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Transplante duplo de rim e pâncreas: evolução clínica e metabólica e significado da expressão de autoimunidade anti-ilhota pancreática

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Abreviaturas:

AGE – Advanced Glycosilation End-Products

Anti-GAD – Anti-Glutamic Acid Decarboxylase antibodies

Anti-ZnT8 - Anti-cation efflux zinc transporter antibodies

AVC – Acidente Vascular Cerebral

CHP – Centro Hospitalar do Porto

CML – Carboxi-metil-lisina

CMV – Citomegalovírus

CV - Cardiovascular

DCCT - Diabetes Control and Complications Trial

DM1 – Diabetes Mellitus tipo 1

DM2 - Diabetes Mellitus tipo 2

DMO – Densitometria óssea

DP – Diálise Peritoneal

DSA – Donor-Specific (HLA) Antibodies

EAM – Enfarte Agudo do Miocárdio

EBV – Vírus de Epstein-Barr

EDIC – Epidemiology of Diabetes Interventions and Complications

EQ-5D – EuroQoL-5 Dimensions survey

EUA – Estados Unidos da América

FA – Fosfatase Alcalina

GAD – Glutamic Acid Decarboxylase

GIQLI - Gastrointestinal Quality of Life Index

Hb – Hemoglobina (sérica)

HbA1c – Hemoglobina glicosilada

HD - Hemodiálise

HLA – Human Leucocyte Antigens

HSA – Hospital de Santo António, CHP

HTA – Hipertensão Arterial

IAA – Insulin Autoantibodies

IA-2 – insulinoma associated protein 2

ICA – Islet Cell Autoantibodies

IGRP - islet-specific glucose-6-phosphatase subunit-related protein

IL - interleucina

IMC – Índice de Massa Corporal

IPTR – International Pancreas Transplant Registry

KDIGO – Kidney Disease Improving Global Outcomes

LYFT - Life Years From Transplant

MFI – Mean Flourescence Intensity

MHC – Major Histocompatibility Complex

PAK – Pancreas after kidney (transplantation)

PDRI – Pancreas-Donor Risk Index

Pept-C – Peptídeo-C

PKC – Protein-Kinase C

PRA – Panel Reactive Antigens

PTA – Pancreas transplantation alone

PTHi – Hormona paratiroideia (intacta)

PTPN22 – Protein tyrosine phosphatase non-receptor 22

QoL – Quality of Life

SPK – Simultaneous pancreas-kidney (transplantation)

SRTR – Scientific Registry of Transplant Recipients

RAGE – Recetores dos AGE

TR – Transplante Renal (isolado)

TRP – Transplante de Rim-Pâncreas

UNOS – United Network for Organ Sharing

ZnT8 – Cation efflux zinc transporter

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I. Introdução

I.1. A Diabetes Mellitus tipo 1

A prevalência da Diabetes Mellitus tipo 1 (DM1) no escalão dos 0 aos 19 anos em Portugal é de 0,15%, registando-se cerca de 3200 crianças e jovens portugueses que padecem da doença. A incidência da doença no último ano, neste mesmo escalão etário, foi de 16,8/100.000 habitantes. Esta incidência correspondeu a 304 novos casos - dados do Observatório Nacional da Diabetes 2013([1]).

A evolução da diabetes e das lesões nos órgão-alvo (cegueira, doença renal crónica, neuropatia, doença cardiovascular) resulta num significativo número de anos potenciais de vida perdidos e numa importante redução na qualidade de vida. A diabetes constitui uma das principais causas de morte e de consumo de recursos económicos: custos diretos com a saúde; custos associados à abstenção laboral por doença; custos associados à baixa empregabilidade dos indivíduos com esta enfermidade, resultando na perda de produtividade; e custos sociais e familiares dificilmente contabilizáveis. O percurso e a rentabilidade escolar - e consequentemente a valorização pessoal - dos indivíduos com DM1, ficam frequentemente prejudicados.

A DM1 é uma das causas mais frequentes de doença crónica na infância e adolescência e resulta da deficiência absoluta da produção de insulina, em consequência da destruição das células beta pancreáticas. Apesar do aumento nos últimos anos da Diabetes Mellitus tipo 2 (DM2) nos jovens, associada à obesidade e a hábitos de vida menos saudáveis, tem havido também uma tendência para o aumento do número de casos de DM1([2]). Este aumento tem sido particularmente notório em crianças mais jovens, abaixo dos 5 anos([2]).

A distinção entre DM1 e DM2 nos jovens nem sempre é fácil. A DM1 carateriza-se por um início abrupto, frequentemente configurando um quadro de cetoacidose, com défice marcado de insulina e Peptídeo-C (Pept-C) circulantes (ou inapropriadamente baixos para o grau de glicemia), em jovens não obesos. É de natureza autoimune na grande maioria dos casos([3]): os doentes com DM1 apresentam usualmente autoanticorpos pancreáticos detetáveis à data do diagnóstico – diabetes tipo 1-A. Numa baixa percentagem de casos, pode não ser identificada esta autoimunidade e designa-se como diabetes tipo 1-B ou idiopática.

I.1.1. Fatores genéticos

O desenvolvimento da DM1 é de influência poligénica e são múltiplos os genes potencialmente implicados. A associação de maior risco ocorre com genes HLA, em particular com os alelos DR3 (DR3, DQB1*0201, também designado DR3-DQ2) ou DR4 (DR4, DQB1*0302, também designado DR4-DQ8). Mais de 90% dos DM1 são portadores destes alelos([4]); mas isto não faz com que todos os portadores destes 2 alelos tenham DM1. Isto é, esta não é uma condição suficiente para o desenvolvimento da doença. Irmãos gémeos monozigóticos de um doente DM1 não desenvolvem necessariamente a doença, que contudo pode afetar cerca de 50% dos casos([5]). A maior suscetibilidade à doença ocorre nos portadores heterozigóticos DR3/DR4: 80% dos portadores deste genótipo que tenham 1 irmão com DM1 desenvolverão a doença([6]). A prevalência deste genótipo de maior risco (heterozigotia DR3/DR4) entre doentes com DM1 é de cerca de 30%([4]). Contudo, alguns subtipos do alelo HLA-DR4, nomeadamente DR4,DQB1*0602, conferem proteção contra o desenvolvimento da DM1, mesmo na presença de DR3-DQ2 e/ou DR4-DQ8([7]). Assim, as distintas combinações de genes HLA parecem ter um papel preponderante, favorecedor ou protetor, do aparecimento da DM1.

Para além dos genes HLA, reconhecidamente com efeito major, existe também um papel relevante de outros genes fora do MHC na suscetibilidade à DM1. Discute-se se o seu efeito potencial fica condicionado pela coexistência dos genes HLA. De entre os marcadores genéticos com impacto relevante, destacam-se os polimorfismos do gene da proinsulina/insulina([8]) e o gene PTPN22([9]). Este gene PTPN22 (*protein tyrosine phosphatase non-receptor22*) codifica para a tirosina fosfatase linfócito-específica, regulando a função dos linfócitos, e está também associado a outras doenças auto-imunes, como o lupus, artrite reumatóide e arterite de células gigantes, entre outras. Existem muitos outros genes (mais de 60) descritos como aumentando a suscetibilidade à DM1, contudo são considerados genes de menor impacto.

I.1.2. A autoimunidade na diabetes tipo 1

Está de facto comprovado que a DM1 é na grande maioria dos casos uma doença de etiologia autoimune([3,10,11]). Ao contrário dos marcadores genéticos, que estão presentes desde o nascimento e podem ser testados precocemente nos descendentes de famílias de alto risco, os marcadores imunológicos são detetados mais tarde, na

infância ou adolescência, quando começa o processo de destruição autoimune. Existe alguma controvérsia sobre o papel de fatores ambientais no despoletar da autoimunidade pancreática. Foram descritos casos associados a vírus Coxsackie-B([12]), entre outros. Um outro dado a favor desta hipótese ambiental foi o observado em indivíduos oriundos de zona de baixa incidência, que após mudança para zona de alta incidência da DM1 apresentaram um maior risco de desenvolver a doença.

Os marcadores imunológicos mais facilmente detetados, robustos e fiáveis([13]), são os anticorpos contra autoantigénios pancreáticos. Pesquisados sistematicamente em indivíduos de alto risco, podem ser detetados vários anos – por vezes décadas - antes da diabetes clinicamente declarada([13]), correspondendo ao período de destruição das células beta pancreáticas até que o tecido endócrino restante seja insuficiente e surja a hiperglicemia.

Além dos autoanticorpos pancreáticos, foi demonstrado em biópsias pancreáticas, à data do diagnóstico de DM1, um infiltrado linfocitário de células T envolvendo as células beta das ilhotas de Langerhans ([14]) e poupando o tecido pancreático exócrino. A biópsia pancreática, como procedimento invasivo que é e dada a sua dificuldade técnica, não é habitualmente realizada – exceto em investigação – para o diagnóstico da DM1. Normalmente são os marcadores serológicos, os autoanticorpos pancreáticos circulantes, facilmente exequíveis, os mais utilizados como teste auxiliar para a confirmação do diagnóstico de DM1. Dada a antecedência com que podem ser detetados, a sua monitorização facilita a identificação de doentes de risco para DM1 e pode antecipar a expressão clínica da doença[(15,16)]. Podem também ser usados como indicadores de evolução da destruição imunológica das células beta pancreáticas[(15)].

