE OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETIC PATIENTS

Marcia Murussi[1]
Nicole Campagnolo[1]
Jorge Luiz Gross[1]
Sandra Pinho Silveiro[1]

[1]Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre
Universidade Federal do Rio Grande do Sul, RS, Brasil

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Resumo

A nefropatia diabética (ND) é uma complicação microvascular freqüente, que acomete cerca de 40% dos indivíduos com diabete melito (DM). A ND associa-se a significativo aumento de morte por doença cardiovascular. É a principal causa de insuficiência renal terminal em países desenvolvidos e em desenvolvimento, representando, dessa forma, um custo elevado para o sistema de saúde.

Os fatores de risco para o desenvolvimento e a progressão da ND mais definidos na literatura são a hiperglicemia e a hipertensão arterial sistêmica. Outros fatores descritos são o fumo, a dislipidemia, o tipo e a quantidade de proteína ingerida na dieta e a presença da retinopatia diabética. Alguns parâmetros de função renal também têm sido estudados como fatores de risco, tais como a excreção urinária de albumina (EUA) normal-alta e a taxa de filtração glomerular excessivamente elevada ou reduzida. Alguns genes candidatos têm sido postulados como risco, mas sem um marcador definitivo.

O diagnóstico da ND é estabelecido pela presença de microalbuminúria (nefropatia incipiente: EUA 20-199 μg/min) e macroalbuminúria (nefropatia clínica: EUA ≥ 200 μg/min).

À medida que progride a ND, aumenta mais a chance de o paciente morrer de cardiopatia isquêmica. Quando o paciente evolui com perda de função renal, há necessidade de terapia de substituição renal e, em diálise, a mortalidade dos pacientes com DM é muito mais significativa do que nos não-diabéticos, com predomínio das causas cardiovasculares.

A progressão nos diferentes estágios da ND não é, no entanto, inexorável. Há estudos de intervenção que demonstram a possibilidade de prevenção e de retardo na evolução da ND principalmente com o uso dos inibidores da enzima conversora da angiotensina, dos bloqueadores da angiotensina II e do tratamento intensivo da hipertensão arterial. Os pacientes podem entrar em remissão, ou até mesmo regredir de estágio.

A importância da detecção precoce e da compreensão do curso clínico da ND tem ganhado cada vez mais ênfase, porque a doença renal do DM é a principal causa de diálise no mundo e está associada ao progressivo aumento de morte por causas cardiovasculares.

Descritores: fatores de risco, nefropatia diabética, diabete melito tipo 2, prevenção, curso clínico

Abstract

Diabetic nephropathy (DN) is a frequent microvascular complication, which affects about 40% of diabetes mellitus (DM) patients. DN is associated with an increased cardiovascular death rate. DN is the major cause of kidney failure in developing as well as in developed countries, and it is, therefore, associated with increased health system costs.

The more defined risk factors for the development and progression of DN are sustained hyperglycemia and hypertension. Other putative risk factors are smoking, dyslipidemia, the amount and source of protein in the diet, and the presence of diabetic retinopathy. Some renal function parameters have also been studied as risk factors, such as high normal urinary albumin excretion (UAE) and extremely high or low glomerular filtration rate levels. Some candidate genes have been analyzed as risk factors, but without any definitive marker.

DN diagnosis is established by the presence of microalbuminuria (incipient nephropathy: UAE 20-199 μg/min), and macroalbuminuria (overt nephropathy: UAE ≥200 μg/min).

As DN progress, the chance of death from coronary artery disease increases. When patients progress to kidney failure with uremia, renal replacement therapy becomes necessary, and when on dialysis, diabetic patients have higher mortality rates in comparison to non-diabetic ones, primarily from cardiovascular causes.

DN progression through stages is not always the rule. Intervention studies demonstrate that DN prevention and remission are possible, mainly with angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, and intensive hypertension treatment.

The importance of the earlier detection, and the understanding of clinical course of DN, have progressively grown, because it is the leading cause of dialysis in the world, and is

associated with increased cardiovascular mortality.

Keywords: Risk factors, diabetic nephropathy, type 2 diabetes mellitus, prevention, clinical course

INTRODUÇÃO

A nefropatia diabética (ND) é uma complicação crônica microvascular freqüente que acomete cerca de 40% dos pacientes com diabete melito (DM) (1,2). Em indivíduos brasileiros com DM tipo 2, observou-se uma incidência cumulativa em 10 anos de 31% (3), semelhante à incidência de 34% em finlandeses (4) e de 35% em israelenses (5). No estudo UKPDS (*United Kingdom Prospective Diabetes Study*), a prevalência após 10 anos de DM foi de 31% (6). Na Ásia, a prevalência chega aos alarmantes índices de 58,6% (7).

Atualmente, a ND é a principal causa de insuficiência renal terminal (IRT), variando de 25% no Reino Unido (8), 24% na Suécia (9), 40% nos Estados Unidos (10) até 51% no Canadá (11). No Rio Grande do Sul, no ano de 1996, 26% dos pacientes admitidos em programas de diálise tinham DM (12). Como o DM tipo 2 representa aproximadamente 90% dos casos de DM, a maioria dos indivíduos admitidos em programas de diálise são pacientes com DM tipo 2 (10).

Além de uma prevalência elevada, a ND associa-se com aumento na taxa de mortalidade, principalmente por doença cardiovascular (13-15). No estudo UKPDS, os pacientes com ND têm risco progressivo de morte cardiovascular à medida que progride a ND (6). O aumento da mortalidade dos pacientes com IRT é significativo (16) e ainda mais dramático em pacientes com DM tipo 2 (6,17). A sobrevida de pacientes brasileiros com DM tipo 2 em 2 anos é de apenas 50%, e a principal causa de morte é a doença cardiovascular (12).

A prevalência global do DM está aumentando em proporções epidêmicas, com estimativas de aumentar o número total de pacientes com DM de 171 milhões em 2000 para 366 milhões em 2030 (18) e, por conseguinte, de aumentar a prevalência da ND. No Brasil,

o DM é uma doença de alta prevalência, atingindo níveis de 7,6% na década de 90 (19). Um estudo recente de base populacional, realizando *screening* em 22,1 milhões de brasileiros acima de 40 anos, em 5.301 municípios, apontou que 15,7% dos indivíduos testados apresentaram *screening* positivo para DM (20). Dessa forma, é esperado que a prevalência da ND aumente sensivelmente. Considerando que o custo do tratamento do DM aumenta em 65% na nefropatia incipiente, 195% na nefropatia clínica e 771% na IRT (21), é previsível que os gastos com o tratamento na ND sejam ainda maiores nos próximos anos.

Portanto, a detecção precoce e a instituição de medidas preventivas eficazes apresentam elevada relação custo-benefício, estando relacionadas à diminuição de mortalidade (22).

DEFINIÇÃO

A ND é dividida, didaticamente, em estágios evolutivos (Tabela 1) conforme os valores de excreção urinária de albumina (EUA) e de função renal. O primeiro estágio é a nefropatia incipiente, que se caracteriza pela presença de microalbuminúria (EUA 20-199 μg/min). O segundo estágio é a nefropatia clínica, que é definida pela presença de macroalbuminúria (EUA ≥200 μg/min) ou de proteinúria (valores de proteínas totais na urina ≥500 mg/24 h) (23). Alguns pacientes podem progredir com perda de função renal para o terceiro estágio, que se define pela IRT (24).

FATORES DE RISCO PARA NEFROPATIA DIABÉTICA

Vários fatores de risco genéticos e não-genéticos têm sido implicados no desenvolvimento e na progressão da ND.

Fatores de Risco Genéticos

Existe uma predisposição genética para a ND, já que apenas 40% dos pacientes com DM são afetados independentemente do controle metabólico (25). Há estudos demonstrando uma agregação familiar da ND (26,27), o que também foi observado em pacientes brasileiros com DM tipo 2 provenientes de uma população geneticamente heterogênea (28). A influência de fatores genéticos no desenvolvimento da síndrome metabólica, na qual existe associação de DM, hipertensão, dislipidemia e microalbuminúria, também foi demonstrada (29).

Sexo masculino (5,30) e algumas etnias, como negros (31), hispânicos (32) e índios americanos (33) têm maior chance de apresentar ND. A predisposição familiar para hipertensão, nefropatia e doença cardiovascular também tem sido documentada (25,34).

Vários polimorfismos candidatos têm sido analisados em relação à predisposição genética para ND. Alguns exemplos são os dos genes da enzima conversora da angiotensina (ECA) (35-37), do *ENPP-1 (PC-1: ecto-nucleotide pyrophosphatase/phosphodiesterase-1 ou plasma cell differentiation antigen*) (38,39), da paraoxonase 2 (*PON 2*) (40), do PAI-1 (plasminogen activator inhibitor-1) (41), da aldose redutase (*Z-2*) (42), e do contratransporte sódio-lítio (43). No entanto, nenhum marcador genético específico foi identificado de forma definitiva (44).

Fatores de Risco Não-Genéticos

Os fatores de risco não-genéticos (tabela 2) são aqueles potencialmente modificáveis com intervenção. A intervenção médica pode ter como objetivo a prevenção primária (evitar a instalação da ND, ou seja, de normoalbuminúria para ND), a prevenção secundária (de micro para macroalbuminúria) e a prevenção terciária (de macroalbuminúria para IRT). Os fatores de risco que estão plenamente estabelecidos na literatura são a

hiperglicemia (3,4,30,45,46) e a hipertensão arterial sistêmica (5,46-48). Outros possíveis fatores de risco são o fumo (49-51), a dislipidemia (5,30,52), os fatores dietéticos (23,53,54) e a presença da retinopatia diabética (3,30,55). Alguns parâmetros de função renal têm sido estudados como fatores de risco, tais como a EUA normal-alta (3,30) e a taxa de filtração glomerular excessivamente elevada (56,57) ou diminuída (58,59).

O mau controle glicêmico tem-se mostrado como fator de risco para o desenvolvimento da ND através de estudos observacionais tanto em pacientes com DM tipo 1 (60-62) quanto em pacientes com DM tipo 2 (3,4,30,45). Além disso, estudos randomizados demonstraram que o tratamento da hiperglicemia evitou o surgimento da microalbuminúria nos pacientes com DM tipo 1 (63,64) e tipo 2 (45,65,66). O DCCT (*Diabetes Control and Complications Trial*) (67) e o UKPDS (45) estabeleceram a eficácia do melhor controle metabólico na prevenção da ND e das outras complicações microvasculares. Em análise observacional, o UKPDS demonstrou que qualquer redução da glico-hemoglobina implica redução de risco de complicações, sendo o menor risco observado quando a glico-hemoglobina encontra-se em níveis normais (<6%). A redução de 1% da glico-hemoglobina associa-se à diminuição significativa do risco para qualquer desfecho relacionado ao DM em 21% e para complicações microvasculares em 37% (45). Em relação à prevenção secundária no DM tipo 1, os estudos disponíveis envolvem menor número de pacientes (63,67,68), mas o estudo EDIC (63) e o seu seguimento (69) mostraram resultados promissores de que o tratamento intensivo da glicemia diminui a chance de progressão de microalbuminúria para nefropatia clínica. Finalmente, ainda não está totalmente esclarecido o papel do controle da glicemia na progressão da ND (prevenção terciária), em fase de declínio da TFG. Estudos não-controlados de pacientes com DM tipo 1 sugerem que o controle da glicemia preserva a taxa de filtração glomerular (TFG) mesmo em pacientes com nefropatia clínica e perda de função renal (70,71). Além disso, após 10

anos de transplante de pâncreas, a normalização da glicemia pode, inclusive, reverter as lesões da ND (72).

Estudos prospectivos confirmam que a hipertensão arterial sistêmica (HAS) é um fator de risco importante para o desenvolvimento da ND em pacientes com DM tipo 2 (5,55) e também o fator de progressão mais relevante (73). Estudos de intervenção têm confirmado os benefícios do tratamento da HAS. No UKPDS 38, que avaliou o controle rigoroso da pressão arterial em pacientes com DM tipo 2, houve diminuição do surgimento das complicações microvasculares (74). A análise observacional do UKPDS demonstra que, para cada 10 mmHg de diminuição na pressão sistólica média, houve uma diminuição significativa de 13% do risco de complicações microvasculares (48). O tratamento da HAS tem se mostrado como um importante aliado na prevenção primária (74-76), secundária (77-79) e terciária (77,80) da ND, principalmente com o uso dos inibidores da ECA e dos bloqueadores do receptor da angiotensina II (BRA-II). O estudo BENEDICT, prospectivo, multicêntrico, randomizado, com o maior número de pacientes com DM tipo 2 normoalbuminúricos já avaliado por 3 anos, comparou o uso de inibidor da ECA (trandolapril) com bloqueador do canal de cálcio não-dihidropiridínico (verapamil), e demonstrou que a progressão para microalbuminúria foi a metade no grupo que usou inibidor da ECA em comparação à outra, comprovando o benefício dessa droga no arsenal da prevenção primária da ND (76).

O fumo afeta tanto a estrutura como a função glomerular (50). Pacientes com DM tipo 2 fumantes têm maior risco de apresentar microalbuminúria do que pacientes não-fumantes e, além disso, a velocidade de progressão para IRT é 2 vezes mais rápida (49). Em estudo da população com DM na Suécia, foi observado, em pacientes com DM tipo 1 e tipo 2, que o fumo relacionava-se a níveis mais elevados de glico-hemoglobina e de EUA de forma independente (81). No entanto, em pacientes com DM tipo 1 com nefropatia clínica, o

fumo não foi um fator de progressão do declínio da TFG (82). Por outro lado, foi recentemente demonstrado em pacientes com DM tipo 2 que a suspensão do fumo é efetiva para evitar a progressão da ND (51).

Estudos longitudinais sugerem que o colesterol elevado seja um fator de risco para o desenvolvimento da ND em pacientes com DM tipo 2 (5,30). Essa hipótese é confirmada pelos achados do estudo EURODIAB, onde níveis de triglicerídeos, apolipoproteína B, colesterol total e LDL elevam-se de maneira proporcional ao aumento da albuminúria (83). Embora a relação causal entre dislipidemia e ND ainda não esteja completamente esclarecida, algumas evidências apontam para um papel patogênico dos lipídeos no desenvolvimento e na progressão da ND (84-86). A diminuição do colesterol pelo uso de drogas associa-se à redução do declínio da TFG (85,87) e a uma tendência à diminuição da proteinúria (85) em pacientes com DM. Além disso, o estudo HPS (87), que avaliou o uso de 40 mg de sinvastatina em quase 6 mil pacientes com DM, demonstrou redução de 27% de eventos cardiovasculares independentemente dos níveis basais de colesterol e também preservou a função renal.

Dietas com maior ingestão de proteína oriunda de peixe (88) parecem estar associadas à redução do risco de desenvolver ND em pacientes com DM tipo 1, enquanto que dietas com com restrição protéica (89) estão associadas a menor risco de progressão. Em pacientes com DM tipo 2 microalbuminúricos, demonstrou-se que uma dieta normoprotéica à base de galinha reduziu a TFG, o colesterol total, o LDL, a apolipoproteína B e a EUA (em 46%) em comparação à dieta hipoprotéica (53). Esses efeitos estão possivelmente relacionados a menor quantidade de gordura saturada, em concordância com os achados de outro estudo (54), e à maior proporção de ácidos graxos poliinsaturados encontrados na carne de galinha em relação à carne vermelha. Já foi relatado o efeito

benéfico dos ácidos graxos poliinsaturados na função endotelial (90), e isso poderia ser extensivo ao rim, diminuindo a EUA.

Estudos prospectivos envolvendo pacientes com DM tipo 2 têm demonstrado que a presença de retinopatia no início do acompanhamento é preditiva de surgimento futuro de ND (3,30,55). Dados recentes demonstram que a presença de retinopatia precede a ocorrência da ND, sugerindo que a retina seja, possivelmente, mais suscetível aos fatores mencionados ou necessite de menos tempo para sofrer suas influências (3). A retinopatia seria mais um marcador de risco do que um fator de risco para a ND *per se*. Provavelmente, essas duas complicações microvasculares compartilhem os mesmos fatores de risco, e talvez a retinopatia seja detectada mais precocemente.

Níveis de EUA mais elevados, ainda que dentro da faixa de normoalbuminúria, têm sido indicados como marcadores de risco para o desenvolvimento da ND. Estudos prospectivos de pacientes com DM tipo 2 (3,4,30,91,92) e de pacientes com DM tipo 1 (62,68) sugerem que mesmo níveis “normais-altos” de EUA podem ser preditivos de progressão ou refletir o processo patológico que leva à ND. Forsblom e colaboradores demonstraram que a progressão para micro ou macroalbuminúria foi mais prevalente nos pacientes com DM tipo 2 com EUA inicial superior à mediana (2,5 mg/24 h) (4). Um estudo dinamarquês observou uma média geométrica basal de EUA significativamente maior nos pacientes com DM tipo 2 que progrediram para micro ou macroalbuminúria quando comparada à dos que permaneceram normoalbuminúricos (14 vs. 7 mg/24 h) (30). Em estudo prospectivo de 10 anos, a análise multivariada demonstrou um risco 29 vezes maior de desenvolvimento da ND em pacientes com DM tipo 2 portadores de EUA acima de 10 µg/min (3). Da mesma forma, em pacientes com DM tipo 1 seguidos por 7 anos, esse mesmo valor de EUA aumentou o risco em 19 vezes (93). Além disso, um estudo realizado em pacientes normoalbuminúricos com DM tipo 2 demonstrou que valores de EUA

≥5μg/min estão associados a valores mais altos da pressão arterial avaliado por MAPA (medida ambulatorial da pressão arterial) (94). Portanto, níveis de EUA ainda que abaixo do valor crítico de 20 μg/min já poderiam ser preditivos de doença renal futura e, portanto, poderiam identificar os pacientes com risco de desenvolvimento de ND. Em pacientes com nefropatia clínica, níveis de proteinúria acima de 2g/24h estão relacionados a um maior risco de progressão para IRT (1,95), sendo que o risco dobra cada vez que duplica a proteinúria basal (96). Esses dados sugerem que, seja na fase inicial do surgimento da ND, seja nas etapas mais avançadas, à medida que aumenta a EUA, aumenta o risco de progressão para estágios evolutivos subseqüentes de lesão renal. Isso confirma o papel direto da perda urinária de albumina no mecanismo de promoção de dano renal através de indução de mecanismos inflamatórios (97).