Os ICA (*islet cell autoantibodies*), descritos inicialmente em doentes com deficiência poliendócrina autoimune, foram posteriormente descritos em 85% dos doentes com DM1([3]). Um estudo sueco mostrou que a presença de ICA circulantes tinha uma sensibilidade, especificidade e valor preditivo positivo de 72%, 96% e 84% para o diagnóstico de DM1, respetivamente([17]). Assim, a sua monitorização facilita a identificação de indivíduos em risco para DM1([15]). Existem vários autoantigénios pancreáticos – e consequentemente autoanticorpos - identificados: os anti-GAD (*anti - Glutamic Acid Decarboxylase 65*) [(18,19)]; os anti-IA2 (*insulinoma-associated protein 2,* ou *anti-tyrosine phosphatase antibodies*) [(20,21)]; anticorpos anti-Insulina (IAA) [(22,23)]; e anti-ZnT8 (*anti-cation efflux zinc transporter*) [(24,25)]. Vários outros autoantigénios têm sido descritos mais recentemente, como potencial alvo do ataque imune da DM1,

como o IGRP – ou *islet-specific glucose-6-phosphatase subunit-related protein*([26]), sendo o seu efeito patogénico isolado ainda discutível.

Os IAA são habitualmente os primeiros a surgir no decurso do processo autoimune. A sua deteção após o início da doença e sob administração de insulina exógena não tem a mesma utilidade diagnóstica, uma vez que um número significativo de doentes sob terapêutica com insulina desenvolve estes anticorpos([23]). Os anti-GAD, contra um antigénio presente nas ilhotas de Langerhans (mas também noutros tecidos, como sistema nervoso central)([19]), podem encontrar-se em cerca de 70% dos doentes com DM1. Os anticorpos anti-IA2, contra uma proteína da membrana dos grânulos secretores da insulina, surgem habitualmente mais tardiamente que os anteriores e podem encontrar-se em 58% destes doentes([27]). A associação de múltiplos autoanticorpos pancreáticos aumenta o valor preditivo de DM1([13,16]). Estudados 882 familiares de doentes com DM1 na Suécia([16]), verificou-se que 98% dos que vieram a desenvolver a doença eram positivos para qualquer 1 destes anticorpos, 80% eram positivos para 2 ou mais anticorpos distintos, estimando-se que a doença se manifestaria em 100% dos que apresentassem 3 anticorpos positivos. Os anticorpos anti-ZnT8, também integrando a membrana dos grânulos([13]), são igualmente de aparecimento mais tardio que os IAA e são dos primeiros a tornar-se indetetáveis após a expressão clínica da doença([25]). Ainda assim, podem ser encontrados em 60 a 80% dos doentes com DM1, dependendo da precocidade da sua pesquisa. Tal como descrito para os autoanticorpos acima enumerados, também os anti-ZnT8 podem ser usados como preditores ou para o diagnóstico da doença([13,28]).

Aproximadamente 94% dos doentes com DM1 expressam pelo menos um dos 4 autoanticorpos acima descritos([13]), o que permite fazer o diagnóstico de DM1-A nestes doentes, restando cerca de 6% de casos de DM1-B. No entanto, não se pode excluir que existam outros autoantigénios relevantes ainda por identificar e que a prevalência real de casos de DM1-B, ou idiopática, seja ainda inferior. O facto de existirem alguns casos de doentes que são ICA+, mas que são negativos para os autoantigénios major já identificados (GAD, IA2, IAA e ZnT8), faz supor que existam autoantigénios adicionais com algum peso na DM1([13]). Estes anticorpos pancreáticos podem persistir positivos ao longo de muitos anos da vida do doente, mesmo após a destruição total do pâncreas e a dependência de insulina, em títulos variáveis; ou desaparecer e tornarem-se indoseáveis. Esta evolução varia de doente para doente, sem correlação clinico-laboratorial evidente.

Existe seguramente também um papel para a imunidade celular neste processo autoimune([29]). Sabe-se, da experimentação animal, que as células Th1 (interferon gama-positivas) são mediadoras do processo de insulite e que as interleucinas IL-18 e IL-12 aceleram o processo; enquanto que anticorpos anti-interferon gama atrasam a destruição das ilhotas pancreáticas. Do balanço entre as células T reguladoras e as células T patogénicas, dependerá a evolução da DM1([30]).

I.1.3. Complicações secundárias da diabetes

As complicações secundárias da diabetes, micro e macrovasculares, representam a causa major de mortalidade e morbilidade também nos doentes com DM1. Os resultados mais relevantes e mais consistentes sobre a evolução das complicações secundárias da diabetes emergiram do estudo *Diabetes Control and Complications Trial* (DCCT). Neste estudo multicêntrico, internacional, observacional, foram incluídos 1441 diabéticos tipo 1, em fase precoce do seu diagnóstico, e foram randomizados em 2 grupos: um sob insulinoterapia em esquema convencional (1 a 2 administrações diárias de insulina e glicemia capilar controlada 4 ou mais vezes/dia), outro sob insulinoterapia "intensiva" (3 ou mais administrações/dia, glicemias controladas 4 ou mais vezes/dia). Foram avaliadas prospetivamente as complicações micro e macrovasculares da DM1. Concluído este estudo em 1993, com um follow-up até 9 anos e médio de 6,5 anos, foi proposto a todos os doentes passarem para o esquema de insulina intensiva. Neste novo estudo, uma extensão do estudo DCCT, que passou a designar-se EDIC (*Epidemiology of Diabetes Interventions and Complications*) aceitaram participar 1375 doentes. Este estudo prolongou-se por mais 11 anos de follow-up, até 2005.

Os resultados dos estudos DCCT/EDIC permitiram relacionar diretamente a glicemia e os níveis de hemoglobina glicosilada (HbA1c) com estas complicações: pior controlo glicémico e HbA1c mais alta associaram-se a evolução mais rápida e mais severa da doença microvascular, a retinopatia, nefropatia e neuropatia([31,32,33,34]). Pelo contrário, atividade residual das células beta pancreáticas, ainda que modesta, e níveis mais altos de Pept-C – co-secretado conjuntamente com a insulina pelas células beta e usado como marcador da secreção de insulina – associou-se a incidência mais baixa de retinopatia e nefropatia no decurso da DM1([35]). Assim, ficou estabelecido o benefício do estrito controlo da glicemia e da redução da HbA1c-alvo na prevenção, ou eventual reversão([36]), da doença microvascular da diabetes. Contudo, este controlo

estrito da glicemia conduz a uma maior incidência de episódios de hipoglicemia([31]), não isentos de riscos e morbilidade.

A retinopatia tem sido a forma de doença microvascular mais estudada, não só porque é uma complicação frequente mas porque permite a sua observação direta através do exame do fundo ocular, tornando-a de mais fácil quantificação e seguimento. No estudo EDIC, com um follow-up muito mais amplo, e apesar de ambos os subgrupos apresentarem valores finais de HbA1c similares, os resultados a 7([37]) e a 10([38]) anos continuaram a mostrar menor incidência de progressão da retinopatia nos doentes que haviam estado previamente sob insulinoterapia intensiva durante a fase DCCT do estudo.

De notar que, numa fase inicial após correção da glicemia para valores mais baixos, pode até haver redução da acuidade visual, consequência da oclusão de pequenos vasos retinianos de lúmen muito estreito. A sua patência mantinha-se à custa da hiperglicemia e de um volume plasmático expandido; a correção deste mecanismo pode levar ao encerramento destes vasos. Esta deterioração inicial é normalmente transitória. Logicamente, as formas avançadas de retinopatia com perda significativa da acuidade visual, não terão benefício claro com o controlo estrito da glicemia([39]).

Tal como para a retinopatia, também na nefropatia se verificou um atraso na sua progressão nos doentes sob insulinoterapia intensiva no estudo DCCT([34]). No estudo EDIC, verificou-se que a taxa de evolução para microalbuminúria era mais baixa no subgrupo que havia estado previamente sob insulinoterapia intensiva durante a fase DCCT do estudo([40]). Este fenómeno foi designado de “memória metabólica”. Uma meta-análise concluiu que mesmo em doentes com microalbuminúria, a probabilidade de progressão para macroalbuminúria foi francamente mais baixa no ramo de insulina intensiva (OR=0,34), quando comparado com o convencional([41]). A insulina intensiva foi também eficaz em atrasar a progressão da doença renal crónica: a evolução para estadio 3 (depuração da creatinina <60ml/min /1,73m2 de superfície corporal), após 22 anos de seguimento cumulativo (estudo DCCT e EDIC), foi menos frequente no subgrupo que tinha feito desde o início insulina intensiva([42]).