A prevalência de hiperfiltração glomerular, isto é, elevação supranormal da TFG >137ml/min/1,73m^2 (98) é de 20% a 40% em pacientes com DM tipo 2 (99,100). Nesses pacientes, os que se apresentam normoalbuminúricos com hiperfiltração glomerular têm uma redução da TFG ao longo do tempo significativamente maior do que aqueles com TFG normal (57) sem, no entanto, aumentar o risco de desenvolver ND durante 10 anos de acompanhamento (3), em concordância com estudos realizados em índios Pima (33) e em afro-americanos (101). A favor de um papel da hiperfiltração no desenvolvimento da ND, está a observação de que os pacientes com rim único (um modelo de marcada hiperfiltração glomerular) e DM tipo 2 apresentam mais freqüentemente microalbuminúria do que os não-diabéticos (56). Portanto, o papel da hiperfiltração na patogênese da ND ainda não está completamente esclarecido, mas parece ser um contribuinte entre os demais fatores. Por outro lado, níveis de TFG <60 ml/min/1,73m^2 (24) têm sido associados a maior risco de insuficiência renal em pacientes com DM tipo 2 mesmo na ausência de albuminúria em

estudos transversais (58,59), alertando para a possibilidade de que outras causas de lesão parenquimatosa renal, além da glomerulopatia clássica avaliada por EUA, possam estar associadas à progressão para IRT, principalmente quando o paciente com DM tipo 2 não apresenta retinopatia diabética concomitante (59). Foi observado que cerca de 30% dos pacientes com DM tipo 2 e insuficiência renal não apresentam albuminúria (58,59,102,103) e que a redução da TFG nesses pacientes está associada com aumento de eventos cardiovasculares (102).

DIAGNÓSTICO

A nefropatia incipiente é caracterizada por aumento da EUA em níveis de 20 a 199 µg/min, que se denomina microalbuminúria (tabela 1). Outros valores recomendados pela Associação Americana de Diabetes (ADA) são de 30 a 299 mg em urina de 24 horas, ou de 30 a 299 mg/g de creatinina em amostra urinária (23). As diretrizes da *National Kidney Foundation* (NKF) de 2003 estabelecem um ponto de corte sexo-específico da proporção albumina/creatinina de 17 mg/g para homens e de 25 mg/g para mulheres (24). Uma forma prática e simples de se realizar o rastreamento é medir a EUA em uma amostra de urina coletada ao acaso, como, por exemplo, durante a consulta médica. Valores de albumina entre 17 e 174 mg/l, em amostra casual de urina, apresentam sensibilidade de 100% e especificidade de 80% para o diagnóstico de microalbuminúria (104).

A nefropatia clínica é definida por valores de EUA ≥200 µg/min (macroalbuminúria) ou por proteinúria persistente superior a 500 mg/24 h (23).

A variação diária da EUA é de 40 a 50 % (105) e, por essa razão, o diagnóstico necessita de confirmação com uma segunda medida, idealmente utilizando-se a urina com tempo marcado (23). Há inúmeros fatores (tabela 3) que interferem na medida da EUA e

devem ser, portanto, considerados quando o exame for solicitado (106).

Os pontos de corte que tradicionalmente definem os estágios da ND foram determinados a partir de estudos prospectivos da década de 80, que definiram risco aumentado de progressão acima desses limites de albuminúria (2). No entanto, esses pontos de corte possivelmente venham a sofrer uma diminuição, já que tem sido observada associação de níveis "normais-altos" com risco de progressão para ND e mortalidade (3,4,30,92,94). Uma possível explicação para justificar que níveis relativamente baixos de EUA venham a trazer risco renal e cardiovascular, são os estudos recentes que têm relatado a presença de uma fração não-imunorreativa detectada por HPLC (*high performance liquid chromatography*) da albumina intacta total (que é composta pelas frações imunorreativa mais não-imunorreativa) (107,108). Esses estudos sugerem, inclusive, que esse método poderia detectar mais precocemente a nefropatia incipiente do que os métodos convencionais (107-109). Talvez esses dados também expliquem parcialmente que alterações histopalotógicas não se relacionem precisamente aos valores de EUA nos pacientes com DM tipo 2 (110). Os métodos convencionais de imunoensaio [imunonefelometria (IN), imunoturbidimetria (IT), radioimunoensaio (RIA)] detectam apenas a albumina intacta total imunorreativa. Esses são os métodos que estão bem estabelecidos na literatura para avaliação da albuminúria (23) com excelente correlação entre si (111). Além disso, os estudos observacionais e de intervenção existentes em relação à ND foram embasados nesses métodos. Ainda não é conhecido o significado da fração não-imunorreativa da albumina intacta total (109,112), mas pode envolver alterações conformacionais da albumina devido a reações bioquímicas durante a passagem através dos túbulos renais (112).

A EUA ainda é o método mais precoce para detectar a presença da ND, porém o papel da microalbuminúria como fator preditivo isolado da ND tem sido questionado, com a

necessidade da busca de marcadores complementares (110). Isso se deve às constatações de que apenas um terço dos pacientes microalbuminúricos de fato progridem para estágios mais avançados da ND (110), e que alguns regridem, inclusive, para normoalbuminúria (91,113). No entanto, não há, até o presente momento, um marcador de risco melhor para ND do que a microalbuminúria, que parece refletir a lesão endotelial a nível renal (22). Além disso, a microalbuminúria é um marcador de risco independente para cardiopatia isquêmica e para aumento da mortalidade tanto em pacientes com DM (114,115) como sem DM (116-118).

No entanto, uma proporção significativa de pacientes com DM tipo 2 já mostra declínio da função renal (23% com TFG <60 ml/min/1,73 m^2) mesmo na presença de normoalbuminúria (58). A redução da TFG nesses pacientes já se associa ao aumento de eventos cardiovasculares (102). Por conseguinte, a EUA não deve ser o único método de avaliação das alterações renais no DM. A TFG é a melhor medida da função renal e deve ser avaliada nesses pacientes (24). O NKF estipula que a creatinina sérica não deva ser usada de forma isolada para avaliar a função renal, visto que é afetada por outros fatores que não a TFG, tais como formação e secreção de creatinina e excreção extra-renal, mas, sim, inserida em equação incluindo idade, sexo, etnia e índice de massa corporal (24). A avaliação da TFG é realizada por meio de equações de estimativa, como a fórmula de Cockroft-Gault em ml/min = [(140-idade) × peso/ 72 × Creatinina sérica] × 0.85 se mulher, e a equação da MDRD (*Modification of Diet in Renal Disease*) em ml/min/1,73 m^2 = 186 × (Creatinina sérica)$^{-1,154}$ × (idade)$^{-0,203}$ × (0,742 se mulher) × (1,210 se afro-americano) (24). A fórmula de Cockroft-Gault no DM superestima (119,120), e a equação da MDRD, por sua vez, subestima a TFG (24). Apesar dessas limitações, elas são aceitas como métodos de monitorização da TFG (24,121) que, por sua vez, é usada pela NKF (24) para classificar os estágios da doença renal crônica (DRC). A DRC é definida por lesão renal (avaliada pela

proteinúria persistente) ou pela diminuição da função renal por mais de 3 meses (avaliada pela TFG), e está classificada em 5 estágios: **estágio 1 -** lesão renal com TFG normal ou aumentada (≥ 90 ml/min/1,73 m^2), **estágio 2 -** lesão renal com leve diminuição da TFG (60-90 ml/min/1,73 m^2), **estágio 3 -** moderada diminuição da TFG (30-59 ml/min/1,73 m^2), **estágio 4 -** grave diminuição da TFG (15-29 ml/min/1,73 m^2) e **estágio 5 -** IRT (<15 ml/min/1,73 m^2 ou diálise) (24).

CURSO CLÍNICO

A história natural representa o acompanhamento de uma doença sem intervenção terapêutica, o que não é mais viável na ND, já que estudos de intervenção na glicemia (65,66) e na hipertensão (48,74-80,122) claramente demonstraram redução das complicações microvasculares nos pacientes com DM tipo 1 e tipo 2 com os tratamentos empregados. Portanto, será descrito o curso clínico da ND, que é definido pela evolução de uma doença com intervenção médica.

Estágio de Nefropatia Incipiente = Microalbuminúria

No maior estudo já realizado em pacientes com DM tipo 2, o UKPDS (6), envolvendo mais de 5 mil pacientes do Reino Unido, a incidência de microalbuminuria foi de 2% ao ano, e a prevalência após 10 anos de diagnóstico do DM foi de 25%. Em populações européias, a prevalência de microalbuminúria varia de 13% a 26% (123) e, em indivíduos brasileiros, chega a 24% (124). A prevalência de microalbuminúria na população geral é de 7 a 12% (116,125), cerca da metade da prevalência descrita para os pacientes com DM (6,123). No entanto, a prevalência da microalbuminúria no momento do diagnóstico do DM é de 7,2% no estudo UKPDS (6), semelhante à da população geral.

Os pacientes com DM tipo 2 microalbuminúricos estão sob risco de desenvolver

macroalbuminúria ao redor de 2,8% ao ano (6). A presença de microalbuminúria prediz o desenvolvimento de proteinúria franca em cerca de 20% a 50% dos pacientes com DM tipo 2 (110). Em estudo de base populacional italiano de 7 anos, a presença de microalbuminúria estava associada ao aumento do risco de progressão para nefropatia clínica de 42% quando comparado ao da normoalbuminúria (126).

A função renal, avaliada pela TFG, usualmente mantém-se estável na fase de nefropatia incipiente, com um declínio ao redor de 0,1 ml/min/mês (33,127,128). Entretanto, em estudo prospectivo, os pacientes que desenvolveram microalbuminúria apresentaram um declínio marcado da TFG (-0,39 ml/min/mês) (129) em relação aos normoalbuminúricos e aos indivíduos não-diabéticos (Figura 1).

A microalbuminúria é um fator de risco independente para doença cardiovascular tanto para pacientes com DM tipo 2 (15,14,130,131) e tipo 1 (132) como para indivíduos não-diabéticos (116,117). Inclusive, em indivíduos não-diabéticos hipertensos, à medida que aumenta a EUA (mesmo para valores considerados normais), aumenta o risco cardiovascular (133,134).

O aumento da mortalidade nos pacientes é de 3% ao ano (6). Isso pode ser explicado pela associação da microalbuminúria com níveis pressóricos mais elevados e alterações da função endotelial e dos fatores de coagulação (115). Há também, freqüentemente, alterações dos lipídios séricos, como aumento do LDL colesterol, da apolipoproteína B e dos triglicerídeos e diminuição do HDL2 colesterol (135). As alterações da função endotelial e hemostática caracterizam-se por aumento do fator de von Willebrand, da atividade do PAI-1 e do fibrinogênio plasmático (136,137).

O mecanismo fisiopatológico que relaciona a microalbuminúria com a doença cardiovascular ainda não é conhecido. Tem sido sugerido que o aumento da EUA, além de

refletir um estado de disfunção endotelial, possa estar associado à inflamação crônica de baixo grau (138,139).

Os marcadores de disfunção endotelial (fator de von Willebrand, PAI-1, selectina-E solúvel, molécula de adesão vascular celular solúvel-1, fator de crescimento vascular endotelial, endotelina-1) (138,140,141) e os marcadores inflamatórios (proteína-C-reativa e fibrinogênio) (138,142) são fatores implicados na patogênese da aterosclerose (143), têm sido relacionados ao aumento da EUA (138) e são considerados potenciais mediadores da relação entre microalbuminúria e doença macrovascular.

Stehouwer e colegas descreveram os dados de uma coorte constituída por 328 indivíduos com DM tipo 2, cujo objetivo foi avaliar se disfunção endotelial e inflamação crônica poderiam explicar a associação entre microalbuminúria e mortalidade (138). Após um período médio de 9 anos, a taxa de mortalidade era maior nos indivíduos micro- (OR 1,78) e macroalbuminúricos (OR 2,86) no início do estudo, em comparação aos normoalbuminúricos. A mortalidade também era maior nos pacientes que apresentavam níveis séricos mais altos de molécula de adesão vascular celular solúvel-1 e de proteína-C-reativa. Além disso, os marcadores de disfunção endotelial e de inflamação estavam envolvidos no aumento da EUA. Esse estudo sugere que a disfunção endotelial e a atividade inflamatória estão envolvidas na patogênese da microalbuminúria e que esses fatores poderiam explicar em parte a associação entre microalbuminúria e risco cardiovascular.

O achado de microalbuminúria parece, portanto, representar um estado generalizado de disfunção endotelial do rim (139). Esta, por sua vez, pode ser a causa da aterosclerose acelerada e, por conseguinte, do aumento do risco cardiovascular e renal (22,144). Um estudo que avaliou a espessura da íntima das artérias carótidas comuns por ultra-sonografia, em pacientes com DM tipo 2, demonstrou que os indivíduos com

microalbuminúria apresentavam aumento da espessura da íntima, sendo que a associação da albuminúria era independente de outros fatores de risco cardiovasculares (145), reforçando a relação entre microalbuminúria e aterosclerose.

Outra possível explicação para o aumento do risco cardiovascular é a presença da síndrome metabólica, cujos critérios, de acordo com a Organização Mundial da Saúde, incluem, além da presença de DM ou tolerância diminuída à glicose, mais dois dos seguintes: HAS, obesidade, dislipidemia e microalbuminúria. A prevalência dessa síndrome chega a mais de 75% em italianos (146) e de 85% em brasileiros com DM tipo 2 (147) e está associada com aumento da prevalência de complicações micro e macrovasculares no DM tipo 2 (147). Em um estudo brasileiro de base populacional da síndrome metabólica, realizado em indivíduos de origem japonesa, observou-se que a prevalência de microalbuminúria era maior quando os pacientes apresentavam associação de HAS e tolerância diminuída aos carboidratos (148).

No entanto, em relação à evolução da ND, os pacientes com microalbuminúria não progridem necessariamente para macroalbuminúria. Alguns estudos da década de 90, apesar do pequeno número de pacientes, já demonstravam que parte dos pacientes permanecia no estágio de microalbuminúria e que outros regrediam para normoalbuminúria (9% a 15% dos pacientes) (91,113).

Atualmente, o curso clínico da microalbuminúria sofre as influências dos efeitos favoráveis das intervenções disponíveis. Um estudo de intervenção de 2 anos com BRA-II (79) mostrou que 34% dos pacientes regrediram de micro para normoalbuminúria. O uso do inibidor da ECA, comparado a outras drogas em pacientes com (149) ou sem (150) HAS, também mostrou regressão dos níveis de EUA em pacientes microalbuminúricos.

Um estudo realizado em pacientes com DM tipo 1 (151) demonstrou a regressão de

micro para normoalbuminúria em cerca de 40% dos pacientes em 6 anos de acompanhamento, sendo que 45% dos pacientes permaneceram microalbuminúricos, enquanto que 15% progrediram para macroalbuminúria. Em estudo prospectivo de 7,8 anos em pacientes com DM tipo 2, houve remissão para normoalbuminúria em 30% dos pacientes, sendo que os fatores preditores da remissão foram o melhor controle glicêmico e pressórico. Além disso, os pacientes que entraram em remissão tiveram diminuição do declínio da TFG (152).

Outras drogas têm mostrado efeito na redução da microalbuminúria, como a eplerenona (bloqueador seletivo da aldosterona) (153,154), a indapamida (diurético) (155), a rosiglitazona (tiazolidinediona) (156) e a sulodexida (glicosaminoglicano) (157).

Portanto, os pacientes microalbuminúricos têm a mesma chance de progredir para nefropatia clínica ou morte cardiovascular, em torno de 3% ao ano. Contudo, cerca de um terço desses pacientes ainda podem regredir para normoalbuminúria, principalmente com o uso de drogas que bloqueiam o sistema renina-angiotensina, que, no caso do uso do inibidor da ECA, está associado inclusive à diminuição de morte cardiovascular (158).

Estágio de Nefropatia Clínica = Macroalbuminúria ou Proteinúria

A prevalência de macroalbuminúria em pacientes com DM tipo 2 varia de 5% a 20% (6,159), sendo maior nos afro-americanos, asiáticos e índios americanos do que nos caucasianos (10,160). No Rio Grande do Sul, um estudo multicêntrico, envolvendo 927 pacientes com DM tipo 2, observou uma prevalência de macroalbuminúria de 12% (124).

Uma vez estabelecida a proteinúria, há uma perda progressiva de função renal, de modo que 10% dos pacientes evoluem para IRT em 10 anos (161). Em estudo prospectivo observacional de população caucasiana seguida por uma média de 6,5 anos, 7% dos pacientes progrediram para IRT e outros 28% dobraram o valor da creatinina inicial (162).

A incidência anual de progressão de macroalbuminúria para IRT ou de duplicação da creatinina sérica é de 2,3% ao ano, mas a chance de morte por ano desses pacientes é duas vezes maior (6). Outro estudo de base populacional também encontrou significativo aumento da mortalidade nos pacientes com macroalbuminúria acompanhados por 12 anos, tanto por causas cardiovasculares como por todas as outras causas (159). Esse aumento significativo da mortalidade pode ser devido à piora dos fatores de risco pré-existentes (163).

Estudos demonstram que quanto mais elevada a proteinúria de pacientes com DM tipo 2, (≥3g/g de creatinina) maior é o risco de doença cardiovascular e de desenvolver insuficiência cardíaca (14). Para níveis acima de 2 g/24 h, maior é a chance de progressão para IRT (95).

Nos pacientes com DM tipo 2, o declínio da TFG é heterogêneo, sendo que o declínio da função renal pode variar de 0,43 ml/min/mês (162) até 1,8 ml/min/mês (164) (Figura 2), podendo, inclusive, manter-se estável por longos períodos (165). Os pacientes que apresentam um declínio mais acentuado da função renal geralmente são os pacientes com glomerulopatia diabética mais grave e com pior controle metabólico (127). Um estudo observacional de pacientes caucasianos com DM tipo 2 de 6,5 anos de acompanhamento demonstrou que os fatores associados ao acentuado declínio da TFG foram médias mais elevadas ao longo do tempo de EUA, de pressão arterial sistólica e de HbA1c, e mais baixos de hemoglobina, além da presença de retinopatia diabética e do hábito do tabagismo (162).

A TFG inicialmente mais baixa prediz risco de progressão para IRT (166) e para doença cardiovascular (102) em pacientes com DM tipo 2, além de aumentar o risco de morte tanto em pacientes com DM tipo 2 (6) como também na população geral (16). Nessa, houve um progressivo aumento da representatividade de pacientes com DM à medida que caía a TFG.