No rim, pela especificidade do próprio órgão, outros fatores (associados à diabetes ou independentes dela, por ex. a hipertensão arterial), podem conduzir à hipertensão intra-glomerular e à proliferação mesangial, concorrendo conjuntamente com a hiperglicemia para a disfunção renal. Tal facto determina que o estrito controlo glicémico na nefropatia declarada possa não ser suficiente para parar a sua

progressão ou revertê-la. Contudo, a sua reversão em estádios precoces é possível com o restabelecimento da normoglicemia([36]).

A evolução da neuropatia é mais difícil de monitorizar, dado que não tem marcadores bioquímicos que a traduzam (como o rim), nem pode ser avaliada por observação direta (como o fundo ocular). São, por conseguinte, necessários exames neurofisiológicos para avaliar e medir a sua expressão e progressão. Na neuropatia, houve mais uma vez evidência do benefício do controlo glicémico no atraso da sua progressão([31]). O tratamento com insulina intensiva, aos 5 anos, reduziu a incidência de neuropatia confirmada por testes em 64% (de 13% para 5%), quando comparado com o esquema insulínico convencional([43]). Alterações na condução nervosa foram observadas em 46% vs 26% e disfunção autonómica em 9% vs 4%, comparando insulina convencional vs intensiva([43]). Um estudo norueguês([44]) permitiu concluir que por cada 1% de subida na HbA1c se assistia a uma deterioração da velocidade de condução motora nervosa, aos 8 anos, de 1,3m/segundo. Resultados ainda do estudo DCCT aos 5 anos([45]), demonstraram que as anormalidades eletrofisiológicas observadas em indivíduos já com algum grau de neuropatia, podem ser atenuadas com controlo glicémico mais estrito: o grupo intensivo com melhores valores de condução motora no nervo peroneal.

Uma meta-análise recentemente publicada([46]), mantém como conclusão que o controlo glicémico rigoroso, em doentes com DM1 jovens e em fase precoce da doença, permite reduzir o risco de desenvolvimento de doença microvascular.

As doenças coronária, cerebrovascular e vascular periférica, são expressões da doença macrovascular associada à DM1. Existem outros fatores que se interligam, como a dislipidemia e a hipertensão arterial (HTA), e que em adição à diabetes contribuem para a aterosclerose, condição subjacente à doença macrovascular.

Resultados do estudo EDIC([47]) mostraram uma incidência de eventos cardiovasculares (CV) não-fatais e fatais [angor; revascularização coronária; enfarte agudo do miocárdio (EAM) não-fatal; acidente cerebrovascular (AVC); morte de causa CV] 42% mais baixa no grupo que tinha feito insulinoterapia intensiva. Essa redução atingia mesmo os 57%, considerando apenas os eventos CV graves([47]) - EAM, AVC, ou morte CV. Um outro estudo com 879 doentes e seguimento durante 20 anos encontrou resultados consistentes com estes: um risco relativo de morte CV mais alto na DM1 com HbA1c mais elevadas([48]).

A doença arterial periférica em associação com outras condições, como a neuropatia e infeção, conduzem às lesões de pé diabético. Estas condicionam seriamente a sobrevivência e a qualidade de vida dos doentes com DM1, contribuindo significativamente para a sua morbilidade e mortalidade, além do custo social de um atualmente ainda elevado número de amputados.

I.1.4. O papel dos AGE na patogénese das complicações secundárias da diabetes

A exposição à glicose, nas situações de hiperglicemia mantida, como é a DM1, é a condição basal para o desenvolvimento das complicações secundárias. Existem alguns mecanismos propostos de lesão tecidular induzida pela hiperglicemia. Destacam-se de entre eles 3 mecanismos([49]): a acumulação do sorbitol, pela ativação da via polióis; o aumento da atividade da proteína-cínase C (PKC); e, como mecanismo major, a formação dos "advanced glycosylation end-products" (AGE).

A hiperglicemia conduz ao aumento da atividade da aldose redutase, que leva à acumulação do sorbitol, à depleção do mioinositol neural e alteração da bomba sódio-potássio ATPase. O aumento do diacilglicerol e da atividade da PKC levam à alteração da contratilidade do músculo liso vascular e à alteração da permeabilidade da célula endotelial. Estes são dois dos mecanismos propostos de lesão microvascular. O terceiro mecanismo, mais estudado e com dados consistentes sobre o seu efeito na lesão dos tecidos – como adiante se descreve mais em detalhe - é a formação dos AGE.

Mais recentemente foi descrito um quarto mecanismo potencial de lesão, que é o fluxo aumentado pela via da hexosamina. Excedida a atividade glicolítica intracelular é ativada esta via, que (através da glucosamina-6-fosfatase) conduz à produção de N-acetilglucosamina, capaz de induzir modificação proteica([50]).

Na diabetes mellitus, a glicosilação não enzimática das proteínas de vida longa com a consequente formação dos AGE, parece estar associada ao aparecimento e evolução das complicações da diabetes([50,51]). O provável papel dos AGE na patogénese da nefropatia([52,53,54,55]), da vasculopatia([55,56,57]), da retinopatia diabética([50,58,59]) e da neuropatia([60]), foi estudado em doentes no decurso da evolução da sua diabetes. Mesmo em doentes não diabéticos, com insuficiência renal crónica em diálise, há acumulação de AGE pela deficiente filtração glomerular, chegando a ser superior a 7,5 vezes os níveis da população controlo saudável([61]). A acumulação dos AGE poderá

ser um dos fatores associados à elevada taxa de complicações CV e mortalidade dos doentes em diálise([61,62]), diabéticos e não diabéticos.

Estudos experimentais em animais sobre o efeito de drogas inibidoras da produção e da ação dos AGE, os "AGE-inhibitors" e os "AGE-crosslink breakers", revelaram resultados promissores na prevenção e até reversão da lesão em órgãos alvo da diabetes, como os rins, os vasos, o coração e os olhos([59,63,64,65]). A nível ultraestrutural, conseguiu provar-se a redução do espessamento da membrana basal capilar da retina, após o uso de "AGE-inhibitors", em modelos animais diabéticos([59]).

Existem métodos bioquímicos para a determinação sérica dos níveis dos AGE e de outros produtos relacionados com este processo. Os AGE são um grupo heterogéneo de vários compostos. A pentosidina([66]), com propriedades fluorescentes; e a carboximetil-lisina ([67]), sem propriedades fluorescentes, são dos mais estudados. É também possível avaliar a sua expressão histológica em tecidos e órgãos como a pele([50,51]), utilizando anticorpos anti-AGE([68]). Nos últimos anos surgiu um novo método de avaliação de deposição dos AGE, não invasivo, através da leitura da auto-fluorescência cutânea utilizando a luz ultra-violeta, pelo "AGE Reader"([61,69]). Os seus resultados foram validados pela comparação com biópsias cutâneas, e este poderá ser um método promissor para avaliação dos AGE. Tem algumas limitações, como o ajuste à idade e sexo, à etnia, ao fototipo cutâneo e à função renal.

Os AGE, a sua ação, e formas de inibir ou bloquear a sua ação, continuam a ser objeto atual de estudo([70]), uma vez que a intervenção na sua produção e regulação parece ser central para o controlo de algumas das complicações da diabetes. O uso de antagonistas dos recetores pró-oxidantes dos AGE, os RAGE, tem merecido particular enfoque nos últimos anos ([71]), como mais um potencial alvo terapêutico.

I.2. Transplante renal e pancreático – a experiência do Hospital de Santo António, CHP

O programa de transplante renal (TR) iniciou-se no Hospital de Santo António (HSA), Centro Hospitalar do Porto (CHP), em 1983. Até final de 2013 tinham-se realizado 2234 TR, 189 de dador vivo. Nos últimos anos este centro tem realizado uma média de 100 TR/ano; é ainda o centro responsável pelo TR pediátrico na região Norte do país. As taxas de sobrevivência globais do TR isolado são, para o doente e para o enxerto renal, respetivamente: 98% e 89% ao 1º ano; 93% e 83% aos 5 anos; 76% e

59% aos 10 anos; 65% e 48% aos 20 anos. Estes são resultados similares aos de outros centros mundiais de referência.

O programa de transplante de pâncreas foi implementado no HSA em Maio de 2000. Previamente, em Portugal, apenas haviam sido realizados 3 transplantes de pâncreas nos Hospitais da Universidade de Coimbra nos anos de 1993/1994, sem continuidade desse programa. Em 2000, quando se iniciou a transplantação de pâncreas no HSA, este era o único centro de transplantes no país a promover este tipo de transplante. Em 2011 iniciou também a transplantação de pâncreas o Hospital Curry Cabral, que mantém este programa ativo.

A implementação do transplante de pâncreas resultou de um esforço coletivo, que envolveu cirurgiões e nefrologistas, após estágios destes profissionais em centros de referência mundial (Hospital da Universidade de Minnesota, Minneapolis, Estados Unidos da América - EUA; e Hospital Clínic, Barcelona, Espanha), onde adquiriram o treino necessário. Estes conhecimentos foram transmitidos aos restantes elementos integrantes do programa, incluindo o corpo de enfermagem. O início da transplantação de pâncreas representou, assim, o culminar do empenho de um grupo de profissionais que encararam com determinação mais esta tarefa, a somar às que desempenhavam previamente.

O responsável pelo programa foi desde 2000 a 2010 o Dr. Manuel Teixeira, cirurgião geral. Após a sua aposentação, assumiu essas funções o Dr. José Davide Silva, também cirurgião geral. A equipa cirúrgica é composta pela Cirurgia Geral, Cirurgia Vascular (responsável o Dr. Rui Almeida), e ainda pela Urologia. A seleção e estudo do recetor pré-transplante, bem como o seguimento durante o internamento e em ambulatório após alta, está a cargo da Nefrologia, mais concretamente dos nefrologistas que integram a Unidade de Transplante Renal - o Dr. Castro Henriques (responsável pelo transplante renal), o Dr. Leonídio Dias, a Drª La Salete Martins desde o seu início, e posteriormente a Drª Manuela Almeida e a Drª Sofia Pedroso.

Além de ter sido o único programa de transplante de pâncreas ativo em Portugal durante mais de 10 anos, soma também o maior número cumulativo de doentes tratados, perfazendo 173 transplantes a 31/12/2013: 165 duplos de rim-pâncreas simultâneo e 8 transplantes de pâncreas isolado (após transplante renal prévio), em diabéticos tipo 1. Os resultados de sobrevivência do transplante de rim-pâncreas no HSA são, para o doente, enxerto renal e enxerto pancreático, respetivamente: 97%, 97% e 87% ao 1º ano; 95%, 95% e 84% aos 3 anos; 94%, 93% e 79% aos 5 anos; e

90%, 84% e 70% aos 10 anos. Na última publicação disponível do registo internacional de transplante de pâncreas([72]) estão reportados resultados, para o doente, rim e pâncreas de: 95%, 93% e 86% ao 1º ano; e 93%, 88% e 80% aos 3 anos. Assim, pode dizer-se que os resultados do HSA estão a par dos resultados internacionais.

I.3. As várias modalidades de Transplante de Pâncreas (ou de ilhotas)

O transplante de pâncreas pode fazer-se isoladamente, designado internacionalmente como "pancreas transplantation alone" (PTA); após um transplante prévio de rim, designado como "pancreas after kidney" (PAK); ou simultaneamente a um transplante renal ("simultaneous pancreas-kidney" ou SPK), que por comodidade, traduzindo para português, se designará daqui por diante como transplante de rim-pâncreas (TRP).

O transplante de ilhotas permanece atualmente no campo experimental, ainda que em evolução([73]). A dificuldade em extrair de apenas 1 pâncreas o número de ilhotas suficientes para serem infundidas através da veia porta e permitir a insulino-independência a longo-prazo, é um dos fatores limitantes. A manutenção da viabilidade das células infundidas nos espaços-porta hepáticos (sem irrigação e oxigenação própria e sem a proteção do tecido circundante, como no transplante do órgão pancreático completo), é outro dos obstáculos do seu sucesso a longo-prazo. A necessidade de imunossupressão mantida no transplante de ilhotas para prevenir a sua destruição imunológica, não lhes confere vantagem sobre o transplante do órgão completo. Estas considerações têm estado na base dos "position statement" da *American Diabetes Association*, que mantêm a recomendação de que o transplante de ilhotas seja realizado apenas no âmbito de estudos de investigação([74]). Os resultados do *Collaborative Islet Transplant Registry* apontam para resultados de insulino-independência de 70% a 1 ano e 35% a 3 anos([75]).

O transplante de pâncreas, sem um transplante renal associado, tem resultados inferiores ao TRP simultâneo([76]). O enxerto renal, quando transplantado conjuntamente com o pâncreas, funciona como órgão-sentinela: é frequente na rejeição no TRP haver manifestações renais antes das pancreáticas([77]); e a rejeição isolada do pâncreas no transplante TRP (com ambos os órgãos do mesmo dador cadáver, como habitual) é incomum, ainda que possível (<15% de todos os casos de rejeição). A disfunção renal, ainda que ligeira, traduzida pela subida da creatinina sérica, funciona como um alerta para a possível rejeição de ambos os enxertos, que

deve ser confirmada idealmente por biópsia. No transplante de pâncreas isolado, a inexistência de um marcador bioquímico precoce e fiável, preditor de rejeição antes de ocorrer disfunção endócrina pancreática, representa a maior dificuldade. A subida das enzimas pancreáticas, sobretudo a lípase mais do que a amílase, ainda que se possa correlacionar com rejeição e severidade da rejeição, é pouco específica([78]). O aparecimento da hiperglicemia é uma manifestação demasiado tardia para intervenção, uma vez que ocorre quando a perda de massa endócrina funcionante já é insuficiente para manter normoglicemia.

O "position statement" da *American Diabetes Association*, emitido periodicamente, tem reiterado a sua posição desde o ano 2000([74,79,80]): nos diabéticos com doença renal crónica avançada que sejam candidatos a transplante, a melhor opção é o TRP simultâneo. Na realidade, já em 1996 a *American Society of Transplant Physicians* recomendava o TRP nos doentes com DM1([81]). O PTA deve ficar reservado para os indivíduos sem doença renal crónica importante, com diabetes lábil e complicações agudas severas. O PAK é uma opção alternativa para diabéticos com doença renal crónica, sobretudo se tiver um dador vivo de rim. Se existir esta possibilidade, podendo obviar ou minorar o tempo de uremia e de diálise, deve ser considerada esta opção, sobretudo se o tempo de espera para TRP de cadáver é longo([82]). A desproporção entre a demanda e a oferta de órgãos de dador cadáver para transplante, tem levado à procura da expansão do pool de dadores, devendo nesta situação ser equacionado o TR de dador vivo([82]).

Alguns estudos analisando grandes bases de dados norte-americanas (UNOS – *United Network for Organ Sharing*; e SRTR – *Scientific Registry of Transplant Recipients*), comparam os resultados do TRP com o TR de dador vivo e TR de dador cadáver([83,84]). No curto e médio-prazo, até aos 5 anos, o TR tem resultados de sobrevivência do recetor superiores, se o transplante for de dador vivo. A maior morbilidade do TRP (e também maior mortalidade) no imediato, inerente ao maior risco operatório deste transplante, justifica estes resultados. Contudo, se analisarmos dentro do TRP apenas os que mantiveram o pâncreas funcionante (além do rim), então a sobrevivência aos 7 anos é melhor no TRP do que no TR isolado, ainda que este tenha sido de dador vivo([84]). Numa análise a mais longo prazo realizada pelo *Collaborative Transplant Study* – que recolhe dados de 46 países nos 5 continentes – pode observar-se que no TRP após os 10 anos, a sobrevivência do doente é sempre maior([82]). Estes resultados são indicadores do benefício do pâncreas adicional([82,84]), do controlo da glicemia e provavelmente da redução dos fatores de risco CV.

No registo internacional do transplante de pâncreas (IPTR – *International Pancreas Transplant Registry*) até 2011, constavam mais de 35000 transplantes realizados em todo o mundo, cerca de 25000 nos EUA([76]). Na sua última publicação disponível, comprova-se a descida nos últimos anos do nº global de transplantes de pâncreas/ano, depois de um máximo anual atingido em 2004. Contudo, os resultados de sobrevivência do doente e do(s) enxerto(s) têm vindo a melhorar progressivamente, comparados com dados de relatórios anteriores do IPTR([85,86,87,88]). Esta melhoria dos resultados nos últimos anos observou-se não só no curto prazo([72]), mas também a longo prazo([76,89]). Das distintas modalidades de transplante de pâncreas, o TRP simultâneo é largamente mais frequente, representando cerca de 90% dos que se realizam em todo o mundo([72]), ainda que nos EUA a sua prevalência não seja tão alta (75%), uma vez que as percentagens de PAK (17%) e PTA (8%) somadas representaram cerca de 25% nos últimos anos([76]).

No que se refere à taxa de sobrevivência do recetor, ao 1º ano, para qualquer das modalidades, ela situa-se nos 95%([76]). Aos 5 anos, é de 89% para o PTA, 87% no TRP e 83% no PAK. Aos 10 anos, a sobrevivência do recetor é superior a 70%, registando-se um máximo de 82% no PTA([76]). Salienta-se que os recetores de PTA são em regra mais jovens, com menos tempo de evolução da sua DM1 e sem nefropatia significativa, o que por si só explica a maior sobrevivência destes doentes. Quanto à sobrevivência dos enxertos no TRP, os resultados para enxerto renal e pancreático são, respetivamente: ao 1º ano de 93% e 86%([76]); aos 5 anos de 80% e 72%([89]); e aos 10 anos de 59% e 66%([89]). De notar, a longo prazo (10 anos ou mais), a menor sobrevivência do enxerto renal no TRP, quando comparada com a sobrevivência do enxerto pancreático([89]). Dados dos registos internacionais permitiram calcular uma semi-vida média para o rim de 128 meses, enquanto que para o pâncreas a semi-vida média calculada foi de 146 meses([76]). No PAK, a sobrevivência do enxerto pancreático é de 83% ao 1º ano([76]); 62% aos 5 anos; e 46% aos 10 anos([89]). No PTA, a sobrevivência do pâncreas é de 80% ao 1º ano([76]); 59% aos 5 anos; e 39% aos 10 anos([89]).