No entanto, também para os pacientes com macroalbuminúria, a progressão para IRT não é inexorável. Parving e colaboradores já haviam demonstrado que o tratamento agressivo da hipertensão diminuía o declínio da TFG na ND (167,168). Estudos usando BRA-II, como o irbesartan (80) e o losartan (169), diminuíram a progressão para IRT em 23 a 28%, respectivamente. Um estudo multicêntrico (96) avaliou pacientes com DM tipo 2, HAS e proteinúria de pelo menos 0,9 g/24 h, tratados por 4 anos com o uso de irbesartan, amlodipina ou placebo. Esses pacientes foram mantidos com níveis semelhantes de pressão arterial. Foi observado que o risco de incidência de novos casos de IRT foi reduzido à metade (36%) no grupo que usou irbesartan, que também apresentou os maiores benefícios na proteinúria. Esse estudo, além de demonstrar as vantagens do bloqueio do sistema renina-angiotensina para retardar a progressão da DRC, também salienta a importância do tratamento da proteinúria como meta terapêutica para evitar a progressão da ND.

Em estudo de coorte envolvendo pacientes com DM tipo 1 proteinúricos (mediana de albumina >2.500 mg/24 h), seguidos por 3 anos, observou-se que 22% dos pacientes entraram em remissão (albuminúria < 600 mg/24 h por pelo menos 1 ano). Esses pacientes eram em sua maioria mulheres, e foi observado maior redução da pressão arterial média e do colesterol e menor declínio da TFG nos pacientes que obtiveram remissão, o que caracteriza um perfil cardiovascular melhor (170). Com o mesmo grupo de pacientes seguidos por mais 3 anos, demonstrou-se que somente 25% dos pacientes que entraram em remissão, comparados a 74% dos que não entraram em remissão, evoluíram para IRT ou morte. O grupo que obteve redução de albuminúria tinha menor chance de progredir para diálise, transplante renal ou morte (171).

Estágio de Uremia = Insuficiência Renal Terminal

A progressiva perda de função renal leva à uremia. Os pacientes com DM que

iniciam terapia de substituição renal têm alta prevalência de doença cardiovascular e de HAS (34). O método de diálise mais freqüentemente utilizado para pacientes com DM é a hemodiálise, seguido do transplante renal e do diálise peritoneal ambulatorial contínua (10,172,173). Nesse último método, a proporção de pacientes com DM é significativamente menor no Rio Grande do Sul (172). A sobrevida em 5 anos de pacientes com DM em diálise é de 20% a 40%, consideravelmente mais baixa em pacientes com DM em comparação a indivíduos não-diabéticos (Figura 3) em vários países (12,173). Uma vez em hemodiálise, a taxa de mortalidade é muito alta, atingindo, no terceiro ano, 55% em população do Canadá (11) e 72% no Rio Grande do Sul (12). O aumento da mortalidade está associado principalmente a alterações cardiovasculares relacionadas à aterosclerose (cardiopatia isquêmica) e à disfunção ventricular (cardiomiopatia). Segundo o UKPDS, os pacientes com aumento da creatinina plasmática ou em terapia de substituição renal estão sob chance de 19% ao ano de morrer, principalmente de doença cardiovascular. O tempo médio que um paciente permanece com elevação de creatinina até finalmente seguir para diálise é de 2,5 anos (6).

Dados do *United States Renal Data System* (10) revelam que a prevalência de pacientes com DM que evoluem para IRT aumentou 68% desde 1992. Para os pacientes entre 20 e 44 anos, a prevalência segue estável nos últimos anos, mas triplicou para pacientes com mais de 75 anos. Apesar de os índices de IRT, devido ao DM, terem estabilizado nos últimos anos (10), esse índice ainda é duas vezes maior do que as metas projetadas de 78 casos por milhão da população para o ano de 2010 nos Estados Unidos. Considerando que a perspectiva é de dobrar o número de pacientes com DM tipo 2 até o ano de 2030 (18), devido ao aumento da sobrevida geral dos pacientes e da epidemia de obesidade, a previsão é de que a ND será ainda mais onerosa ao sistema de saúde pública em todo o mundo. Os dados atuais do *United States Renal Data System* (10) já mostram o

progressivo aumento do índice de massa corporal (IMC em kg/m^2) que vem ocorrendo desde 1996, sendo que a média já é de 25 kg/m^2. A média de IMC dos pacientes com DM tipo 2 no Rio Grande do Sul é de 24 kg/m^2 (12).

Entre os pacientes com DM tipo 2, 80% deles são também hipertensos (34). Quando os pacientes com IRT, além do DM, têm HAS, eles têm o dobro de chance de complicações cardiovasculares do que o paciente com uma condição isolada (174). E já que a HAS é também muito comum nos pacientes com DM (95% dos pacientes com DM tipo 2 em Porto Alegre) (12) e que é um fator de risco independente para doença cardiovascular, é uma condição que deve ser tratada agressivamente em qualquer paciente com DM, independente da presença ou não de ND (174,175).

CONSIDERAÇÕES FINAIS

A nefropatia diabética (ND) é diagnosticada pela medida da excreção urinária de albumina (EUA) por métodos de imunoensaio que detectam a porção imunorreativa da albumina total. A medida da EUA classifica a ND em nefropatia incipiente ou microalbuminúria (EUA 20-199 μg/min) e nefropatia clínica ou macroalbuminúria (EUA ≥200 μg/min). No entanto, diversas evidências apontam para a necessidade de redução do atual ponto de corte da EUA de ≥20 μg/min, a partir do qual se define doença, visto que valores em torno de 5 a 10 μg/min já têm sido associados com risco de progressão para ND e para doença cardiovascular. Esses valores mais baixos estariam diretamente relacionados à detecção e à intervenção mais precoces nos pacientes com diabete melito (DM).

Adicionalmente, a introdução recente de novos métodos laboratoriais de detecção de albumina urinária intacta total (imunorreativa mais não-imunorreativa) parece ser promissora. Esses métodos poderiam identificar mais precocemente os indivíduos sob maior

risco de desenvolver ND.

Outro dado importante é a recomendação da avaliação da taxa de filtração glomerular (TFG) no DM, além da EUA, visto que uma significativa parcela desses pacientes pode apresentar redução da TFG mesmo na presença de valores normais de EUA. Nesses casos, a própria redução da TFG já está associada à evolução desfavorável com maior risco de insuficiência renal e de morte cardiovascular.

Portanto, a busca de marcadores de risco de instalação e de progressão da ND é plenamente justificada para identificar os pacientes mais suscetíveis. O uso dos marcadores disponíveis e a pesquisa de outros elementos são extremamente relevantes para o diagnóstico mais precoce. Isso pode contribuir para aumentar o desempenho das intervenções terapêuticas nos pacientes com DM.

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Tabela 1. Diagnóstico de Nefropatia Diabética

Estágios da ND	Albuminúria em urina de 24 h com tempo marcado	Albuminúria em amostra casual* de urina	Albuminúria em amostra - proporção albumina/creatinina
Nefropatia Incipiente: Microalbuminúria	20 a 199 μg/min	17 a 174 mg/l	30-299 mg/g
Nefropatia Clínica: Macroalbuminúria	≥200 μg/min (≥ 500 mg/24h **)	>174 mg/l (≥ 430mg/l **)	≥300 mg/g
Insuficiência Renal Terminal	TFG <15 ml/min/1,73 m^2 ou diálise***		

TFG = taxa de filtração glomerular.
American Diabetes Association. Diabetes Care 2004; 27 (suppl 1): s79-s83 (23)
* Zelmanovitz T et al. Diabetes Care 20:516-519, 1997 (104)
** proteinúria
. *** Levey AS et al. NKF. Ann Intern Med 139: 137-147, 2003 (24)

Tabela 2. Fatores de risco não-genéticos para nefropatia diabética

FATORES BEM ESTABELECIDOS
Hiperglicemia
Hipertensão arterial sistêmica
FATORES MENOS ESTABELECIDOS
Hiperfiltração glomerular ou diminuição da TFG
Fumo
Dislipidemia
Retinopatia Diabética
Elevada ingestão de carne vermelha
Excreção urinária de albumina normal-alta

TFG: taxa de filtração glomerular

Tabela 3. Fatores que aumentam os valores de albuminúria

Mau controle glicêmico
Infecção do trato urinário
Exercício físico rigoroso
Hipertensão arterial sistêmica não-controlada
Obesidade mórbida
Insuficiência cardíaca descompensada
Doença aguda ou febre
Hematúria
Sobrecarca protéica
Sobrecarga hídrica
Menstruação, leucorréia
Gestação

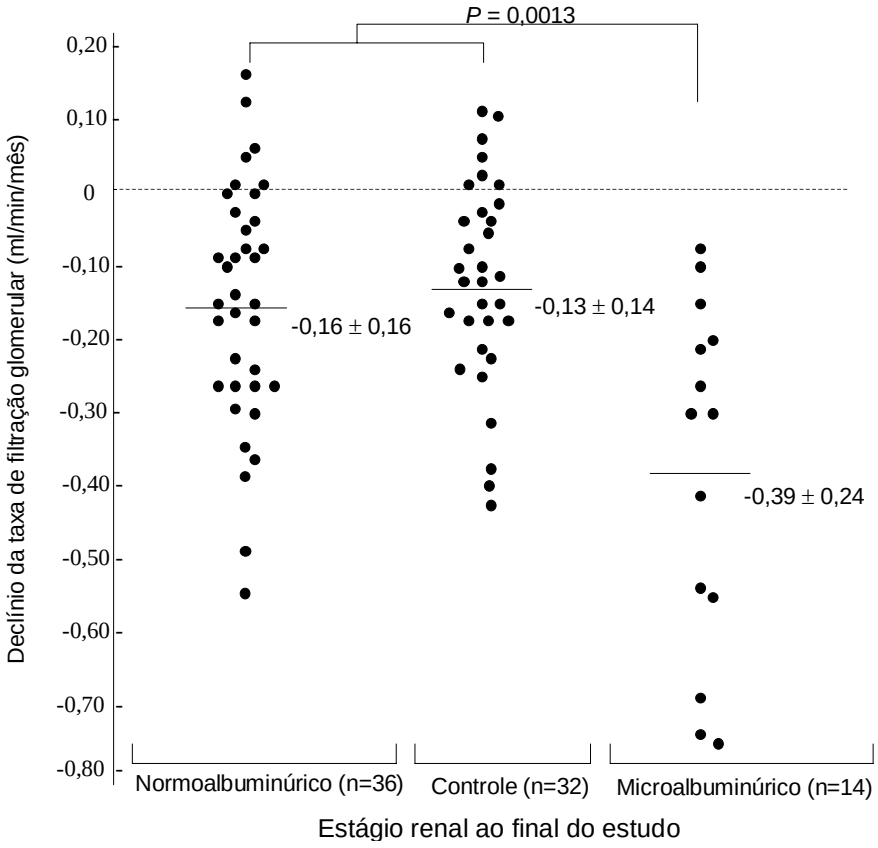

Figura 1. Declínio da taxa de filtração glomerular (média ± DP) em pacientes com DM 2 persistentemente normoalbuminúricos, em indivíduos não-diabéticos (grupo controle) e em pacientes que desenvolvem microalbuminúria após 10 anos de acompanhamento (129).

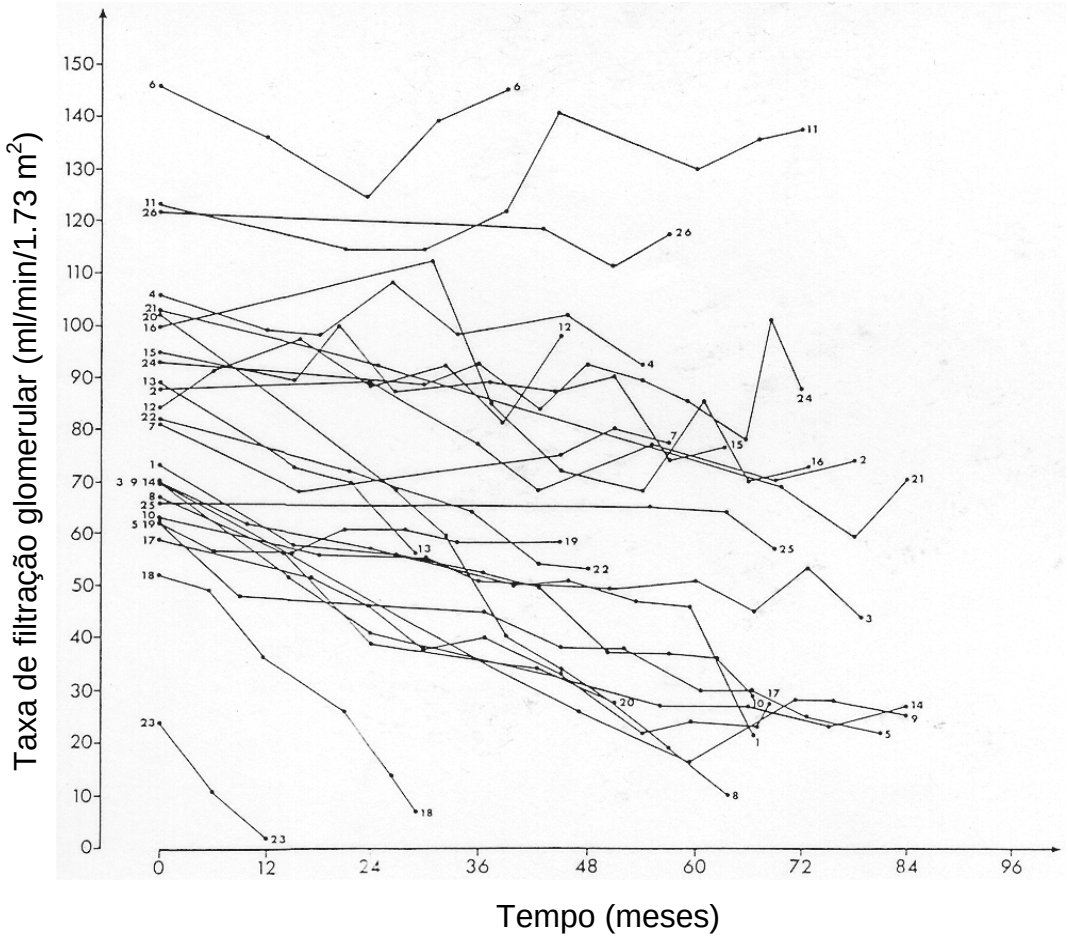

Figura 2. Declínio da taxa de filtração glomerular em pacientes macroalbuminúricos com DM tipo 2 (referência 164).

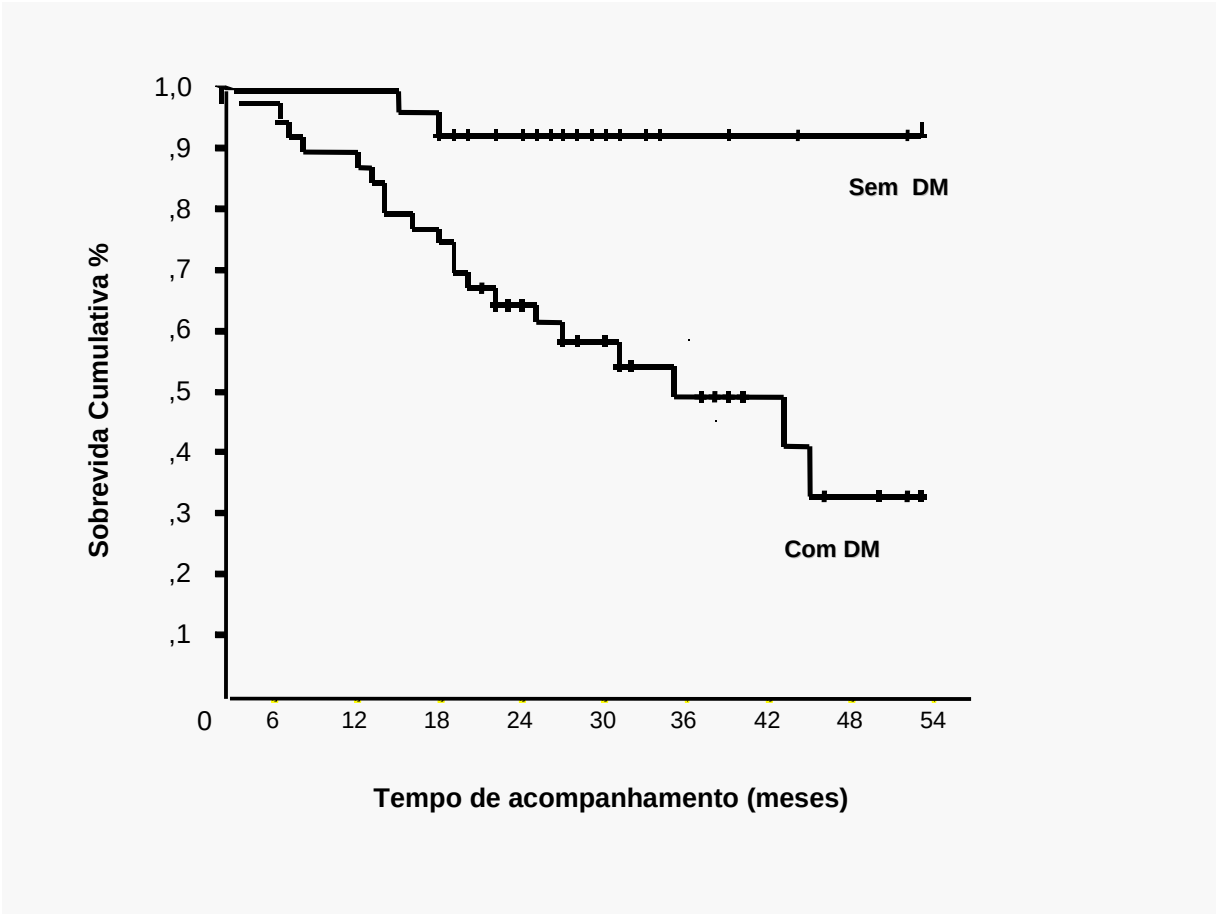

Figura 3. Sobrevida de pacientes com e sem DM em estudo de 18 centros de diálise na área metropolitana de Porto Alegre (referência 12, comunicação pessoal).