O TRP é realizado na quase totalidade dos casos com órgãos de dador cadáver. A colheita de pâncreas no dador vivo obriga a pancreatectomia parcial, cirurgia de risco e morbilidade consideráveis. Por este motivo, o transplante de pâncreas a partir de dador vivo representa uma percentagem residual destes transplantes (0,3%), sendo que atualmente, nos EUA, apenas 3 centros realizam este tipo de transplante([76]).

I.3.1. O Transplante de rim-pâncreas (TRP)

I.3.1.1. Candidatos, imunossupressão e técnica cirúrgica

A transplantação simultânea de rim e pâncreas, em diabéticos tipo 1 com doença renal crónica avançada, é hoje aceite como a melhor opção terapêutica para estes doentes, tal como assumido desde 2000 pela *American Diabetes Association*[74,79,80]. É a única capaz de estabelecer um estado euglicémico e insulino-independência a longo prazo, e a que permite o tratamento simultâneo das duas patologias, diabetes e insuficiência renal, libertando os doentes da insulina e da diálise[90,91,92].

O TRP também tem sido realizado em alguns doentes com DM2, criteriosamente selecionados([93,94,95,96,97]). É um facto que a incidência de transplantes de pâncreas em DM2 tem vindo a aumentar([76]), correspondendo a cerca de 8% destes transplantes atualmente. Não obstante, os critérios de seleção destes doentes são muito restritivos([93,97]): idade <60 anos, índice de massa corporal (IMC) <30 (ou <32) kg/m^2; sem doença CV ou vascular periférica importante; ausência de tabagismo; requerendo insulina há mais de 5 anos; dose total diária de insulina <1U/kg; Pept-C <1.0 (ou <1.8) ng/ml; capacidade de cumprimento de restrições dietéticas, além da medicação. Alguns autores advogam que doentes com DM2 que cumpram estes critérios podem ser propostos para transplante de pâncreas, nomeadamente para TRP([94,97]). Porém, e ao contrário dos resultados na DM1, não está provado que o transplante adicional do pâncreas tenha benefício acrescido de sobrevivência a longo prazo quando comparado com um TR de dador vivo para esses recetores([96]).

Em termos de técnica cirúrgica, o TRP pode ser realizado com derivação venosa sistémica (usualmente anastomose aos vasos ilíacos) ou com derivação portal. A derivação à veia porta, que se implementou há uns anos com entusiasmo inicial, tem sido usada em <20% dos transplantes de pâncreas - cerca de 18% dos TRP([76]). O objetivo da drenagem venosa portal é o de restabelecer o "efeito de 1ª passagem" pelo fígado, aproximando o transplante mais da fisiologia normal, e diminuindo o hiperinsulinismo resultante da técnica de derivação sistémica. Contudo, a derivação portal aumenta a dificuldade técnica e, por outro lado, não está comprovado o benefício da resolução do hiperinsulinismo([98]). A derivação da secreção exócrina pode ser entérica (anastomose do arco duodenal, que inclui a ampola de Vater, ao intestino) ou vesical (anastomose do arco duodenal à bexiga). A derivação entérica, mais fisiológica, veio substituir progressivamente a derivação vesical que era a mais usada no início, evitando a morbilidade e as complicações que lhe estavam associadas([99]) –

nomeadamente formas graves de cistite, a desidratação e acidose pela perda de bicarbonato pela urina. Atualmente, mais de 90% dos TRP são realizados utilizando a derivação entérica([76]). No HSA-CHP todos os transplantes de pâncreas foram efetuados com recurso à derivação venosa sistémica e derivação exócrina entérica.

A imunossupressão usada no TRP foi evoluindo ao longo das diferentes décadas, tal como para os outros tipos de transplante. Na década de 80, houve de facto uma expansão do transplante pancreático, graças aos melhores resultados com a introdução da ciclosporina. Desde essa altura, em que se usava um esquema imunossupressor triplo com ciclosporina, azatioprina e prednisolona([100,101]), passou-se progressivamente para um esquema que inclui atualmente, na maioria dos casos, tacrolimus e ácido micofenólico([100,101]). Nos estudos de sobrevivência do transplante a longo-prazo, tornou-se evidente que este esquema imunossupressor - usado em mais de 80% dos transplantes de pâncreas([76]) – se associou a melhores resultados a longo-prazo([76,89]). A indução com anticorpos passou também a ser a regra, com mais de 90% dos transplantes usando indução([76]). Apesar do entusiasmo inicial na utilização de outros anticorpos não depletores linfocitários, nomeadamente o basiliximab, estes representam nos registos atuais <10% dos casos de indução com anticorpos([76]). Assim, os anticorpos anti-linfocitários (globulina anti-timocítica) constituem a escolha em mais de 90% dos esquemas de indução com recurso a anticorpos. A indução com estes anticorpos anti-linfocitários associou-se também a melhores resultados a longo-prazo, no TRP([76]) ou no pâncreas solitário([76,89]). Tem havido também uma tendência crescente à suspensão precoce ou mesmo eliminação dos corticóides dos esquemas imunossupressores, sem que isso se tenha associado a pior prognóstico([76]).

No HSA o esquema imunossupressor usado inclui globulina anti-timocítica, tacrolimus, ácido micofenólico e corticóides, sendo estes suspensos sempre que possível, decorridos os primeiros 6 meses pós-transplante.

I.3.1.2. Evolução clínica e complicações (precoces e tardias)

O desfecho do TRP depende muito da evolução clínica inicial. Tem havido melhorias significativas, quer na técnica cirúrgica, quer nos cuidados médicos precoces, que resultaram num decréscimo progressivo das complicações no pós-transplante imediato([102,103]). Dois artigos publicados na década passada provenientes dos 2 maiores centros nos EUA, com mais de 1000 transplantes de pâncreas realizados em cada centro, são as mais sólidas referências sobre a evolução deste tipo de transplante([100,101]).

Relativamente ao enxerto pancreático temos a considerar: a trombose; a hemorragia; as fístulas pancreáticas ou do segmento duodenal; a pancreatite; e a infeção, como complicações precoces que podem levar à remoção do pâncreas. São coletivamente designadas como perdas técnicas([102,103,104,105]). Estas intercorrências, que conduzem na maioria das vezes à relaparotomia([92,101,106,107,108]) e que no caso das fístulas e da pancreatite frequentemente complicam com a formação de coleções peri-pancreáticas e infeção([107,108]), são a causa major de perda do pâncreas([100,101,107]). De todas estas complicações, a trombose continua a ser a causa principal de falência do pâncreas([100,101,106,108]). A incidência destas complicações tem vindo a diminuir progressivamente ao longo do tempo, reflexo do refinamento da técnica cirúrgica e do melhor manejo no pós-operatório imediato([76,100,102,103]). A perda técnica global no TRP, reportada até final dos anos 90 como causa de perda de mais de 20% dos pâncreas([100]), foi na última década responsável pela perda de 13%([100,104]), e nos últimos registos por 8-9% das perdas de pâncreas([76]). A mortalidade precoce do recetor tem também vindo a diminuir([76,106]), consequência da redução destas complicações.

Ainda que a incidência de rejeição aguda após o TRP seja na maioria das séries reportada como >10% (entre 10-20%) com a terapêutica atual([100,101]), ela é rara como causa da falência precoce do pâncreas (ao 1º ano), correspondendo a 1,8% nos dados mais recentes do IPTR([76]). As outras modalidades de transplante de pâncreas, PAK e PTA apresentam taxas de perda por rejeição mais altas: 3,7% e 6%, respetivamente([76]). A vantagem do enxerto renal simultâneo no TRP transparece nos melhores resultados quanto a perda imunológica no TRP, uma vez que a rejeição dissociada (apenas pâncreas sem rim afetado) é rara([101]) e as alterações dos marcadores de função renal são mais precoces e fiáveis nesta situação, aumentando a capacidade de deteção de rejeição([92]) e a probabilidade do seu tratamento eficaz. As causas mais frequentes de perda tardia do enxerto pancreático são a morte com enxerto funcionante, seguida da perda crónica do enxerto/ rejeição([101]).

As causas de perda do enxerto renal no TRP não são muito distintas das constatadas no TR isolado. A perda precoce é mais rara do que para o enxerto pancreático e a sobrevivência ao 1º ano de 93%([76]) também não é díspar da observada do TR isolado. A rejeição crónica é a causa mais frequente de perda tardia do enxerto renal([100,101]), seguida da morte com enxerto funcionante ([101]).

As principais causas de morte do recetor no TRP são a doença CV /cerebrovascular e a infeção([76,101]). A existência de doença CV (coronária ou vascular periférica) prévia ao transplante é um fator de risco muito importante para a morte CV([100]). A idade do recetor superior a 44 anos; o tempo em diálise (vs *preemptive*); e a falência de um dos enxertos, são fatores de risco para a morte do recetor([76]). Mais especificamente, a perda do enxerto renal nos recetores de TRP representou um risco relativo de morte >17 vezes superior, quando comparada com os que mantinham o rim funcionante; e a perda do pâncreas nesses doentes representou um risco relativo de morte >3 vezes superior, comparada com os que mantinham o enxerto pancreático funcionante([76]).

No TRP, a qualidade do dador é um dos principais determinantes tanto da taxa de complicações cirúrgicas e das falências técnicas a curto prazo([104,106,107]), como da manutenção do enxerto pancreático funcionante a longo prazo([76,89]). O IMC do dador >30kg/m^2 é apontado como mais um fator de risco para as complicações cirúrgicas precoces, com impacto na sobrevivência do transplante([104,107]). A idade do dador acima de 44 anos ([76,89,106]) – ou mesmo acima dos 30 anos em alguns estudos([76,107]); o tempo de isquemia fria do enxerto pancreático >12h([76,89,107]); e a morte do dador de causa cerebrovascular - ou não traumática([76,89,104,106]) são importantes fatores de risco para a falência do pâncreas transplantado. A sobrevivência do enxerto pancreático a longo-prazo é superior quando o dador não apresenta estes fatores([76,89]). O reconhecimento da influência das caraterísticas do dador levou à criação de índices de risco do dador, para determinar a aceitação ou não do pâncreas, como o "Pancreas-Donor Risk Index" ou PDRI([109]). Outros Aspetos, como a idade do recetor < 45 anos([89]); ou grau de alosensibilização (PRA<20%) do recetor ([76]); bem como a utilização de esquemas de imunossupressão que incluam anticorpos anti-linfocitários([76]); e a associação de tacrolimus com ácido micofenólico([76,89]), associaram-se a menor risco de falência do pâncreas.

Não restam grandes dúvidas sobre as vantagens do TRP nos doentes com DM1 e doença renal crónica, comparativamente com os que permanecem em diálise, ou que recebem um TR isolado([101]). Os receios da maior mortalidade no período inicial do TRP vs TR isolado foram-se progressivamente desvanecendo nas últimas décadas, com claros benefícios a médio e longo prazo para o TRP([110,111,112]). Em função destes resultados, este é considerado o tratamento com melhor relação custo-eficácia nos doentes com DM1 e doença renal crónica([113]). Wolfe et al([114]) desenvolveram o score LYFT (Life Years From Transplant) que permite calcular o nº de anos ganho por cada transplante, sendo este favorável ao TRP (vs TR isolado) para estes doentes. Assim, o

TRP é considerado não só "life enhancing"([115]), mas também "life saving"([116]), e este conceito deve ser tido em conta numa perspetiva de melhor gestão de um recurso que é escasso([114,117]), como a disponibilidade de órgãos para transplante.

A transplantação simultânea de rim e pâncreas na DM1 com doença renal crónica é a terapêutica que confere não só melhor sobrevivência a médio e longo prazo([76,89,100,101,112,118]), mas também a que oferece melhor qualidade de vida([102,103,112,119,120]).

I.3.1.3. Impacto nas complicações secundárias da diabetes

- na doença microvascular (neuropatia, nefropatia, retinopatia)

A maioria dos estudos mostra uma melhoria significativa das complicações da diabetes após o TRP, superior à adquirida com o TR isolado([112,118,119]). A resolução da diabetes com um transplante de pâncreas funcionante previne o aparecimento da nefropatia diabética no rim transplantado[(121,122,123)]. Nos doentes com DM1 com nefropatia ligeira e submetidos apenas a transplante de pâncreas (PTA) pôde observar-se uma reversão das alterações histológicas[(36,124,125)] e tradução clínica com remissão da albuminúria[(126)]. A presença adicional de um enxerto pancreático funcionante, quando comparados os resultados dos TRP com os dos diabéticos tipo 1 que tendo sido submetidos a TRP tiveram falência do pâncreas ou receberam um TR isolado, associou-se a melhor sobrevivência do enxerto renal[(127,128)], ou mesmo do recetor[(128)].

É na polineuropatia diabética que existem os dados mais consistentes sobre o efeito do transplante de pâncreas na sua prevenção, atraso na progressão, ou mesmo reversão. O TRP pode resultar na melhoria da neuropatia sensitivo-motora([129,130,131,132,133,134]) e autonómica([129,132,135,136]). Estudos realizados em doentes com DM1 sem doença renal crónica (antes do seu aparecimento ou corrigida por um enxerto renal normofuncionante) confirmam melhoria da neuropatia diabética após restabelecimento da euglicemia através de um transplante de pâncreas([129]). Ou seja, excluído o fator urémico - causa adicional de neuropatia – ficou demonstrado o benefício independente da euglicemia mantida na correção da neuropatia diabética. Este impacto positivo pode ser sentido no 1º ano após o transplante, sobretudo a neuropatia sensitivo-motora([112,134]), mas no caso da neuropatia autonómica pode ser percebido e medido a mais longo prazo([112,132]). O TRP permite ainda melhorar alterações metabólicas e funcionais do sistema nervoso central observadas nos doentes com DM1, traduzindo-se por uma melhor capacidade cognitiva e performance intelectual([137]).

Existem dados controversos sobre o impacto do transplante de pâncreas na retinopatia. Enquanto que alguns estudos não demonstram benefício claro, outros descrevem uma estabilização ou até melhoria da retinopatia([138,139,140]). A severidade da retinopatia à data do transplante, frequentemente proliferativa difusa pan-fotocoagulada, obvia a obtenção de melhores resultados([141,142]). O efeito adicional da corticoterapia e dos inibidores da calcineurina, podendo precipitar a formação de cataratas e o glaucoma, deve ser tido também em conta na avaliação oftalmológica pós-transplante([143]). A reversão da uremia pode por si só melhorar a acuidade visual. Contudo, nos doentes com DM1 submetidos a TRP, quando comparados com os que receberam TR isolado, verificou-se menor deterioração da retinopatia([142,144]).

- na doença macrovascular (cardiopatia e vasculopatia)

Os resultados são menos consistentes no que se refere à evolução da macrovasculopatia pós-transplante: estudos mais precoces não mostraram essa melhoria([145]); outros estudos mais recentes evidenciaram que pode haver melhoria na doença microvascular([100,146,147]) e macrovascular([148,149]), traduzido por menor incidência de úlceras diabéticas e amputações após TRP([148,149]) e também menor doença coronária([149]). Foi demonstrado com o TRP benefício na função e geometria cardíacas([149,150,151,152,153]) e melhoria do perfil lipídico([126,154,155,156]) e controlo metabólico([157]).

É relevante a perda tardia do TRP por morte com o enxerto funcionante([92,100,101,136]). A morbilidade e mortalidade CV representam um problema major a longo prazo nestes doentes([76,100,101,118,158]), não só pela sua condição de diabéticos durante muitos anos, mas também pela adição de novos fatores de risco CV após o transplante, como a imunossupressão([102]). Também neste aspeto será o TRP mais favorável que o TR isolado([148,159,160]).

I.3.1.4. A recidiva da autoimunidade pancreática

A recidiva da DM1 no pâncreas transplantado foi documentada por David Sutherland *et al* há cerca de 30 anos[(161)]. Os primeiros casos foram descritos em diabéticos que receberam o enxerto de pâncreas (segmentar) a partir de irmãos HLA-idênticos ou haplo-idênticos, e que estavam sob imunossupressão mínima[(161,162)]. Essa constatação levou à hipótese de que o maior match HLA poderia ser um fator de risco para a recidiva da doença, facilitada pela redução da imunossupressão[(162)]. Sibley et al[(163)],

em 100 exames histopatológicos de enxertos pancreáticos, viria a dar fundamento a essa hipótese, não encontrando evidência de recidiva em transplantes sob imunossupressão mantida e sem elevado match HLA. Estudos posteriores mostraram que a recidiva podia ocorrer independentemente do match HLA[164].

A suspeição da recidiva da DM1 surgiu em situações de deterioração ou falência do transplante de pâncreas, após exclusão de outras causas (nomeadamente vasculares e infeciosas), mantendo-se a função do enxerto renal inalterada e sem evidência de rejeição na biópsia renal. Na biópsia pancreática, a ausência de sinais de rejeição aguda ou crónica[165] e a presença um infiltrado linfocitário envolvendo especificamente as células beta das ilhotas de Langerhans (com marcação com anti-soros positiva para insulina), e poupando outras células (alfa e delta - produtoras de glucagon e somatostatina) e o tecido pancreático exócrino[166], estabelece o diagnóstico.

A biopsia pancreática permanece o “gold standard” para o diagnóstico. Contudo, como método invasivo que é, não é o ideal para o rastreio. A pesquisa dos autoanticorpos pancreáticos que usualmente são positivos por altura do diagnóstico da DM1 inaugural (ICA, anti-GAD, IAA, IA2, anti-ZnT8) é usada após o transplante de pâncreas como método de rastreio. O reaparecimento ou subida do título destes anticorpos é um potencial indicador da recidiva da doença[167].