HIGH NORMAL LEVELS OF ALBUMINURIA ARE PREDICTORS OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETIC PATIENTS: AN 8-YEAR FOLLOW-UP STUDY

Short running title: Diabetic nephropathy predictors in type 2 diabetic patients

Marcia Murussi, MD[1]

Nicole Campagnolo[1]

Maristela de Oliveira Beck, MD[1]

Jorge L Gross, MD[1]

Sandra P Silveiro, MD[1]

[1]Endocrine Division, Hospital de Clínicas de Porto Alegre,

Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

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Abstract

OBJECTIVE: The aim of this study was to analyze the risk factors for the development of diabetic nephropathy (DN) in a cohort of normoalbuminuric type 2 diabetes mellitus (DM 2) patients.

RESEARCH DESIGN AND METHODS: In this prospective study, 193 DM 2 patients with urinary albumin excretion (UAE) <20 μg/min (immunoturbidimetry), 96 men (50%), 162 (84%) white and 31 mixed or black (16%), aged 56.5 ± 8.6 years, with diabetes duration of 8.2 ± 6.4 years, were followed for a mean period of 8 ± 3 years. Renal function was assessed at baseline and at the end of the follow-up.

RESULTS: Eighteen patients (9.3%) were lost to follow up and 2 were excluded due to the lack of baseline 24-h UAE measurements (1%). Among the 173 remaining patients, 15 died. Therefore, 158 were re-examined regarding DN: 34 developed microalbuminuria (22%) and 7 macroalbuminuria (4%). Patients who developed DN were more frequently men (61% vs. 42%, *P* = 0.045), and, at baseline, had a higher proportion of diabetic retinopathy (54% vs. 23%, *P* = 0.0001), and arterial hypertension (73% vs. 49%, *P* = 0.01). Baseline UAE was significantly higher in the progressors (8.2 [2.0-19.0] vs. 4.8 [0.1-19.2] μg/min, *P* <0.0001), who also had higher baseline fasting plasma glucose (198 ± 74 vs. 163 ± 55 mg/dl, *P* = 0.007), higher triglycerides (202 [32-646] vs. 125 [27-1292] mg/dl, *P* = 0.006), and lower estimated glomerular filtration rate (GFR) levels (78±17 vs. 84±18 ml/min/1.73m^2, *P* = 0.046). In a Cox proportional hazard analysis (hazard ratio [HR], 95% confidence interval [CI]) the variables significantly related to the later development of DN were a baseline UAE >5.1 μg/min (above the median, HR 2.85; 95% CI, 1.41-5.74; *P* = 0.0035), diabetic

retinopathy (HR 2.68; 95% CI, 1.37-5.12; *P* = 0.0036), fasting plasma glucose (HR 1.007; 95% CI, 1.001-1.012; *P* = 0.012), male sex (HR 2.89; 95% CI, 1.38-6.06; *P* = 0.0049), and lower estimated GFR (HR 0.98; 95% CI, 0.96-1.00; *P* = 0.0514). The presence of hypertension at baseline was excluded from the model (*P* = 0.16).

CONCLUSIONS: An UAE level >5.1 μg/min, even though within the normal range, is a strong predictor of progression for DN. Male sex, diabetic retinopathy, higher glucose levels and worse renal function are also related to the development of DN. Therefore, DM 2 patients with this profile deserve a program of intensified risk intervention.

Keywords: diabetic nephropathy, risk factors, microalbuminuria, albumin cutoff values, type 2 diabetes mellitus

Approximately 31% of type 2 diabetes mellitus (DM 2) patients are affected by diabetic nephropathy (DN) after 10 years of disease (1). According to the annual US Renal Data System's report, although overall rates of end stage renal disease (ESRD) due to diabetes have leveled off in recent years, diabetes is still the leading cause of chronic kidney disease, accounting for about 40% of all new cases starting renal replacement therapy (RRT) (2). DN was also the most often original kidney disease among ESRD patients in Sweden (24%) (3), and in Canada (51%) (4). In spite of the increasing number of therapeutic tools available to prevent and delay the progression of DN, mainly better blood pressure and glycemic control, the rates of ESRD remain nearly twice as high as the target rate of 78 per million of healthy people population for 2010, and show no signs of decreasing. As DM 2 accounts for the majority of new patients starting RRT (2,5), and considering that World Health Organization (WHO) estimates that the number of people with this type of diabetes will more than double until 2030 (6), it is expected a growing burden of DN for public health system across the world. Furthermore, the survival rate of diabetic patients requiring RRT is tremendously shortened, mainly due to cardiovascular deaths. It was previously demonstrated that among those patients, the 2-year survival rate is only 50% (5).

Genetic predisposition (7-9) and other risk factors have been identified in the development of DN in DM 2 patients. Poor glycemic control, increased blood pressure, dyslipidemia and smoking were described as risk factors in prospective analyses (10-15). Some studies have demonstrated that high UAE levels, even within the normal range, predict the development of DN in both type 1 (16), and type 2 diabetic patients (11,12,17-20).

The aim of this study was to analyze the risk factors for DN in a cohort of normoalbuminuric DM 2 patients, and to evaluate the cutoff value of UAE that would predict the development of DN.

RESEARCH DESIGN AND METHODS

Subjects

Three hundred eight DM 2 patients underwent a baseline clinical and renal evaluation in a university hospital. Among these patients, 116 already presented DN diagnosis (52 micro-, 60 macroalbuminuria and 3 kidney failure) and were not evaluated. The remaining 193 normoalbuminuric patients (UAE <20 μg/min) were re-examined after a mean period of 8 ± 3 years (range 1-16 years), and were enrolled in a prospective study to identify the main risk factors for the development of DN (Fig. 1). From these patients, 18 (9.3%) were lost to follow-up (14 patients could not be located and 4 refused to participate), and 2 (1%) were excluded due to the lack of baseline 24-h UAE measurements. Their baseline data were not different from those of the 173 re-evaluated patients in terms of male gender, age, diabetes duration, HbA1c, systolic and diastolic blood pressures, cholesterol, triglycerides, and UAE. Fasting plasma glucose and calculated GFR were lower in the lost patients (data not shown).

The primary endpoint of the study was the development of persistent micro- (UAE 20-199 μg/min) or macroalbuminuria (UAE ≥200 μg/min) in the period between the first evaluation and the moment the primary endpoint of the study was reached, or until the time of the last evaluation or death.

The diagnosis of DM 2 was established according to WHO criteria (21), without insulin use during the first 5 years after diagnosis.

Baseline evaluation

At baseline, DM 2 patients underwent a complete clinical interview and physical examination. Height and weight (light clothes without shoes) were measured and body mass index (BMI) was calculated (kg/m^2). Blood pressure was measured twice in the sitting position, after a 5-minute rest with a standard 12.5-cm cuff mercury sphygmomanometer (phases I-V). Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or as any value in patients using antihypertensive drugs. The presence of diabetic retinopathy (DR) was assessed by fundus examination performed by an ophthalmologist after mydriasis. Distal sensory neuropathy was investigated by testing vibratory perception (tuning fork test) along with the presence of compatible symptoms, abnormal results on Achilles tendon reflexes, and sensory perception by a 10-g Semmes-Weinstein monofilament at the hallux of each foot. Coronary artery disease was diagnosed on the presence of any of the following: symptoms of angina, previous heart attack, possible infarct (WHO Cardiovascular Questionnaire) (22), presence of resting ECG abnormalities (Minnesota Code) (23), revascularization procedures, perfusion abnormalities (fixed or variable) upon myocardial scintigraphy. Cerebrovascular disease was established by history of stroke, presence of compatible findings or evident damage. Peripheral vascular disease was diagnosed by intermittent claudication (WHO Cardiovascular Questionnaire) (22), absence of posterior tibial pulse upon clinical examination, or presence of inferior limb amputations. Cardiovascular disease was diagnosed if coronary artery disease, cerebrovascular disease or peripheral vascular disease were diagnosed. Smokers were defined as those smoking any kind of smoke at the beginning of the study, and former smokers were defined as those who had smoked for ≥1 year and had quit before starting the study. Both were analyzed as one group. Non-smokers were patients who had never smoked. Patients were classified as white or non-white (mixed or black) according to their own self-report.

Follow-up evaluation

At follow-up evaluation, the patients underwent the same procedures of baseline examination. Information regarding cause of death was collected from medical records, death certificates (recording the primary cause of death), individuals' relatives, and from the Health Information System. The interview and physical examination were performed at the same time of the renal evaluation and complemented as necessary in subsequent visits or in their medical records.

All patients gave their written informed consent to participate. The study protocol was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre.

Methods

At baseline, UAE was measured in 24-hour collections of sterile urine (2 to 3 samples) over a period of six months by radioimmunoassay (RIA) (DPC, Los Angeles), with an intra- and interassay coefficient of variation (CV) of 2.8% and 2.3%, respectively. At follow-up, UAE was measured by immunoturbidimetry (IT) (Microalb; Ames-Bayer, Tarrytown, NY, USA) in random urine samples (24), and confirmed by two 24-hour collections of sterile urine over a six-month period (coefficient of correlation with RIA: $r = 0.99$, interassay CV = 6.9%, and intra-assay CV = 3.8% for values around 20 μg/ml, and 1.9% for values around 150 μg/ml). Persistent micro- and macroalbuminuria were defined by at least two out of three 24-h collections with UAE values of 20-199 μg/min, and ≥200 μg/min, respectively (25). Administration of angiotensin-converting enzyme inhibitors was interrupted a week before UAE measurements.

Glomerular filtration rate (GFR) was estimated by MDRD (Modification of Diet in Renal Disease) study equation: $GFR\ (ml/min/1.73m^2) = 186 \times (Serum\ Creatinine_{\ mg/dl})^{-1.154} \times (Age_{\ years})^{-0.203} \times (0.742\ if\ female) \times (1.210\ if\ African\text{-}American)$ (26).

Fasting plasma glucose (FPG) was measured by the glucose oxidase UV enzymatic method. HbA1 levels were measured at baseline by ion-exchange chromatography (normal range: 6.5-8.5 %, intra-assay CV 4.1-5.8%). At follow-up, HbA1c levels were measured by high-performance liquid chromatography (HPLC) procedure (Merck-Hitachi L-9100 Glycated Haemoglobin Analyzer; reference range: 3.2-4.5%**,** inter- and intra-assay CV= 2.4% and 0.5%, respectively). Baseline HbA_1 levels (y) were converted to HbA1c levels (x) using the formula y = 1.09x + 1.95 (27). Urea, cholesterol, and triglycerides were measured by enzymatic methods. LDL cholesterol was calculated using the Friedewald equation. Creatinine was measured by the Jaffé reaction.

Statistical analysis

Results are expressed as mean ± SD, median (ranges) or number of cases and percentage. Incidence was expressed as the number of subjects who developed persistent micro- or macroalbuminuria per 1,000 person-years at risk, and as cumulative rate. A Cox proportional-hazard multiple regression model (backward stepwise method) was used to examine predictors of progression to micro- or macroalbuminuria. Results are expressed as relative risk (Hazard Ratio) and 95% Confidence Interval (CI). The model included baseline variables that were found to be statistically significant in univariate analyses, as well as those implicated *a priori* as potential risk factors (even though not significant in the univariate analysis). Kaplan-Meier curves were employed to estimate the probability of surviving without the development of DN or death according to the presence of a given risk factor. A *P*-value <0.05 (two-sided) was considered to be statistically significant. All data were analyzed using the Statistical Package the Social Sciences (SPSS 10.0–Professional Statistics^{TM}, SPSS Inc., Chicago, IL, USA).

RESULTS

Risk factors for micro- or macroalbuminuria

Among the 173 DM 2 patients, 15 (8.7%) died before renal status re-evaluation (3 from cancer, 2 from respiratory failure, 5 from cardiovascular disease, 1 from rheumatoid arthritis, 1 from sepsis and 3 from unknown causes). Their data were not different (data not shown) from the survivors, except for their older age (63.5 ± 6.6 vs. 56.2 ± 8.4, $P = 0.001$). The data of the deceased patients were considered in the survival rate analysis.

From the 158 eligible normoalbuminuric patients followed for a mean period of 8 ± 3 years (median 8.6 years [1-16]), forty-one patients (26%) developed DN: 34 (22%) developed microalbuminuria and 7 (4%) developed macroalbuminuria (2 of them progressed to kidney failure). This represents an incidence density of micro- and macroalbuminuria of 31/1,000 person-years.

The baseline data of persistently normoalbuminuric patients (non-progressors) and of those who developed micro- or macroalbuminuria (progressors) are shown in Table 1.

There was no difference regarding ethnicity. Patients who progressed were more frequently men than non-progressors. They had higher baseline FPG, although HbA1c was not different between groups. Baseline cholesterol and HDL levels were not different, but serum triglycerides were higher in patients who later developed DN. The proportion of initially hypertensive patients was higher in progressors than in non-progressors, although systolic and diastolic blood pressure values did not differ between groups at baseline. There were also no differences between the groups regarding the use of antihypertensive treatment (76% vs. 74%, $P = 1.00$), or the use of specific drugs like β-blockers (25% vs. 22%, $P = 0.79$), and ACE inhibitors (28% vs. 22%, $P = 0.60$) in the patients who developed DN and those who did not, respectively.

Regarding renal function evaluation, calculated baseline GFR values were lower among progressors, but the proportion of patients with a GFR <60 ml/min/1.73m^2 was not different between groups. Accordingly, serum creatinine was higher among patients who developed DN. Baseline UAE was higher in the patients who developed DN at the end of the study. It was observed that patients with a baseline UAE >5.1 μg/min (that is, above the median value of the entire normoalbuminuric cohort group) progressed more frequently (66% vs. 44%, *P* = 0.029) than the patients with lower UAE values. These patients had a 2.4-fold (1.15-5.06) increased risk of developing DN (Fig. 2). Furthermore, when data of the 15 deceased patients were included, this value was related to an increased risk of death (Fig. 3) (log rank *P*=0.027), mainly from cardiovascular disease (14 deaths out of 20 [70%] to the UAE > 5.1 μg/min group, and 3 out of 13 (23%) to the UAE ≤5.1 μg/min group, *P* = 0.02).

There were no differences in the presence of pre-existing coronary artery disease (12 [29%] vs. 17 [15%], *P* = 0.058), peripheral vascular disease (14 [36%] vs. 30 [26%], *P* = 0.31), and distal sensory neuropathy (13 [33%] vs. 27 [23%], *P* = 0.29) at baseline between the patients who developed and those who did not develop micro- or macroalbuminuria, respectively. However, more patients who progressed presented diabetic retinopathy at the beginning of the study (22 [54%] vs. 27 [23%], *P* = 0.001) (Fig. 4).

In a multivariate Cox proportional hazard analysis (hazard ratio [HR], 95% confidence interval [CI]) the variables significantly related to the later development of DN in this model were a baseline UAE >5.1 μg/min (above the median), diabetic retinopathy, male sex, lower GFR, and fasting plasma glucose (Table 2). The presence of hypertension at baseline was excluded from the model (*P* = 0.16).

Characteristics of micro- and macroalbuminuric patients at follow-up

At the end of the study (Table 3), there was no difference between progressors and non-progressors concerning the presence of hypertension. However, the final systolic blood

pressures were higher among micro- and macroalbuminuric than in the normoalbuminuric patients, while diastolic blood pressures were not statistically different. The number of patients in use of antihypertensive treatment was not different between (33 [97%] vs. 64 patients [96%], *P* = 1.00), as well as the number in use of β-blocker (18 [53%] vs. 30 [45%], *P* = 0.52) at the end of the study. On the other hand, the number of patients receiving ACE inhibitors was significantly higher in progressors (27 [79%] vs. 27 patients [40%], *P* <0.0001).

At follow-up, we observed that a higher number of patients in the micro-macroalbuminuric group were taking insulin. There were no differences between the groups in terms of final BMI, and current smoking (7 % vs. 16%, *P* = 0.24). In spite of no difference in the final FPG values, HbA_{1c} was higher in the progressors. Micro- and macroalbuminuric patients presented lower HDL cholesterol and higher triglyceride levels at the end of the study. Conversely, total cholesterol and LDL cholesterol were not different between groups.

When we analyzed the evolution of other chronic complications, we observed that, at the end of the study, progressors had a higher prevalence of diabetic retinopathy (83% vs. 43%, *P*< 0.0001), coronary artery disease (66% vs. 34%, *P* = 0.001), peripheral vascular disease (55% vs. 36%, *P* = 0.04), distal sensory neuropathy (56% vs. 34%, *P* = 0.016), and cardiovascular disease (76% vs. 50%, *P* = 0.006).

UAE increased significantly even in DM 2 patients who remained normoalbuminuric during the follow-up (4.8 μg/min [0.1-19.2] to 5.76 μg/min [2.6-19.8], *P* <0.0001; n = 109 patients).

Eight out of 41 (19.5%) micro- and macroalbuminuric patients and 10 out of 117 (8.5%) normoalbuminuric patients died during the follow-up evaluation, and these proportions were not different (*P* = 0.083). Death causes were cardiovascular in 5 (63%) of

the progressors and in 7 (70%) of the non-progressors (P = 1.00). In each group, the other causes of death were sepsis, cancer, and unknown reason.

CONCLUSIONS

The cumulative incidence of micro- and macroalbuminuria was 26% in 8 years, similar to that reported in other studies after comparable periods: 34% in Finland (12), and 35% in Israel (15). Other prospective studies found cumulative incidence of 24% in a Danish population (11), 25% in Korean patients (14), and 42% in Pima Indians (13), but the follow-up periods were shorter (about 5 years). The UKPDS (1) found that after 10 years following the diagnosis of diabetes, the prevalence was 31%.

The main risk factors for the development of micro- and macroalbuminuria in our study were higher baseline UAE levels (even though within the normal range), the presence of diabetic retinopathy, male sex, lower GFR and higher FPG at baseline.