Vários estudos reportam uma associação entre estes anticorpos positivos e a disfunção das células beta pancreáticas após o transplante[164,165,166,167,168,169,170,171]. Seria lógico pensar que os referidos autoanticorpos são um fator de risco para perda do enxerto pancreático. Porém, esta não é uma hipótese consensual. Outros estudos[172] não documentam essa ação deletéria dos anticorpos sobre a função do enxerto.

Alguns autores estimaram que de entre as perdas imunológicas (cerca de 10%), metade podem ser devidas à autoimunidade e outra metade à aloimunidade[173]. O desconhecimento desta entidade ou a sua subvalorização contribuirá para a falta de diagnóstico em muitos casos. Dado que a monitorização destes anticorpos é facilmente exequível e está disponível na maioria das unidades de transplantação, tem sido recomendado que o seu rastreio faça parte da metodologia de seguimento dos doentes transplantados de pâncreas[173].

O papel da autoimunidade celular neste processo de autoimunidade pancreática também tem sido alvo de investigação mais intensa nos últimos anos. Os linfócitos T

autoreativos CD4+ e GAD-específicos, observados nos enxertos pancreáticos com recidiva de DM1, foram também identificados no sangue periférico e puderam ser clonados[173,174,175]. Este grupo investigador observou, em experimentação animal, que esses linfócitos quando transplantados conjuntamente com ilhotas pancreáticas, levavam à destruição das células beta pancreáticas[175]. Nos doentes que apresentaram recidiva da DM1 comprovada por biópsia, foi tentado o seu tratamento com intensificação da imunossupressão, nomeadamente com anticorpos anti-linfocitários[174,175]. Verificaram que após uma resposta transitória, estas células reapareceram meses a anos depois, correlacionando-se com a perda definitiva da produção de insulina[174,175]. Desta investigação resultaram várias conclusões. A patogenicidade destas células T autoreacitvas GAD-específicas ficou comprovada[175]. Demonstraram também que após o transplante de pâncreas a exposição a antigénios das ilhotas pancreáticas pode reativar essas células T de memória[173,174,175]; e que a imunossupressão usualmente utilizada para controlar a aloimunidade não consegue controlar a autoimunidade[173,174,175].

A participação das células T CD8+ na autoimunidade pancreática tem sido também enfatizada[29,30]. A procura de uma estratégia terapêutica eficaz no tratamento, ou até na prevenção desta recidiva tem-se intensificado. Os diversos esquemas imunossupressores tentados até ao momento têm falhado em termos de eficácia a médio e longo prazo. Progredir no reconhecimento da autoimunidade pancreática recidivada no enxerto, no estudo dos seus mecanismos patogénicos e na sua evolução, poderá trazer algumas respostas que apontem para novas possibilidades terapêuticas[173].

I.4. Objetivos da tese

Sendo o HSA um hospital pioneiro na promoção da transplantação reno-pancreática em Portugal, o objetivo geral desta tese centrou-se na avaliação da sua eficácia traduzida na evolução clínica e metabólica dos doentes com DM1 que são submetidos a TRP neste centro de transplantação. Pretendeu-se também investigar linhas adicionais de intervenção terapêutica para sua otimização.

Trata-se de um grupo particular de doentes que durante muitos anos foram somando comorbilidades, associadas à sua condição de diabéticos e posteriormente de doentes renais crónicos. A intervenção cirúrgica major do transplante duplo de rim e pâncreas,

e a imunossupressão crónica, acrescem como potenciais fatores de risco com impacto na sua evolução pós-transplante. A avaliação criteriosa deverá ser capaz de selecionar os candidatos em que o risco deste procedimento seja claramente ultrapassado pelos seus benefícios. Esta é, por conseguinte, uma população de doentes de elevada complexidade que requer cuidados clínicos exigentes e diferenciados após o transplante. É fundamental no seu seguimento esta visão global do doente, das complicações da DM1 e da doença renal crónica, para além do enfoque nos órgãos transplantados.

Tem sido extensamente estudado o papel dos AGE na DM1 e na evolução das suas complicações, mas está por esclarecer a sua evolução após TRP. A monitorização seriada destes marcadores, da sua expressão sérica e tecidular, numa população de doentes TRP - que deixaram de ter diabetes e insuficiência renal - poderá permitir tirar ilações sobre a correlação destes com a evolução clínica das complicações secundárias da diabetes após o transplante, ainda que esta se possa vir a apreciar apenas a longo prazo.

A recorrência da autoimunidade anti-ilhota pancreática após o TRP é uma observação no mínimo inquietante, que provavelmente tem sido subvalorizada. Desconhece-se a sua real dimensão e o seu impacto na sobrevivência e função do pâncreas. Na maioria dos casos em que se observa a expressão desta autoimunidade não coexiste um descontrolo da aloimunidade, traduzida por rejeição no exame histológico. Nesta situação, têm sido desencorajadores os resultados das várias estratégias imunossupressoras utilizadas, levando a que não seja consensual a decisão de intensificação da imunossupressão, e muito menos do tipo de agentes a utilizar. Urge conhecer melhor todo este processo da autoimunidade pancreática, começando pelo seu reconhecimento precoce, o estudo da sua evolução, e a análise de possíveis fatores de risco para a sua recorrência que possam vir a permitir uma intervenção eficaz.

Mais especificamente os objetivos desta tese compreendem:

- Avaliação da sobrevivência do doente e dos enxertos, e das causas de morte e de perda dos enxertos;
- Avaliação da evolução da função dos enxertos e do controlo metabólico;
- Avaliação de complicações clínicas pós-TRP nomeadamente infeciosas, complicações da imunossupressão em curso e episódios de rejeição aguda;
- Avaliação da doença cardiovascular e da doença óssea renal após o TRP;

- Avaliação da qualidade de vida pós-TRP;
- Avaliação da evolução da expressão bioquímica e histológica dos AGE, com potencial correlação com a evolução das complicações secundárias da diabetes;
- Avaliação da evolução dos autoanticorpos pancreáticos, identificação de potenciais fatores de risco para a sua expressão, e estudo de implicações da sua positividade na função do enxerto pancreático.

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I – Introdução

II - Evolução clínica e metabólica do doente submetido a TRP

III - Complicações mais frequentes após TRP

IV - Doença cardiovascular no TRP

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X. Resumo. Lista de publicações no âmbito desta tese

XI. Agradecimentos

II - Evolução clínica e metabólica do doente submetido a TRP

- **II.1** - **Martins L**, Pedroso S, Henriques AC, Dias L, Sarmento AM, Seca R, Oliveira F, Dores J, Lhamas A, Coelho T, Ribeiro A, Esteves S, Pereira R, Almeida R, Amil M, Cabrita A, Teixeira M. **Simultaneous Pancreas-Kidney Transplantation: Five-year results from a single center**. *Transplant Proc* 2006; 38(6): 1929-1932.

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- **II.2** - **Martins L**, Henriques AC, Dias L, Pedroso S, Almeida M, Santos J, Dores J, Almeida R, Cabrita A, Teixeira M. **One-hundred eleven simultaneous pancreas-kidney transplantations: 10-year experience from a single center in Portugal**. *Transplant Proc* 2011; 43(1): 205-208.

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  - **II.2.1 – Martins LS,** Fonseca I, Aguiar P, Rocha A, Costa R, Santos C, Malheiro J, Pedroso S, Almeida M, Dias L, Henriques AC, Cabrita A, Davide J. **Pancreas-kidney transplantation: analysis of 150 patients from one centre in Portugal.** *Port J Nephrol Hypert* 2013; 27(3): 173-178.

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- **II.3** - Malheiro J, **Martins L**, Fonseca I, Gomes AM, Santos J, Dias L, Dores J, Oliveira F, Seca R, Almeida R, Henriques A, Cabrita A, Teixeira M. **Steroid withdrawal in pancreas-kidney transplantation: a 7-year report**". *Transplant Proc* 2009; 41(3): 909-912.

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Resumo do artigo II.1

O transplante duplo de rim e pâncreas é reconhecido como o tratamento de eleição para o doente com DM1 e doença renal crónica avançada.

Neste primeiro trabalho fomos avaliar os resultados obtidos após 5 anos de atividade cumulativa da Unidade de TRP do HSA.

Foram estudados 42 TRP, 40 em doentes já em diálise, 2 ainda antes do início da diálise. Descrevemos os nossos critérios de exclusão; a técnica cirúrgica utilizada; o protocolo imunossupressor; bem como as profilaxias anti-infeciosa e anti-trombótica. Este grupo de doentes tinha um longo tempo de evolução da sua DM1 (média 23 anos) e de diálise (média 34 anos), apesar duma média etária de apenas 34 anos. Cerca de 45% não tinha qualquer compatibilidade HLA com o dador.

Observou-se uma incidência de não função imediata do enxerto renal de 9,5%; e de rejeição aguda global (presumida e tratada) de 21,4% - baseada em critérios clínicos e na biópsia renal, sem biópsia pancreática confirmatória. Nos últimos 2 anos deste período, verificou-se uma tendência à redução da incidência de rejeição aguda.