The predictive value of high normal UAE had already been described by other authors in type 1 (16,18) and type 2 diabetes (11-13,17-20). Gilbert et al. (18) observed that type 1 and type 2 diabetic patients at risk for DN could be identified by serial measurements of UAE from diagnosis, even before they reached the 20 μg/min threshold of microalbuminuria. Forsblom et al. demonstrated that the progression to micro- and macroalbuminuria was more frequent in patients with initial UAE above the median (2.5 mg/24 h) (12). Similarly, Gall et al. described a significantly higher baseline UAE in patients who progressed in comparison with those who remained normoalbuminuric (geometric mean, 14 vs. 7 mg/24 h) (11). Nelson et al. found that patients who developed DN had higher median UAE/creatinine concentrations (12.2 vs. 7.4 mg/g) (13). Nielsen et al. confirmed these findings with UAE levels of 10.1 ± 1.1 vs. 5.3 ± 1.1 μg/min (19). We have also previously observed that patients who progressed to DN presented higher median

UAE values (5.9 vs. 3.2 μg/min) (20). In the present study, when UAE was controlled for other risk factors through multivariate analyses, there was a 2.4-fold increased risk for DN in those patients with UAE levels of 5.1 μg/min (above the median). We have earlier demonstrated in a 9-year follow-up study that normoalbuminuric DM 2 patients with UAE values above 10 μg/min had a 29-fold increased chance to develop DN (20). Similarly, in normoalbuminuric type 1 diabetic patients followed for 7 years (16), this same cutoff value conferred a 19-fold chance to develop microalbuminuria. Additionally, in DM 2 normoalbuminuric patients, values ≥5 μg/min were also related to higher ambulatory blood pressure, serum creatinine and cholesterol, and to thicker left ventricle (28), meaning a worse cardiovascular risk profile. Besides that, it has been shown that UAE values, even within the normal range, are related to increased incidence of cardiovascular disease and higher mortality rates in DM 2 (29,30), and even in non-diabetic individuals (31,32). This also applies to our DM 2 patients, who presented an increased risk of death when their UAE was >5.1 μg/min. A plausible explanation for the damage provoked by these relatively low levels of UAE could be the new findings that conventional immunoassays routinely used for UAE measurement may underestimate the actual urinary albumin level (33,34), as it detects only the immunoreactive part of the total intact UAE, composed of immunoreactive and immuno-unreactive albumin. Indeed, Comper et al. (35) have recently observed that this new urinary albumin assay would have detected microalbuminuria 3.9 and 2.4 years earlier in type 1 and type 2 diabetic patients, respectively. Hence, UAE levels below the traditional recommended value of 20 μg/min (25) could already be reflecting a disease in its very initial stage, or signaling a generalized state of endothelial dysfunction (36,37), without, however, specifically be expressing underlying renal damage. Therefore, high normal UAE levels could be a remarkable risk factor for DN and death. On the other hand, UAE may not be the only marker of DN (38,39,40). In fact, some authors have described that low GFR levels can

also predict renal function decline (38), and cardiac events (39) even in the absence of albuminuria. This being so, low GFR levels may already be pointing out the presence of renal damage (40). Accordingly, in our study, baseline GFR values were initially lower in progressors.

As shown by others (11,13,14), diabetic retinopathy was also a strong risk factor for DN in our patients. Retinopathy is probably a marker rather than a risk factor "*per se*," because nephropathy and retinopathy seem to share the same environmental predisposing factors, such as hyperglycemia and arterial hypertension. Our study suggests that the onset of retinopathy occurs earlier than that of DN, probably because the retina would be more sensitive to these environmental risk factors or alternatively would be detected earlier.

Our data showed an increased risk for DN in males, but male sex has been considered a risk factor only in some (11,15), not in all (13,14,20) studies.

Baseline FPG levels were higher in the patients who developed DN. In prospective studies, higher HbA1c levels have been identified as a major risk for the development of microalbuminuria (11,15). Through an observational analysis, UKPDS (41) has demonstrated that any reduction in HbA1c decreases the risks for microvascular complications.

Cholesterol has been disclosed as a risk factor for DN in some (11,13,15), but not in all longitudinal studies (12,14). Our patients did not differ regarding baseline cholesterol levels, although there were higher baseline triglycerides levels among those who developed micro- and macroalbuminuria.

Arterial hypertension has been implicated as a risk factor for DN in several prospective studies (13-15,42). Two of them analyzed blood pressure during the study period, and observed higher levels among patients who progressed to micro- or macroalbuminuria

(14,15). Accordingly, we found a higher prevalence of baseline hypertension among those patients who progressed.

In the UKPDS (1), it was found a trend for increasing risk of death with increasing DN stage. The mortality rate in our study was not different between progressors and non-progressors, probably because of the small number of outcomes in our cohort.

The patients who became micro- and macroalbuminuric during the observation period more often presented other complications, such as retinopathy and cardiovascular disease. Furthermore, they presented higher levels of triglycerides and their metabolic control was worse. The interaction of these factors might have influenced the overall less favorable evolution of these patients. A recent study has demonstrated that intensive multifactorial treatment in type 2 microalbuminuric patients reduces the rate of progression of renal disease (43).

In conclusion, our study demonstrated that over a period of 8 years, 26% of normoalbuminuric type 2 diabetic patients developed DN. Higher UAE (even though within the normal range), the presence of retinopathy, male sex, lower GFR, and baseline FPG were risk factors for the development of micro- and macroalbuminuria. Therefore, these factors are markers to be searched for. According to guidelines, the cutoff value to define microalbuminuria is 20 μg/min (25). However, our patients presented a greater chance to develop DN if they had a baseline UAE >5.1 μg/min. Since growing evidence from literature has shown that patients with lower UAE already are at increased risk for DN, and also at increased risk for cardiovascular disease and mortality, we believe that these patients deserve a program of intensified and multifactorial risk intervention.

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Table 1. Baseline clinical and laboratory characteristics of DM 2 patients with and without progression to DN

Baseline Characteristics	Progressors (n= 41)	Non-progressors (n=117)	*P*
Male sex	25 (61%)	49 (42%)	0.045
Ethnicity (white/non-white)	35/6	100/17	1.00
Age (years)	57 ± 9	56 ± 8	0.49
Duration of diabetes (years)	8.3 ± 6.8	8.8 ± 6.4	0.71
Body mass index (kg/m^2)	27.1 ± 4.4	27.0 ± 4.5	0.91
Smoking (Ex + S)	13 + 8 (51%)	15 + 38 (45%)	0.59
Type of treatment (D/OA/I)	11/23/7	34/68/15	0.79
Arterial hypertension	30 (73%)	57 (49%)	0.01
SBP (mmHg)	145 ± 19	141 ± 25	0.41
DBP (mmHg)	86 ± 11	84 ± 13	0.50
Fasting plasma glucose (mg/dl)	198 ± 74	163 ± 55	0.007
$HbA1_c$ (%)	7.3 ± 2.1	6.8 ± 2.0	0.13
Cholesterol (mg/dl)	219 ± 50	214 ± 45	0.60
HDL (mg/dl)	43 ± 15	48 ± 13	0.10
Triglycerides (mg/dl)	202 (32-646)	125 (27-1292)	0.006
Urinary urea (g/24h)	24.6 ± 14	23.8 ± 9.3	0.79
GFR (ml/min/1.73m^2)	78 ± 17	84 ± 18	0.046
Serum Creatinine (mg/dl)	1.00 ± 0.16	0.90 ± 0.19	0.002
GFR <60 ml/min/1.73m^2	5 (12%)	10 (9%)	0.54
UAE (μg/min)	8.2 (2.0-19.0)	4.8 (0.1-19.2)	<0.0001
Follow-up (years)	8.1 ± 3.4	8.0 ± 3.2	0.90

Mean ± SD (range), median (range), number of cases (percentage). Ex = ex-smoker, S= current smoker, D = diet, OA= oral agents, I = insulin, SBP = systolic blood pressure, DBP = diastolic blood pressure, GFR = glomerular filtration rate, estimated by MDRD (Modification of Diet in Renal Disease) equation, UAE = urinary albumin excretion.

Table 2. Baseline risk factors for diabetic nephropathy development in 158 normoalbuminuric DM 2 patients

Independent Variables	Relative Risk-HR	95% CI	*P*
UAE >5.1 μg/min	2.85	1.14-5.74	0.0035
Retinopathy	2.65	1.37-5.12	0.0036
Male sex	2.89	1.38-6.06	0.0049
GFR	0.98	0.96-1.00	0.0514
Fasting plasma glucose	1.007	1.001-1.012	0.012

Cox multiple regression analysis by backward stepwise. The presence of hypertension at baseline was excluded from the model (P = 0.16). UAE = urinary albumin excretion, GFR = glomerular filtration rate, MDRD (Modification of Diet in Renal Disease equation).

Table 3. Final clinical and laboratory characteristics of DM 2 patients with and without progression to DN

Final characteristics	Progressors (n= 41)	Non-progressors (n=117)	*P**
Body mass index (kg/m^2)	27.5 ± 4.6	27.0 ± 4.5	0.50
With insulin	26 (65%)	39 (33%)†	0.006
Arterial hypertension	35 (85%) †	68 (59%) †	0.002
SBP (mmHg)	149 ± 23	139 ± 21	0.01
DBP (mmHg)	84 ± 11	80 ± 12†	0.09
Fasting plasma glucose (mg/dl)	182 ± 69	166 ± 58	0.15
$HbA1_c$ (%)	7.5 ± 1.9	6.6 ± 1.6	0.004
Cholesterol (mg/dl)	211 ± 58	209 ± 40	0.84
HDL (mg/dl)	43 ± 14	49 ± 11	0.019
Triglycerides (mg/dl)	150 (45-758)	125 (33-599)	0.014
LDL (mg/dl)	127 ± 44	132 ± 33	0.56
UAE (μg/min)	57.1 (20.2-1137) †	5.76 (2.6-19.8) †	0.0001
Diabetic Retinopathy	34 (83%) †	50 (43%) †	<0.0001
Coronary artery disease	27 (66%) †	40 (34%) †	0.001

Mean ± SD (range), median (range), number of cases (percentage). N= never, Ex = ex-smoker, S= current smoker, D = diet, OA= oral agents, I = insulin, SBP = systolic blood pressure, DBP = diastolic blood pressure, UAE = urinary albumin excretion. * *P* values to comparisons between groups regarding final data. † *P* values to comparisons between baseline and final data into the same group ($P < 0.05$).

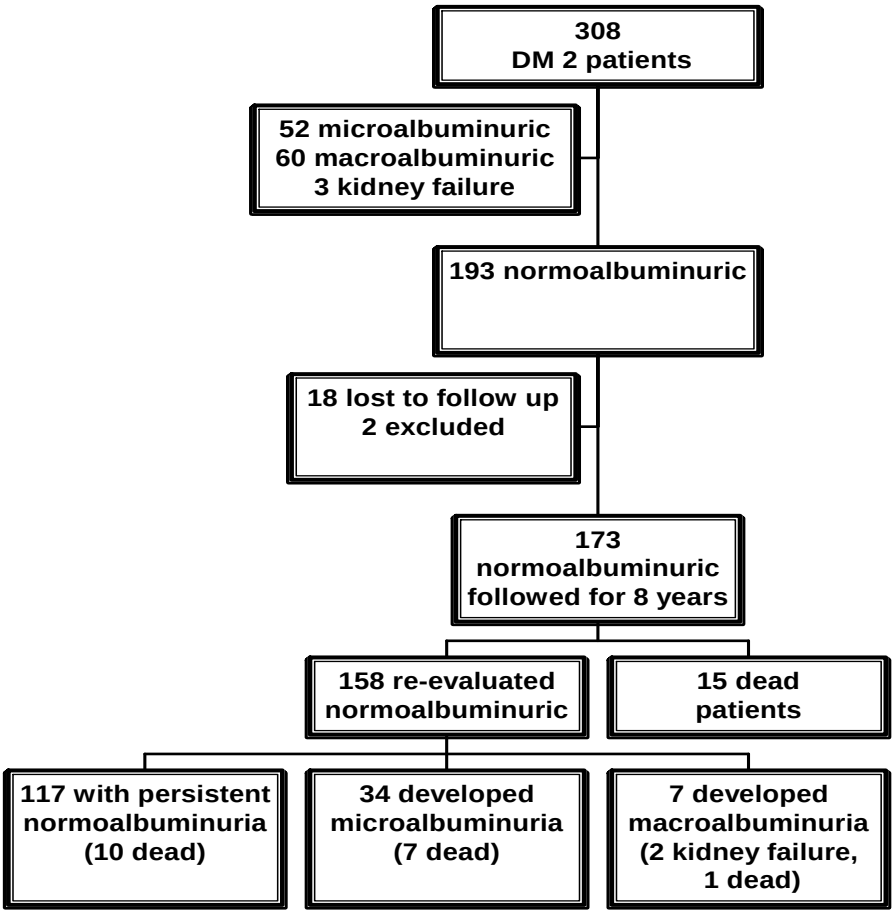

Figure 1. Flow chart of normoalbuminuric DM 2 patients.

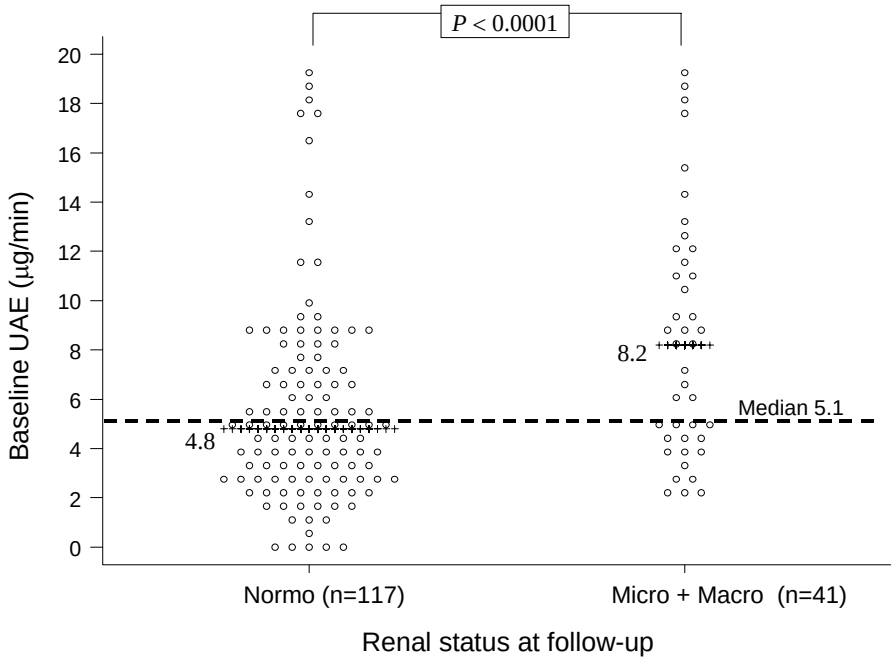

Figure 2. Baseline urinary albumin excretion (UAE) in normoalbuminuric and in micro- plus macroalbuminuric DM 2 patients at follow-up (to UAE >5.1 µg/min, OR 2.4 [1.15-5.06]).

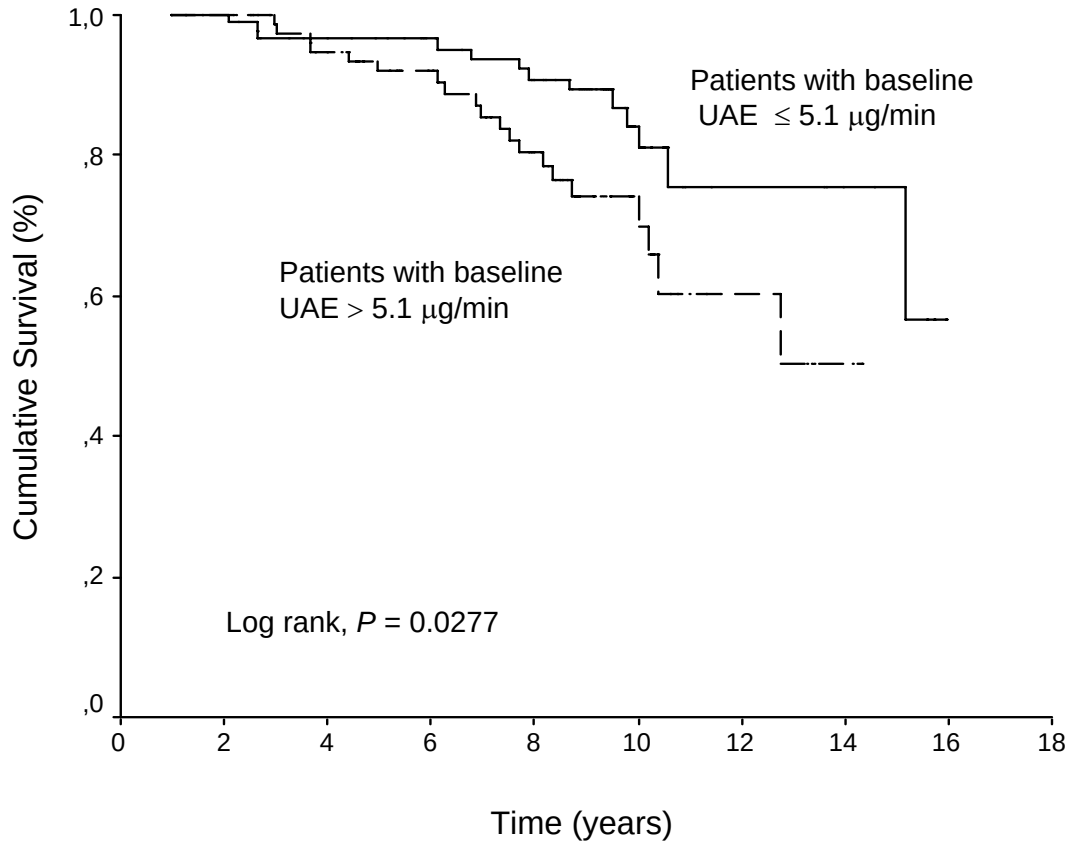

Figure 3. Kaplan-Meier estimates of survival in DM 2 patients (158 re-evaluated + 15 dead before re-evaluation) according to UAE above the median 5.1 μg/min at baseline.

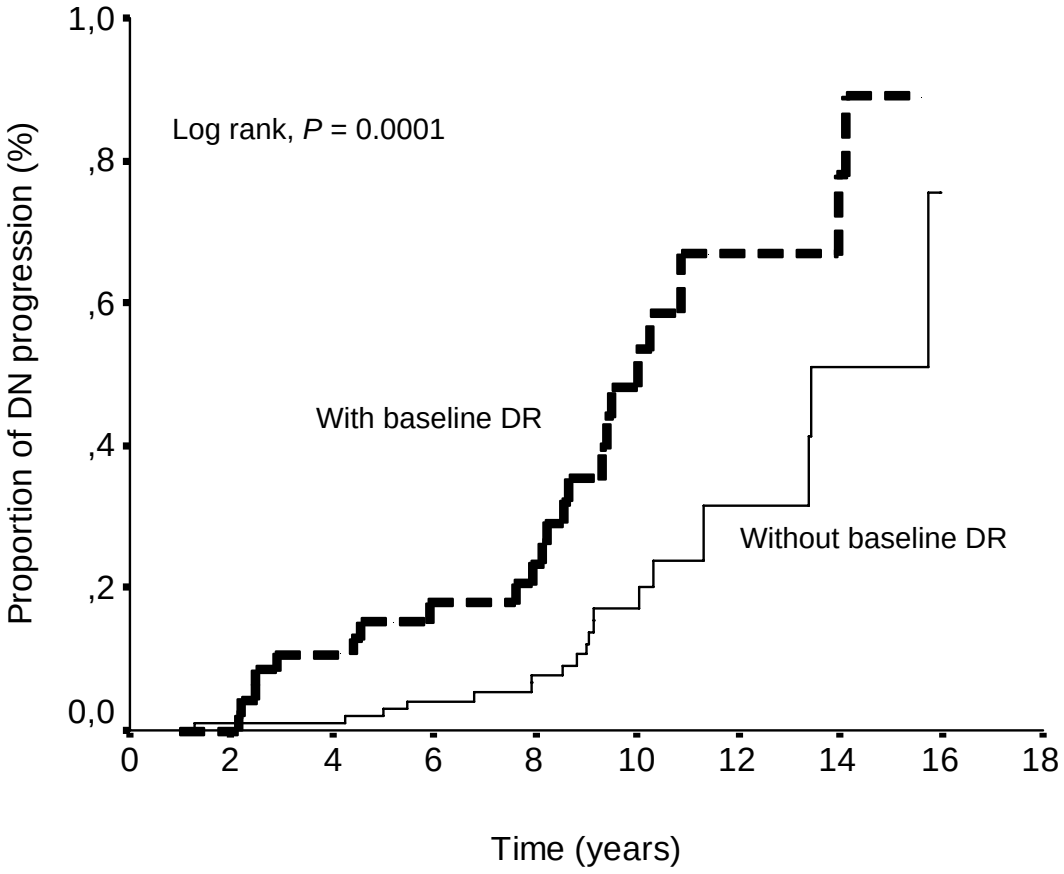

Figure 4. Kaplan-Meier estimates of the development of micro- and macroalbuminuria in DM 2 patients according to the presence of diabetic retinopathy (DR) at baseline, n = 158.

COURSE OF MICROALBUMINURIA IN TYPE 2 DIABETIC PATIENTS: A 6-YEAR FOLLOW-UP STUDY

Short running title: Course of Microalbuminuria in Type 2 Diabetes

Marcia Murussi, MD[1]
Nicole Campagnolo[1]
Ariane Coester[1]
Maristela de Oliveira Beck, MD[1]
Jorge L Gross, MD[1]
Sandra P Silveiro, MD[1]

[1]Endocrine Division, Hospital de Clínicas de Porto Alegre,
Universidade Federal of Rio Grande do Sul, Porto Alegre, Brazil

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Abstract

OBJECTIVE: The aim of this study was to analyze the clinical course of microalbuminuria in a cohort of type 2 diabetic (DM 2) patients, and to evaluate risk factors related to diabetic nephropathy (DN) progression and mortality.

RESEARCH DESIGN AND METHODS: In this prospective study, 52 microalbuminuric (urinary albumin excretion [UAE] 20-199 μg/min) DM 2 patients, 31 men (60%), 43 (83%) white and 9 (17%) mixed or black, aged 59 ± 9 years, with diabetes duration of 12 ± 7 years, and median UAE of 48 μg/min (range 20-193), were followed for a mean period of 6 ± 3 years. They were submitted to renal evaluation by UAE (immunoturbidimetry) and glomerular filtration rate (GFR) estimated by the four-component Modification of Diet in Renal Disease equation, at baseline and at follow-up.

RESULTS: Six patients (11.5%) were lost to follow up. Three (5.5%) died before renal status re-evaluation (from coronary artery disease). Among the 43 (83%) re-examined patients, 14 (32%) progressed to macroalbuminuria (MA) (5 of them developed kidney failure), 17 (40%) remained microalbuminuric (MI), and 12 (28%) regressed to normoalbuminuria (NO). Multiple Cox regression analysis (Hazard Ratio [Confidence Interval]) showed that baseline variables related to DN progression were UAE above the median (≥48 μg/min, 5.16 [1.44-18.58], *P* = 0.012), and diabetic retinopathy (5.39 [1.24-23.43], *P* = 0.025), while age (*P* = 0.07), and GFR (*P* = 0.41) were excluded from the model. At follow-up, 12 (28%) of the patients presented GFR values <60 ml/min/1.73 m^2 (10 in MA, 2 in MI, and none in NO group [*P* = 0.0001]). A Cox proportional hazards analysis disclosed that higher baseline GFR (0.96 [0.94-0.99], *P* = 0.005) protected against GFR values <60 ml/min/1.73m^2 at follow-up, while UAE ≥48 μg/min (4.47 [0.85-23.46], *P* = 0.077), and age (*P* = 0.307) were not significant in the model. The mortality rate tended to be higher in the MA group (log rank, *P* = 0.059).

CONCLUSION: In microalbuminuric DM 2 patients, the progression was not the rule, as a great proportion of patients remained microalbuminuric (40%) or regressed to normoalbuminuria (28%). The main risk factors for progression to overt diabetic nephropathy were higher baseline UAE levels, and the presence of diabetic retinopathy. Higher baseline GFR values prevented the development of GFR values <60 ml/min/1.73m^2 at follow-up.

Keywords: diabetic nephropathy, microalbuminuria, clinical course

There are few observational studies describing the clinical course of microalbuminuria in type 2 diabetic (DM 2) patients (1-3). Since these studies, conducted in the 90 decade, have evaluated specific ethnic populations, they do not allow their conclusions to be applied for different ethnic groups. Additionally, most studies describe only progression rates (4-6), not taking into account the possibility of remission, and when considering remission rates, are usually randomized intervention studies (7-9).

The major factors implicated as risk for progression from micro- to macroalbuminuria are poor glycemic control, systemic hypertension, genetic factors, dyslipidemia, smoking, and albuminuria *per se* (10,11). However, their relative roles are far from being certain, because of the difficulties to appropriately dissect interactive variables.

The definition of microalbuminuria (urinary albumin excretion ≥20 to 199 μg/min or ≥30 to 299 mg/24h) (12) was established in the 80 decade based on studies in both type 1 and type 2 diabetes (5,13-15), where progression rates of these patients to macroalbuminuria were around 80%. However, some recent studies have found lower progression figures of 20% to 50% in DM 2 patients (4,16). This picture has probably changed due to knowledge of the benefits of intensive treatment, mainly regarding glucose and blood pressure levels (10).

Microalbuminuric DM 2 patients are a heterogeneous population, with distinct renal structure lesions (17), and with variable prevalence of 5% to 30% (4,18,19). In addition, these patients present an increased risk of death, essentially from cardiovascular causes (4,18,20). At this stage, glomerular filtration rate course is not clear, as some authors have described a significant decline (21,22), while others have not (3).

As long as the incidence of kidney failure (KF) caused by diabetes mellitus is still rising (23), and as DM 2 patients account for the majority of the diabetic patients starting kidney replacement therapy (23,24), it is imperative to figure out the meaning and clinical course of microalbuminuria in these patients. Furthermore, when requiring dialysis, their survival is remarkably shortened (23,25), usually due to cardiovascular endpoints (25,26). In Brazilian patients, the 2-year survival is only 50% (26).

The aim of this study was to describe the clinical course of microalbuminuria in a cohort of microalbuminuric DM 2 patients, pointing out the putative risk factors for progression to renal impairment and mortality.

RESEARCH DESIGN AND METHODS

Subjects

The population based study was a cohort of 308 DM 2 patients who underwent clinical and renal evaluation in a tertiary referral center. Among these patients, 256 were excluded (193 were normoalbuminuric, 60 were macroalbuminuric, and 3 were on dialysis). Therefore, 52 patients were microalbuminuric (UAE 20-199 μg/min) at baseline, and were enrolled in this prospective study of 6 ± 3 years (range 2-11) in order to evaluate the clinical course of microalbuminuria, and to identify the main risk factors for progression of DN and mortality. Their baseline characteristics are shown in Table 1.

The primary endpoints of the study were progression to persistent macroalbuminuria (MA) (UAE > 200 μg/min) or kidney failure (KF), or remission to normoalbuminuria (NO) (UAE <20 μg/min) in the period between the first evaluation and the moment the primary endpoint of the study was reached, or until the time of the last evaluation if the patient remained microalbuminuric (MI), or until death.

DM 2 diagnosis was established according to WHO criteria (27), without insulin use for at least 5 years after diagnosis.

Baseline evaluation

At baseline, DM 2 patients underwent a complete clinical interview and physical examination. Height and weight (light clothes without shoes) were measured and body mass index (BMI) was calculated (kg/m^2). Blood pressure was measured twice in the sitting position, after 5-minute rest with a standard 12.5-cm cuff mercury sphygmomanometer (phases I-V). Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg, or as any value in patients using antihypertensive drugs. The presence of diabetic retinopathy (DR) was assessed by fundus examination performed by an ophthalmologist after mydriasis. Distal sensory neuropathy was investigated by testing vibratory perception (tuning fork test) along with the presence of compatible symptoms, abnormal results on Achilles tendon reflexes, and sensory perception by a 10-g Semmes-Weinstein monofilament at the hallux on each foot. Coronary artery disease was diagnosed on the presence of any of the following: symptoms of angina, previous heart attack, possible infarct (WHO Cardiovascular Questionnaire) (28), presence of resting ECG abnormalities (Minnesota code) (20), revascularization procedures, perfusion abnormalities (fixed or variable) upon myocardial scintigraphy. Cerebrovascular disease was established by history of stroke, presence of compatible findings or evident damage. Peripheral vascular disease was diagnosed by intermittent claudication (WHO Cardiovascular Questionnaire) (28), absence of posterior tibial pulse upon clinical examination, or presence of inferior limb amputations. Cardiovascular disease was diagnosed if coronary artery disease, cerebrovascular disease or peripheral vascular disease were diagnosed. Smokers were defined as those smoking any kind of smoke at the beginning of the study, and former smokers were defined as those who had smoked for ≥ 1

year and had quit before starting the study. Both were analyzed as one group. Non-smokers were patients who had never smoked. Patients were classified as white or non-white (mixed or black) according to their own self-report.

Follow-up evaluation

At follow-up evaluation, the patients underwent the same procedures of baseline examination. Information regarding causes of death was collected from medical records, death certificates (recording the primary cause of death), individuals' relatives, and from the Health Information System. The interview and physical examination were performed at the same time of the renal evaluation and complemented as necessary in subsequent visits or in their medical records.

All patients gave their written informed consent to participate. The study protocol was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre.

Methods

At baseline, UAE was measured by random and 24-hour collections of sterile urine (2 to 3 samples) over a period of six months by radioimmunoassay (RIA) (DPC, Los Angeles), with an intra and interassay coefficient of variation (CV) of 2.8% and 2.3%, respectively. At follow-up, UAE was measured by immunoturbidimetry (IT) (Microalb; Ames-Bayer, Tarrytown, NY, USA) in random urine samples (29), and confirmed by two 24-hour collections of sterile urine over a six-month period (coefficient of correlation with RIA r = 0.99, interassay CV = 6.9%, and intra-assay CV = 3.8% for values around 20 μg/ml, and 1.9% for values around 150 μg/ml). Persistence of MA, MI, and NO at follow-up were defined by at least two random urine samples with values of ≥173 mg/l, 17-173 mg/l, and <17 mg/l, respectively (29), and confirmed by 24-h UAE values of <20 μg/min, 20-199 μg/min, and ≥200 μg/min, respectively (12). Two patients without 24-h collections had 24-h values calculated by a transforming equation (24-h UAE [μg/min] = 51.759 + 0.297 ×

random UAE [mg/l]), based on a linear correlation (r = 0.55, *P* = 0.0001) obtained from 112 microalbuminuric DM 2 patients with random urine samples and 24-h collections performed at the same cross-sectional evaluation (data not shown). Administration of angiotensin-converting enzyme (ACE) inhibitors was interrupted a week before UAE measurements. Proteinuria was evaluated by pyrogallol red colorimetric method (30).

Glomerular filtration rate (GFR) was estimated by the four-component Modification of Diet in Renal Disease (MDRD) equation: GFR (ml/min/1.73m^2) = 186 × (Serum Creatinine $_{mg/dl}$)$^{-1.154}$ × (Age $_{years}$)$^{-0.203}$ × (0.742 if female) × (1.210 if African-American) (31). GFR change (Δ) was calculated as final GFR - baseline GFR/number of years of follow-up. A significant GFR decline to values <60 ml/min/1.73m^2 at follow-up was defined according to NKF classification for stages of chronic kidney disease [CKD]: stage 3 = moderately decreased GFR [30-59 ml/min/1.73m^2]; stage 4 = severely decreased GFR [15-29 ml/min/1.73m^2]; and stage 5 = kidney failure [KF]) (31).

Fasting plasma glucose (FPG) was measured by the glucose oxidase UV enzymatic method. HbA_1 levels were measured at baseline by ion-exchange chromatography (normal range: 6.5-8.5 %, intra-assay CV 4.1-5.8%). At follow-up, HbA_{1c} was measured by high-performance liquid chromatography (HPLC) procedure (Merck-Hitachi L-9100 Glycated haemoglobin Analyzer; reference range: 3.2-4.5% **(**inter- and intra-assay CV= 2.4% and 0.5%, respectively). Baseline HbA_1 levels by ion-exchange chromatography (y) were converted to HbA_{1c} levels by HPLC (x) using the formula y = 1.09x + 1.95 (32). Urea, cholesterol, and triglycerides were measured by enzymatic methods. LDL cholesterol was calculated using the Friedewald formula. Creatinine was measured by the Jaffé reaction.

Statistical analysis

Results are expressed as mean ± SD, median (range) or number of cases and percentage. Incidence was expressed as the number of subjects who developed persistent

MA or NO per 1,000 person-years at risk, and as cumulative rate. One sample *t* test was done to analyze if changes in GFR were statistically significant. ANOVA and Kruskal-Wallis methods were employed to compare groups. A Cox proportional-hazard multiple regression model (backward stepwise method) was used to examine predictors of DN progression and GFR decline. Variables were excluded from the model if their *P* value was >0.10. Results are expressed as relative risk (Hazard Ratio-HR) and 95% Confidence Interval (CI). The model included baseline variables that were found to be statistically significant in univariate analyses, as well as those implicated *a priori* as potential risk factors (even though not significant in the Cox univariate analysis). Kaplan-Meier curves were employed to estimate the probability of surviving without progression for DN or death according to the presence of a given risk factor. A *P*-value <0.05 (two-sided) was considered to be statistically significant. All data were analyzed using the Statistical Package the Social Sciences (SPSS 10.0–Professional Statistics^{TM}, SPSS Inc., Chicago, IL, USA).

RESULTS

Six patients were lost to follow-up (11.5%) (4 patients could not be located and 2 refused to participate). Their baseline data were not different from those of the 46 re-valuated patients in terms of gender, age, systolic and diastolic blood pressure, cholesterol, median triglycerides and UAE, estimated GFR, serum creatinine, diabetes duration, and fasting plasma glucose. However, HbA1c (9.3 ± 3.1 vs. 7.4 ± 1.8 %, $P = 0.028$) was higher in the lost patients, suggesting that they probably presented worse glucose control. Three (5.5%) patients died before renal status re-evaluation (from ischaemic heart disease). Their baseline data were not different from the survivors (data not shown). Therefore, 43 (83%) of

the microalbuminuric type 2 DM patients (Fig. 1) were examined regarding clinical course of DN.

Baseline risk factors for UAE elevation

Among the 43 microalbuminuric DM 2 patients followed for a mean period of 6.3 ± 2.5 years (median 6.1 years [2-11]), 14 (32%) patients progressed to MA (5 of them developed KF), with a cumulative incidence of progression of 52/1,000 persons-year. Among those who did not progress, 17 (40%) remained MI, and 12 (28%) regressed to NO, with a cumulative incidence of regression of 44/1,000 persons-year.

The baseline data of these patients are shown in Table 2**.** MA patients tended to be older than the other groups. They presented lower baseline GFR and higher serum creatinine, and proteinuria values.

Although baseline UAE levels were not statistically significant among the three groups (Table 2, $P = 0.13$), MA group presented higher levels when compared to MI + NO analyzed as one group ($P = 0.043$), as shown in Fig. 2. Among the 43 patients, 4 out of 21 (19%) with UAE <48 μg/min, and 10 out of 22 (45%) with UAE ≥48 μg/min progressed to MA (Relative risk 3.54 [0.9-14.0], $P = 0.10$). When considering the P_{75} level (UAE ≥110 μg/min, n = 11 patients), 7 (64%) progressed to MA, while 4 (36%) did not progress (3 persisted MI, and 1 regressed to NO, $P = 0.022$), giving a relative risk for progression of 2.91 (1.32-6.43).

A Cox multivariate analysis was performed with DN as the dependent variable (Table 3), and the baseline variables that appeared as risk factors were UAE above the median (≥48 μg/min) and diabetic retinopathy, while age and lower GFR values were excluded from the model.

Kaplan-Meier curves confirmed the predictive value of median 24-h UAE (log rank, *P* = 0.0022) (Fig. 3), and of diabetic retinopathy (DR) (log rank, *P* = 0.0394) (Fig 4) in the progression to MA.

In the 37 patients with available proteinuria, their values were higher among the progressors. Baseline values above the median (176 mg/24 h) were related to a greater chance (6.9 fold) of progression to MA (Cox univariate analysis, *P* = 0.0139). A similar value (Relative risk 6.14 [1.1-34.2]) was detected by χ^2 analysis, where progressors presented more frequently proteinuria above 176 mg/24 h in comparison to the non-progressors (82% [MA] vs. 42% [MI + NO groups], *P* = 0.036), with a sensitivity of 82% to identify the patients with the greater chance to progress. None of the patients with baseline proteinuria <125 mg/24 h progressed to MA (Relative risk 0.65 [0.49-0.87], *P* =0.036).

There were no differences regarding the presence of pre-existing hypertension in the MA, MI and NO groups (79%, 65%, and 86%, *P* = 0.48), as well as in the use of antihypertensive treatment (73%, 89%, and 90%, respectively, *P* = 0.49), or ACE inhibitors (25%, 25%, and 50%, respectively, *P* = 0.24).

Regarding chronic complications in MA, MI and NO groups, respectively, the proportion of diabetic retinopathy was 71%, 53%, and 33% (*P* = 0.15); coronary heart disease was 14%, 12%, and 42% (*P* = 0.11); distal sensory neuropathy was 57%, 35%, and 42% (*P* = 0.47); and peripheral vascular disease was 50%, 38%, and 50% (*P* = 0.73).

Only two patients did not fit the diagnosis of metabolic syndrome, meaning that they had only diabetes (mandatory factor) and microalbuminuria (one criteria), without the other second criteria (hypertension, dyslipidemia or obesity).

Baseline risk factors for GFR decline

Regarding renal function evaluation, baseline GFR values (Table 2) were lower in MA in comparison to the MI and NO groups (64 ± 21 vs. 83 ± 21 and 81 ± 17 ml/min/1.73m^2,

respectively, P = 0.027), although the proportion of patients with baseline GFR levels <60 ml/min/1.73m^2 were not different among groups (P = 0.13). Serum creatinine was also higher in patients who later developed overt DN (P = 0.037). Although no significant GFR changes (ml/min/year) were observed in any of the groups (MA: -2.15 ± 4.34, P = 0.087; MI: +0.33 ± 3.87, P = 0.73; NO: +2.11 ± 3.78, P = 0.079), those patients who developed GFR values <60 ml/min/1.73 m^2 at follow-up (n = 12 [28%], 10 from MA and 2 from MI groups) presented a significative lower baseline GFR values than those who did not (55 ± 17 vs. 85 ± 17 ml/min/1.73 m^2, P <0.0001). They also had higher baseline UAE, proteinuria, creatinine, and systolic blood pressure values. Additionally, they were older and all of them presented hypertension. Baseline clinical and laboratory characteristics of these distinct groups of patients are presented in Table 4. A Cox multivariate analysis (Hazard Ratio [Confidence interval]) identified baseline higher GFR (0.96[0.94-0.99], P = 0.005) as a protector factor against GFR values <60 ml/min/1.73 m^2 at follow-up, while higher baseline median UAE values ≥48 μg/min (4.47[0.85-23.46]), P = 0.077), and age (P = 0.307), were not significant in the model.

Characteristics of microalbuminuric patients at follow-up

At the end of the study, there were no differences among the groups concerning the presence of hypertension, systolic and diastolic blood pressure, the use of antihypertensive treatment, or the number of patients receiving ACE inhibitors or β-blockers (Table 5).

The proportion of patients taking insulin was the same among MA, MI and NO groups (64%, 65%, and 60%, respectively, P = 0.99), and there was an increased number of insulin users at follow-up among MA (P = 0.001) and MI (P = 0.034) groups in comparison to the baseline. There were no differences among MA, MI, and NO patients, respectively, in terms of BMI (27.8 ± 4.1, 29.3 ± 4.2, and 28.4 ± 3.0 kg/m^2, P = 0.53), current smoking (14 %,

12%, and 0%, *P* = 0.93), FPG values (174 ± 84, 206 ± 77, and 141 ± 60 mg/dl, *P* = 0.08), HbA_{1c} (7.2% ± 1.9, 6.7% ± 1.8, and 6.5% ± 2.7, *P* = 0.74), total cholesterol (230 ± 53, 201 ± 60, and 183 ± 46 mg/dl, *P* = 0.10), HDL cholesterol (50 ± 14, 44 ± 12, and 41 ± 9 mg/dl, *P* = 0.16), and triglyceride levels (144 mg/dl [56-361], 122 mg/dl [63-255], 120 mg/dl [86-417], *P* = 0.85). However, FPG levels were lower at follow-up in comparison to baseline levels in the NO group (*P* = 0.01).

When we analyzed the evolution of other chronic complications (Table 5), we observed that, at the end of the study, the prevalence of diabetic retinopathy, coronary artery disease, and peripheral vascular disease were not different among groups, but NO patients presented a higher prevalence of distal sensory neuropathy than the others.

Seven out of 14 (50%) MA, 4 out of 17 (24%) MI, and 3 out of 12 (25%) NO patients died after the last follow-up evaluation, and these proportions were not different (*P* = 0.24). Cardiovascular disease was the cause in 4 (29%), 1 (6%), and 3 (25%) patients, respectively (*P* = 0.28). Kaplan-Meier survival curve demonstrated that there was no difference in the mortality rate between progressors (MA) and non-progressors (MI + NO) (log rank P=0.059) (Fig 5).

CONCLUSIONS

In this prospective study of 43 microalbuminuric DM 2 patients followed for 6 ± 3 years, we found that 32% progressed to macroalbuminuria (MA), 40% remained microalbuminuric (MI), and 28% regressed to normoalbuminuria (NO). The cumulative incidence of progression to MA was of 52/1,000 persons-year, quite similar to another prospective Italian study, with a cumulative incidence of 53.6/1,000 persons-year (33). Twelve (28%) patients out of 43 progressed to CKD (GFR values <60 ml/min/1.73m^2), and

5 out of 43 (11.6%) progressed to KF requiring dialysis. These figures are comparable to other studies (34,35).

Although microalbuminuria is established as risk factor for DN and death, little attention has been paid to the rate of regression to NO without specific intervention approach in microalbuminuric DM 2 patients (1,2). Schmitz et al followed 52 microalbuminuric patients for 6 years and found that only 9% of them regressed to NO (1). John et al followed 61 microalbuminuric DM 2 patients for 5 years, and 15% of them regressed to NO (2). However, these studies were conducted about 10 years ago, when probably less intensive measures were applied to these high-risk patients. We demonstrated a regression of 28% with a cumulative incidence of 44/1,000 persons-year. A recent intervention study showed that 30% of DM 2 patients followed for 7.8 years reached remission to NO during multifactorial treatment (36). Similar remission rates were achieved in intervention trials with irbesartan (7) and enalapril (37), but, although the remission rates with those drugs were greater, the remission rates even in the placebo group (using antihypertensive drugs other than renin-angiotensin system blockers) could not be neglected, as it was 21% in comparison to 34% in the 300-mg irbesartan group ($P = 0.006$) (7), and 15% in comparison to 24% in the enalapril group ($P < 0.05$) (37).

The main risk factors for progression to MA in our study were baseline UAE above the median (≥48 μg/min), and diabetic retinopathy (DR). Baseline UAE also influenced the rate of progression of albuminuria in microalbuminuric DM 2 patients from India (2), and Denmark (36), and also in Pima Indians (3). Conversely, another study could not document the same findings, probably because of the shorter follow-up period (6). Higher UAE values, even in the normal range, have also been documented as a risk factor for the development of incipient diabetic nephropathy in normoalbuminuric patients (1,38), as well as higher

albuminuria (3) and proteinuria (39) values for worsening overt nephropathy in prospective studies. Accordingly, higher proteinuria levels were also a risk factor for progression in our patients.

DR was a risk factor for progression in our microalbuminuric patients, in accordance with other prospective study (36). We have previously shown that DR and high normal UAE were risk factors for the development of incipient diabetic nephropathy in a cohort of normoalbuminuric DM 2 patients (38). DR was also a risk for worsening overt nephropathy (40). Taking this information into account, DR has been claimed as a risk for DN progression independently of DN stage.

Although ADA (12) does not emphasize the performance of GFR measurements as a routine renal evaluation, there is growing evidence that it is necessary to evaluate GFR (31,41). In our cohort, baseline GFR values were lower in those who progressed, and normal baseline GFR values protected patients against GFR values <60 ml/min/1.73m^2 at follow-up. Although these patients presented higher baseline UAE values, we could not confirm the role of UAE as a predictor of lower GFR at follow-up as previously shown in Pima Indians (3), and in Brazilian patients (42). A decreasing GFR has been observed in patients who progress to MA (3,36). Even microalbuminuric (21), and also normoalbuminuric (41) patients may already present a significant GFR decline. In our study, however, we could not detect GFR changes in any group along the time. Most of the previous studies evaluated GFR by a direct measurement, usually ^{51}Cr-EDTA (21,36,42) or iothalamate (3), while our GFR was evaluated by estimation, with the four-component MDRD equation (31). However, the MDRD equation has not yet been well validated in DM patients, and it may indeed underestimate GFR decline in this group of patients. In CKD Chinese patients, it was found that the lower the GFR measured by ^{99m}Tc-DTPA, the greater the overestimation by

MDRD equation, mainly at worse CKD stages (43). In a previous report, we have demonstrated that GFR decline (measured by ^{51}Cr-EDTA method) in microalbuminuric patients was 4.7 ml/min/year (21), similar to the findings of 3.7 ml/min/year of another study employing the same method (36). Therefore, we conclude that in our study, MDRD equation was not able to identify changes in GFR levels, maybe due to intrinsic limitations of the method. This being so, the usefulness of MDRD equation in DM 2 patients remains to be investigated.

We did not find any association between hypertension and progression to overt nephropathy, in accordance with other studies (2,6,33). However, this finding should be considered with caution because most of the microalbuminuric patients were hypertensive at baseline. Additionally, although all patients were under antihypertensive treatment at the end of the study, the regression group presented lower diastolic blood pressure and better glucose control at follow-up in comparison to baseline values, which could have possibly contributed to the observed regression. We could not find any differences in the proportion of ACE inhibitors users among the groups at follow-up, in accordance with a study conducted in type 1 diabetes, where they did not observe a relation between ACE inhibitors and regression to NO (44). Without any question, hypertension treatment has been shown to offer a huge benefit to diabetic patients, leading to microalbuminuria regression (7,36), or UAE remission (8,9).

The mortality rate was slightly higher in the new onset MA group, although not significantly, than in those who remained MI or regressed to NO, probably because of the small number of outcomes in our cohort. As a matter of fact, UKPDS study (4) has definitely demonstrated greater mortality rates as DN progressed to advanced stages, and cardiovascular disease was always the leading cause of death (4,18). In our study, all deaths

were cardiovascular in those who regressed to NO, and their mortality rate was not different from a population cohort of normoalbuminuric patients followed for 8 years who remained NO for the whole period (3 out of 12 [25%] vs. 10 out of 117 [10%], $P = 0.10$) (45).

One possible drawback of our study is the small number of patients evaluated, and the lack of GFR measurements by a more precise method instead of an estimated one.

In conclusion, in this observational study of microalbuminuric DM 2 patients we found that 32% progressed to MA, 40% remained MI and another 28% regressed to NO. The major baseline risk factors related to progression were higher levels of baseline UAE, and diabetic retinopathy. Baseline GFR values were already lower in new onset MA patients, and higher baseline GFR values protected against GFR values <60 ml/min/1.73m^2 at follow-up.

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Table 1. Baseline clinical and laboratory characteristics of the 52 microalbuminuric DM 2 patients.

Clinical Characteristics		Laboratory Characteristics	
Male sex (%)	31 (60%)	**Fasting plasma glucose (mg/dl)**	191 ± 83
Ethnicity (white/mixed or black)	43/9	**HbA1c (%)**	7.6 ± 2.0
Age (years)	59 ± 9	**Cholesterol (mg/dl)**	207 ± 46
Duration of diabetes (years)	12 ± 7	**HDL (mg/dl) (n=48)**	46 ± 14
Body mass index (kg/m^2)	28.6 ± 4.2	**Triglycerides (mg/dl)**	134 (35-423)
Smoking (N/ Ex + S)	32/10/10	**Urinary urea (g/24h) (n=41)**	26.1 ± 11.3
Type of treatment (D/OA/I)	7/27/18	**GFR (ml/min/1.73m^2)**	78 ± 22
Arterial hypertension	37 (71%)	**GFR < 60 ml/min/1.73m^2**	8 (16%)
SBP (mmHg)	153 ± 25	**Serum Creatinine (mg/dl)**	1.04 ± 0.29
DBP (mmHg)	90 ± 15	**24-h UAE (μg/min)**	47.8 (20-193)
Diabetic retinopathy	30 (58%)	**Random UAE (mg/l)**	51 (2.5-504.3)
Coronary heart disease	12 (23%)	**Proteinuria (mg/24 h) (n=45)**	176 (15-1596)

Mean ± SD (range), median (range), number of cases (percentage). N= never, Ex = ex-smoker, S= current smoker, D = diet, OA= oral agents, I = insulin. SBP = systolic blood pressure, DBP = diastolic blood pressure, GFR = glomerular filtration rate (estimated by MDRD equation), UAE = urinary albumin excretion.

Table 2. Baseline clinical and laboratory characteristics of microalbuminuric DM 2 patients according to clinical course of DN at follow-up.

Baseline Characteristics	Progressed to **MACRO** (n= 14)	Remained **MICRO** (n=17)	Regressed to **NORMO** (n=12)	*P*
Male sex	7 (50%)	9 (53%)	8 (67%)	0.66
Ethnicity (white/mixed or black)	11/3	13/4	11/1	0.55
Age (years)	64 ± 6	58 ± 9	57 ± 9	0.054
Duration of diabetes (years)	13 ± 7	13 ± 8	11 ± 6	0.65
Body mass index (kg/m^2)	28 ± 4	30 ± 4	27 ± 4	0.49
Smoking (N/ Ex + S)	9/1/4	12/2/3	8/3/1	0.93
Type of treatment (D/OA/I)	1/6/7	5/8/4	1/6/5	0.33
Arterial hypertension (%)	11 (79%)	11 (65%)	10 (86%)	0.48
SBP (mmHg)	155 ± 24	148 ± 27	160 ± 31	0.49
DBP (mmHg)	88 ± 14	88 ± 12	97 ± 22	0.22
Fasting plasma glucose (mg/dl)	177 ± 87	208 ± 102	178 ± 35	0.50
HbA1$_c$ (%)	7.3 ± 1.44	7.34 ± 1.77	7.23 ± 1.94	0.99
Cholesterol (mg/dl)	211 ± 45	208 ± 49	184 ± 40	0.27
HDL (mg/dl)	53 ± 16	46 ± 13	40 ± 14	0.11
Triglycerides (mg/dl)	129 (51-293)	132 (35-348)	143 (70-386)	0.81
GFR (ml/min/1.73m^2)	64 ± 21*	83 ± 21	81 ± 17	0.027
GFR <60 ml/min/1.73m^2	5 (36%)	2 (12%)	1 (7%)	0.13
Serum creatinine (mg/dl)	1.21 ± 0.36*	0.96 ± 0.25	0.98 ± 0.19	0.037
UAE (μg/min)	104.7 (27-193)	40.5 (20-163)	42.7 (22-171)	0.12
Proteinuria (mg/24 h)	342 (144-893)*	140 (49-715)	130 (29-1596)	0.022
Follow-up (years)	5.7 ± 2.5	6.7 ± 2.6	6.5 ± 2.3	0.51

Mean ± SD (range), median (range), number of cases (percentage). N= never, Ex = ex-smoker, S= current smoker, D = diet, OA= oral agents, I = insulin. SBP = systolic blood pressure, DBP = diastolic blood pressure, GFR = glomerular filtration rate (estimated by MDRD equation), UAE = urinary albumin excretion. * *P* <0.05 to MA regarding the other 2 groups. To proteinuria: n=11, 15, and 11, respectively.

Table 3: Baseline risk factors for diabetic nephropathy progression in 43 microalbuminuric type 2 diabetic patients.

Independent Variables	Relative Risk-HR	95% CI	*P*
Median UAE ≥48 μg/min	5.16	1.44-18.58	0.012
Retinopathy	5.39	1.24-23.43	0.025

Cox multivariate analyses, backward stepwise method. Adjusted for age ($P = 0.07$), and estimated GFR (MDRD) ml/min/1.73 m^2 ($P = 0.405$).

Table 4. Baseline clinical and laboratory characteristics of DM 2 patients with and without GFR values <60 ml/min/1.73 m^2 at follow-up.

Baseline Characteristics	GFR <60 ml/min/1.73 m^2 (n=12)	GFR ≥60 ml/min/1.73 m^2 (n= 31)	*P*
Male sex	6 (50%)	18 (58%)	0.74
Ethnicity (white/non-white)	8/4	27/4	0.19
Age (years)	64 ± 6	58 ± 9	0.037
Duration of diabetes (years)	12.8 ± 6.0	12.5 ± 7.3	0.89
Body mass index (kg/m^2)	29.1 ± 2.5	28.6 ± 4.8	0.65
Arterial hypertension (%)	12 (100%)	20 (65%)	0.019
SBP (mmHg)	165 ± 15	149 ± 29	0.028
DBP (mmHg)	92 ± 13	90 ± 17	0.75
Fasting plasma glucose (mg/dl)	152 ± 77	204 ± 82	0.067
$HbA1_c$ (%)	6.8 ± 1.6	7.5 ± 1.7	0.27
Cholesterol (mg/dl)	202 ± 48	202 ± 46	0.99
HDL (mg/dl)	48 ± 16	46 ± 15	0.74
Triglycerides (mg/dl)	132 (51-293)	135 (35-386)	0.97
GFR (ml/min/1.73m^2)	55 ± 17	85 ± 17	0.0001
Serum Creatinine (mg/dl)	1.36 ± 0.30	0.92 ± 0.18	0.0001
UAE (μg/min)	121.4 (30-193)	42.7 (20-170)	0.001
Median UAE (≥48 μg/min)	10 (83%)	12 (39%)	0.016
Proteinuria (mg/24 h) (n=27 vs. 10)	441 (140-893)	144 (29-1596)	0.001

Mean ± SD (range), median (range), number of cases (percentage). SBP = systolic blood pressure, DBP = diastolic blood pressure, GFR = glomerular filtration rate (estimated by MDRD equation), UAE = urinary albumin excretion.

Table 5: Final characteristics of DM 2 patients according to DN evolution in 6 years.

Final Characteristics	Progressed to **Macro** (n= 14)	Remained **Micro** (n=17)	Regressed to **Normo** (n=12)	*P* *
Arterial hypertension (%)	12 (86%) †	14 (82%) †	11 (92%)	0.775
SBP (mmHg)	154 ± 20	151 ± 21	152 ± 19	0.883
DBP (mmHg)	83 ± 7	86 ± 12	85 ± 15 †	0.689
With antihypertensive treatment	100 %	100 %	100 %	1.00
ACE inhibitor	7 (58%)	11(79%)	9 (82%)	0.375
β-blocker	4 (33%)	6 (43%)	6 (55%)	0.591
GFR (ml/min/1.73m^2)	56 ± 24 ‡	86 ± 21	88 ± 11	0.0001
GFR <60 ml/min/1.73m^2	10 (71%) ‡	2 (12%)	0 (0%)	0.0001
UAE (μg/min)	1104 (250-3056) ‡	77.6 (28-191)	6.4 (3.6-15.0)	0.0001
Proteinuria (mg/24 h)	2083 (158-13000) ‡	304 (107-564)	158 (75-455)	0.0001
Diabetic Retinopathy	12 (86%)	14 (82%)	9 (82%)	0.957
Coronary artery disease	10 (71%)	8 (47%)	9 (75%)	0.222
Distal sensory neuropathy	9 (64%)	8 (47%) †	11 (92%) ¶	0.046
Peripheral vascular disease	11 (79%)	6 (34%)	8 (67%)	0.061

Mean ± SD (range), median (range), number of cases (percentage). SBP = systolic blood pressure, DBP = diastolic blood pressure, ACE = angiotensin-converting enzyme, GFR = glomerular filtration rate, estimated by MDRD equation, UAE = urinary albumin excretion. To NO, MI and MA, respectively: UAE n = 12, 17, and 13; to proteinuria n = 8, 13, and 11; and to peripheral vascular disease n = 12, 16, and 14. * *P* values to comparisons between groups regarding to final data. † *P* values to comparisons between baseline and final data into the same group < 0.05. ‡ Macro vs. Normo and Micro, *P* = 0.001. ¶ Normo vs. Micro and Macro, *P* = 0.001

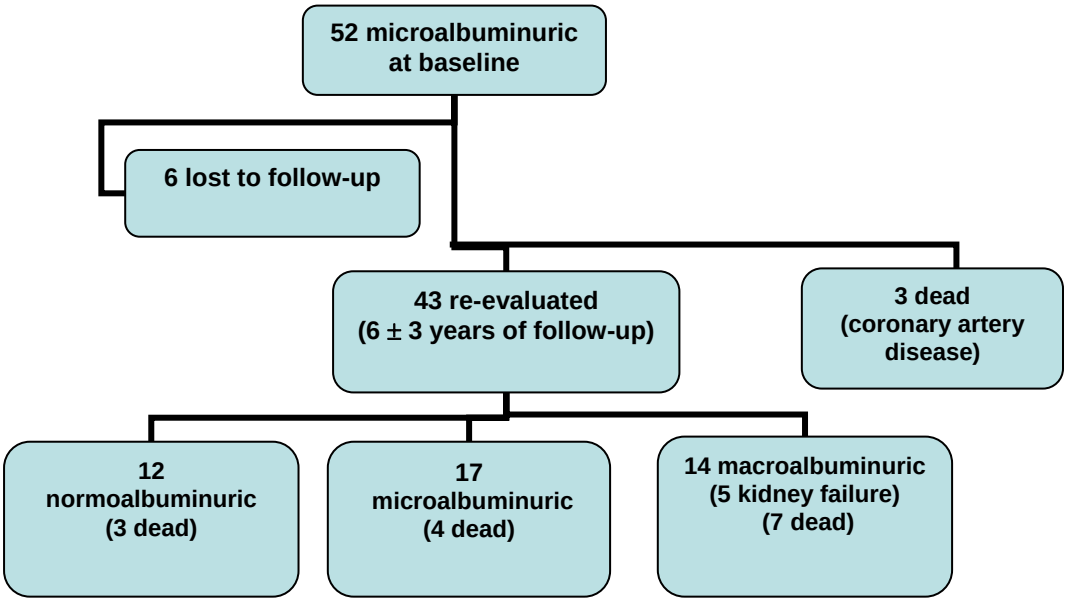

Figure 1. Flow chart of the microalbuminuric DM 2 patients.

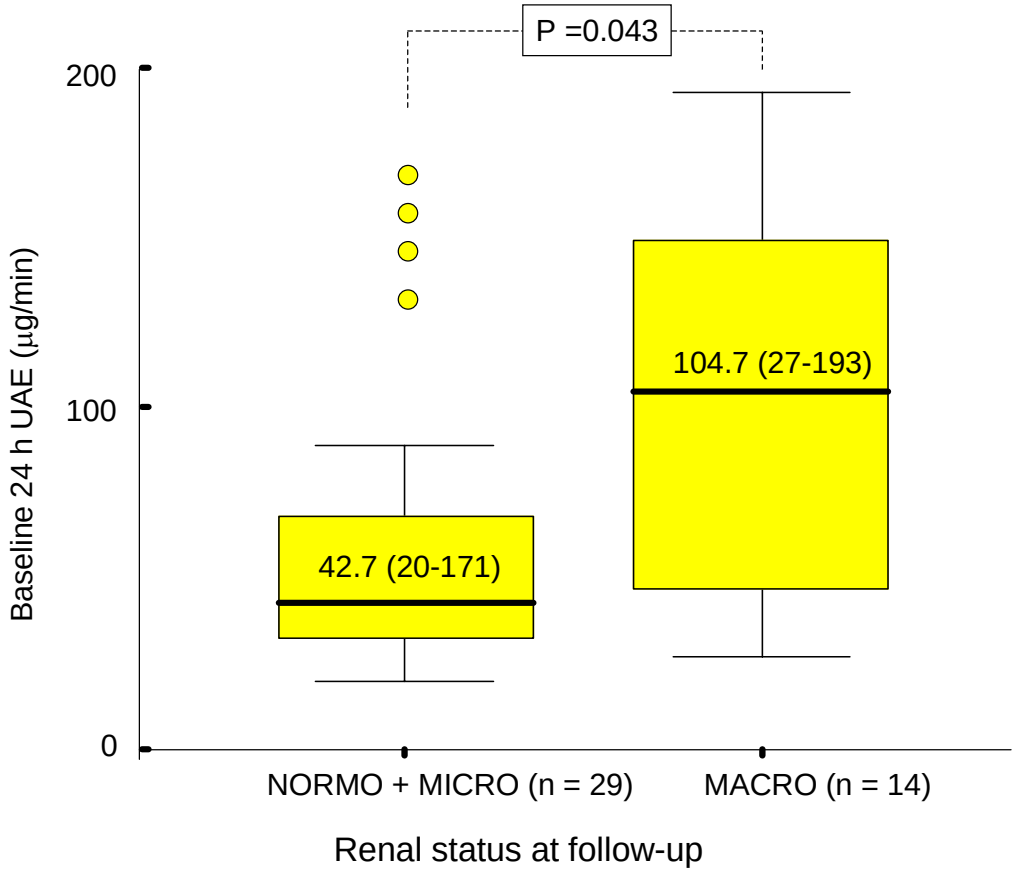

Figure 2. Baseline UAE (µg/min) values according to follow-up renal status: progressor group (MA, n=14) vs. non-progressor group (MI + NO, n = 29). Outliers are indicated in yellow circles.

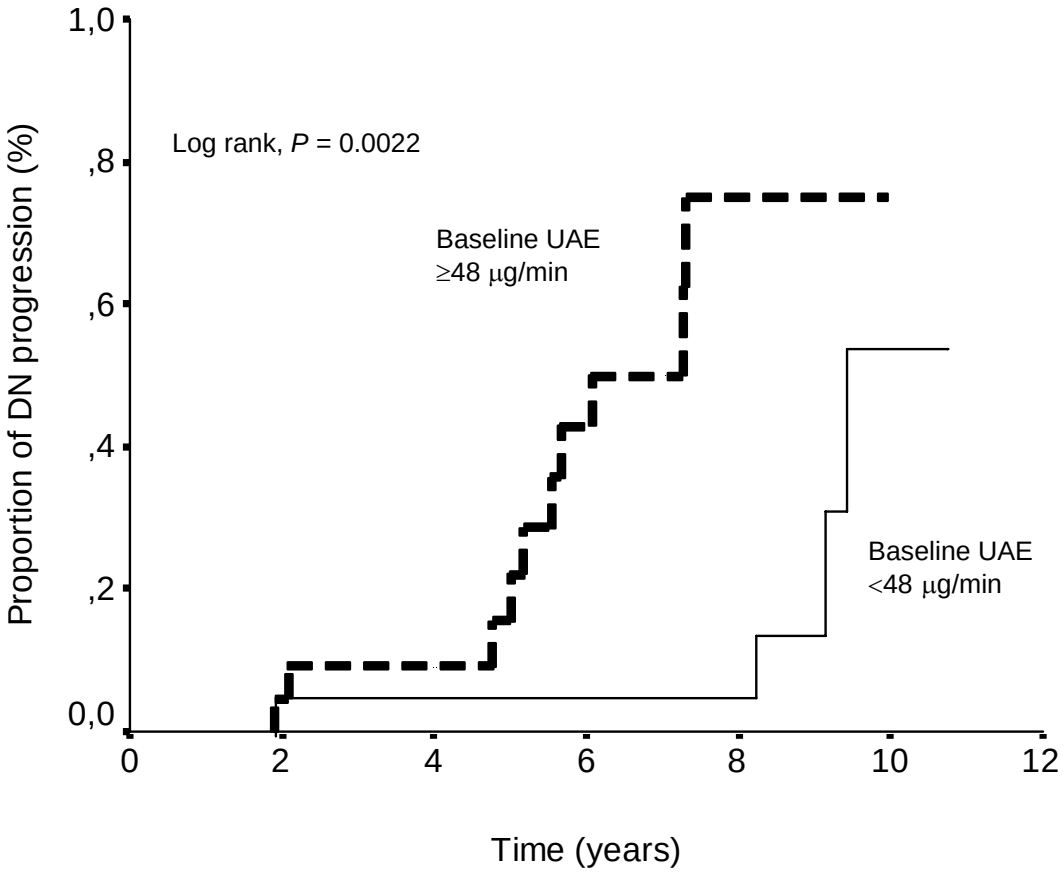

Figure 3. Kaplan-Meier estimates of DN progression according to baseline median UAE (≥48 μg/min), n = 43.

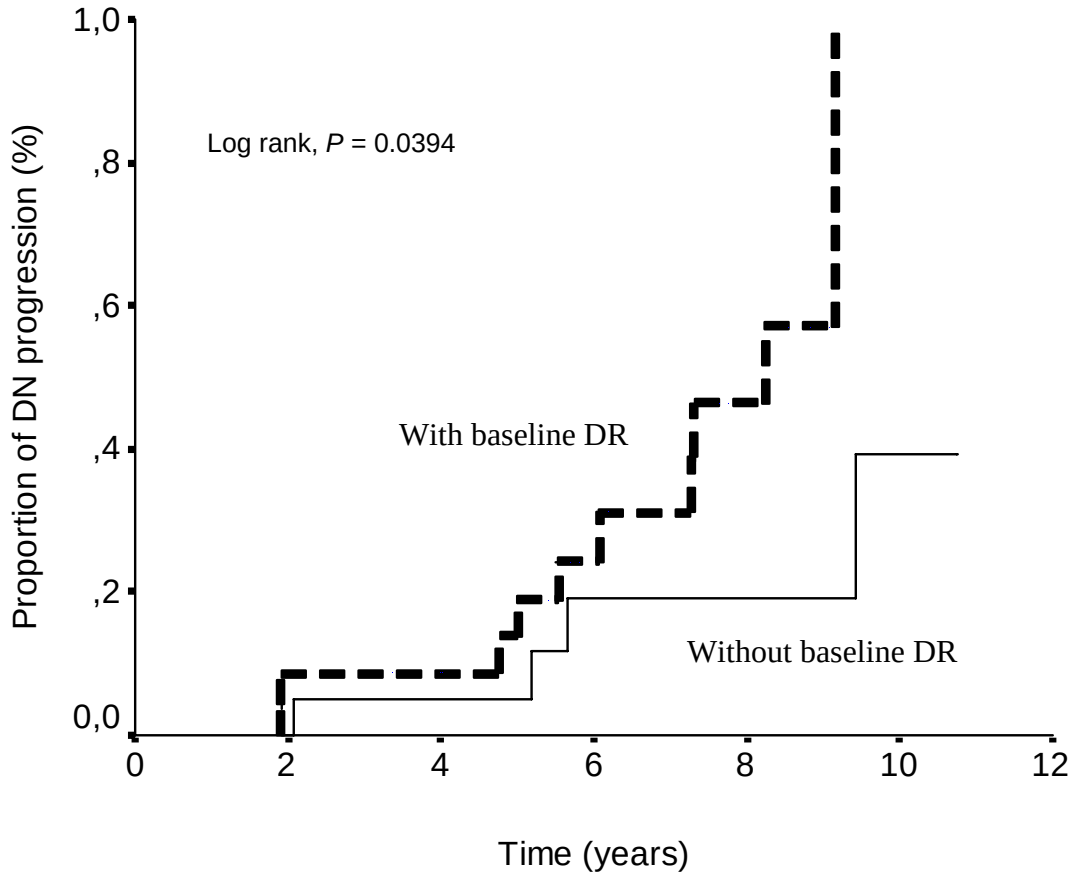

Figure 4. Kaplan-Meier estimates of DN progression according to the presence of diabetic retinopathy (DR) at baseline, n=43.

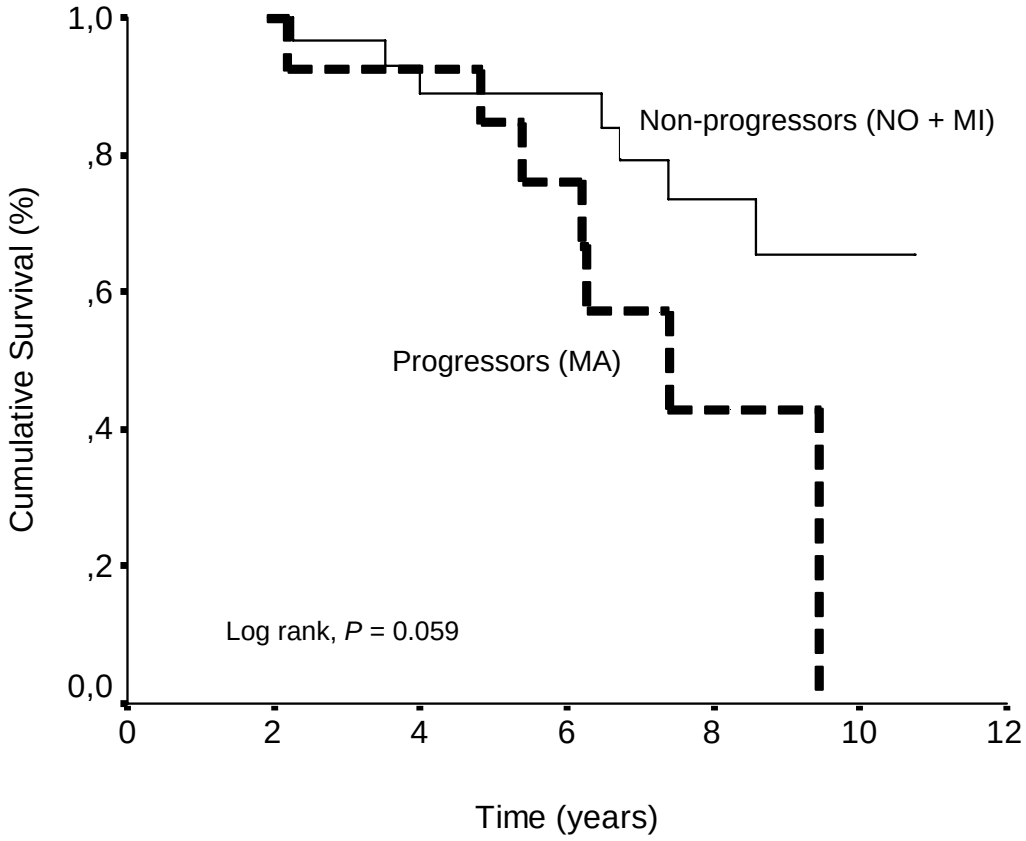

Figure 5. Kaplan-Meier estimates of cumulative survival regarding DN status at follow-up (n=43).

COMENTÁRIOS FINAIS

A nefropatia diabética (ND) é considerada uma entidade médica relevante face ao prognóstico desfavorável que acarreta, especialmente em relação ao aumento do risco cardiovascular. No presente trabalho, observou-se que níveis mais elevados de excreção urinária de albumina (EUA), embora dentro da faixa convencional de normalidade, foram identificados como fator de risco marcante para a progressão e para a instalação da doença, tanto nos pacientes com microalbuminúria, como naqueles com normoalbuminúria, respectivamente, caracterizando um *continuum* de risco conforme aumentam os valores da EUA. Além disso, esses níveis mais elevados de EUA também aumentaram o risco de mortalidade.

Portanto, novos pontos de corte nos valores de albuminúria que definem risco de progressão renal deverão ser instituídos, substituindo os valores atuais de consenso da Associação Americana de Diabetes. Assim, a fase de microalbuminúria, definida tradicionalmente pela presença de valores de EUA de 20-200 μg/min e considerada como fase preditiva de progressão renal e de risco cardiovascular, deverá ser redefinida com adoção de valores mais baixos permitindo a identificação mais precoce dos casos de risco.

Por outro lado, demonstramos também, em concordância com dados da literatura, que não apenas os valores de albuminúria seriam indicativos do risco de progressão da nefropatia, como o seriam também a ocorrência de taxas de filtração glomerular (TFG) relativamente mais baixas, indicando possivelmente um subtipo ou uma apresentação alternativa do quadro de ND. Portanto, é reforçada a indicação formal de pesquisar a presença de ND tanto através da medida da EUA, como também através da avaliação da TFG, cuja análise é atualmente recomendada através de equação sugerida pela *National Kidney Foundation*, disponível *online*.

Adicionalmente, também demonstramos que cerca de 30% dos pacientes microalbuminúricos podem apresentar regressão para normoalbuminúria, sendo essa cifra semelhante à encontrada nos ensaios clínicos que avaliaram especificamente o efeito de drogas antiproteinúricas. Dessa forma, nossos achados reforçam a hipótese de que a presença de microalbuminúria vem sendo considerada não como fase em si do curso da nefropatia, mas sim como um fator dentro do contexto da síndrome metabólica. Esse conjunto de achados, incluindo a alteração renal mais a dislipidemia e a hipertensão arterial, compartilhariam um *background* comum, de predisposição genética, com conseqüentes alterações metabólicas, decorrentes de resistência insulínica.

O entendimento completo dos mecanismos de instalação e do curso da nefropatia diabética são caminho para a instituição de medidas mais eficazes de forma cada vez mais precoce.