Em mais de 40% houve necessidade de reintervenção cirúrgica, principalmente por hemorragia e infeção, que resultaram num tempo de internamento mais prolongado nesses casos.

Nos casos de perda do enxerto pancreático, a trombose e a infeção foram as causas mais frequentes. Contabilizaram-se 2 mortes, 1 de causa infeciosa e outra cardiovascular. A sobrevivência ao 1º e 5º anos foi de 97,3% e 91,7% para o doente, 94,6% e 89,2% para o enxerto renal, e 83,8% e 78,7% para o enxerto pancreático.

Os resultados do registo internacional do transplante de pâncreas (IPTR), publicados meses antes e relativos ao período de 2000-20004, mostraram indicadores de sobrevivência semelhantes ao 1º ano. Também outros resultados, de reputados centros internacionais de transplante de pâncreas, aos 5 anos, não diferiam dos obtidos na nossa Unidade.

Esta constatação constituiu um estímulo adicional à manutenção e ampliação deste programa de transplante de pâncreas, até então o único em atividade em Portugal.

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Simultaneous Pancreas-Kidney Transplantation: Five-Year Results From a Single Center

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ABSTRACT

We report the 5-year results of our simultaneous pancreas-kidney transplantation (SPKT) program, started on May 2, 2000. Forty-two SPKT were performed on 42 type I diabetic patients with chronic renal failure. The procedure was performed with enteric diversion and vascular anastomosis to the iliac vessels. Immunosuppressive protocol included antithymocyte globulin, tacrolimus, mycophenolate mofetil, and steroids. The 24 women and 18 men had a mean age of 33.5 ± 6.3 years and mean 22.8 ± 14.2 years time of diabetes evolution. Forty patients had been on dialysis for 34.3 ± 24.1 months, and two were preemptive transplantations.

Acute rejection episodes were treated in eight patients (19.1%): in three cases they affected both organs; in two only the kidney was affected; and the other three were pancreas graft rejections. The incidence of postoperative complications requiring re-operation was 42.9%, mostly pancreas graft related. Two patients died, one due to cardiovascular disease; the other was transplant related. Three kidney grafts were lost, and the causes were immunologic, thrombosis, and patient death. Pancreas graft loss occurred in seven patients: thrombosis (n = 3); infection (n = 3); immunologic (n = 1).

The patients with surviving grafts were doing well, with normal kidney and pancreas function: serum creatinine = 0.89 ± 0.15 mg/dL; fasting blood glucose = 79 ± 16 mg/dL; HbA1c = 4.7 ± 1.1%. The 1-year patient, kidney, and pancreas survival rates were 97.3%, 94.6%, and 83.8% and 5-year values, 91.7%, 89.2%, and 78.7%, respectively.

In conclusion, these results are similar to the most recent UNOS/IPTR reports, leading us to consider our experience with SPKT very positive.

SIMULTANEOUS PANCREAS-KIDNEY transplantation (SPKT) is the best treatment option for type I diabetic patients with advanced chronic renal failure.[1] The long-lasting independence from dialysis and insulin as well as the avoidance of hypoglycemic episodes after restoration of hormonal counterregulation[2] represents an important improvement in the quality of life of these patients.[1,3] In addition, stabilization or even reversion of diabetic microvascular complications may be achieved after SPKT, reducing cardiovascular risk factors and also enhancing quality of life.[3]

Some years ago, it has been well-established that SPKT provides better long-term patient survival than kidney transplantation alone.[3–6] Moreover, every annual IPTR report has shown steady improvement in SPKT results,[7–9] a natural consequence of several factors, such as progress in surgical technique, immunosuppressive protocols, and post-operative care. With such exciting news, several centers including ours have started SPKT programs. The first SPKT was performed at our center on May 2, 2000. This is the single active center of kidney-pancreas transplantation in Portugal. Without interruption, we have performed 42

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SPKT in 5 years of activity. The purpose of this article was to present the overall results of our program.

PATIENTS AND METHODS

The 42 SPKT were performed in 42 type I diabetic patients. Each case was the first transplant, and all were obtained from cadaveric donors. Exclusion criteria for admission to the active SPKT list, besides those commonly established for other solid organ transplants (active malignancy or infection, drug addiction, uncontrolled psychiatric disease, or noncompliance to the medication), included age above 45 years; significant coronary disease not solved by angioplasty or surgery; and severe and clinically significant peripheral artery disease. In fact, one patient who had a previous myocardial infarction, but reversion of the coronary artery stenosis by angioplasty plus stent placement underwent SPKT. Another recipient was on the very urgent list for transplantation due to exhaustion of vascular accesses for hemodialysis and without conditions for peritoneal dialysis.

Technically, SPKT was performed via a midline incision for intraperitoneal placement on the right side of the duodenopancreatic graft with enteric drainage, without a Y-Roux loop, and vascular anastomosis between splenomesenteric vessels and the common iliac vessels. The kidney graft was placed via a new incision in the left iliac fossa, extraperitoneally using the usual technique for kidney transplantation alone (KTA).

The immunosuppressive protocol included induction with antithymocyte globulin (ATG, Fresenius-S) plus tacrolimus, mycophenolate mofetil (MMF), and steroids as maintenance immunosuppression. For infection prophylaxis we used a third generation cephalosporin, vancomycin, and fluconazole during the first days; thereafter we prescribed oral valgancyclovir and cotrimoxazole for 3 and 6 months, respectively. For thrombosis prophylaxis we used oral aspirin preoperatively, adding subcutaneous low weight heparin in the immediate postoperative period during hospitalization. After discharge, oral aspirin was continued at a daily dose of 50 mg.

Diagnosis of acute rejection of the pancreas graft was inferred based on clinical, laboratory, and imaging data, and a kidney graft biopsy, always performed in cases of pancreas or kidney dysfunction, after exclusion of other etiologies. Pancreas graft biopsies were not done.

All patients were regularly followed up at our outpatient care unit. The permanent need for dialysis or insulin, a retransplant, or patient death, were considered graft loss.

Statistical Analysis

We used SPSS statistical analysis software, version 12.0 for Windows (SPSS Inc, Chicago, Ill). Basic statistics results are expressed as mean values ± standard deviations.

Differences between groups were assessed using Student's t test for independent sample continuous variables. Results were considered statistically significant at $P < .05$. Survival curves for patient, renal, and pancreas grafts were calculated using the Kaplan-Meier method. Graft survival was not censored for patient death.

RESULTS

Our study population of 42 SPKT patients, including 24 women and 18 men, had a mean age at transplantation of 33.5 ± 6.3 years (range 23–48 years). Only two underwent preemptive SPKT. The others had pretransplant times on dialysis of 34.3 ± 24.1 months. The mean time of evolution of type I diabetes was 22.8 ± 4.2 years; eight patients were already blind due to diabetic retinopathy. Mean HbA1c before transplantation was 8.6 ± 1.6%. SPKT was performed without any HLA match with the donor in 19 patients (45.2%).

The incidence of delayed renal graft function, defined as the transient need for dialysis during the first week after transplantation, was 9.5%. It was possible to discontinue insulin administration at 1.4 ± 1.7 days after SPKT. Twelve patients were converted from MMF to sirolimus due to digestive intolerance. Acute rejection was diagnosed and treated in nine patients (21.4%): in three cases both grafts were affected; in four, only the kidney; and in two, the diagnosis was presumed, to affect only the pancreas graft. All rejection episodes were efficiently treated with steroids, except a single patient with late, recurrent rejection who needed antilymphocyte preparations for treatment. The rate of acute rejection in 2003 and 2004 decreased to 9.1% (2 in 22 patients).

Eighteen patients (42.9%) experienced postoperative surgical complications, requiring one (eight patients) or several (10 patients) surgical re-interventions. The complications were: pancreas graft thrombosis (three patients, one preceded by anastomotic leak); kidney graft thrombosis (one patient); anastomotic/intraabdominal bleeding (five patients); urinary fistula (one patient); intraabdominal abscess/anastomotic leak (six patients, one of them followed by thrombosis); wound infection (one patient); and intestinal obstruction (two patients).

For the whole group, the total hospital stay of the transplantation admission was 32.9 ± 20.9 days (range 10–102; median 25 days) and the time in the intensive care unit (ICU) was 5.8 ± 14.3 days (range 1–78; median 2 days). The length of hospitalization for the 18 patients who had surgical complications (43.8 ± 26.2 days; range 12–102; median 25 days) was significantly longer than the other 24 patients who did not have re-operations (24.8 ± 10.4 days; range 10–52; median 21.5 days); ($P = .002$). This difference was also evident when we compared ICU admission between the two groups (11.3 ± 20.9 days, range 1–78, median 3 days; and 1.7 ± 0.8 days, range 1–4; median 2 days, respectively; $P = .03$).

Two patients died. One patient's sudden death occurred 34 months after transplantation and 5 months after he became dialysis- and insulin-dependent due to late, recurrent acute rejection. This patient had a previous myocardial infarction. The other death was a consequence of sepsis due to intraabdominal abscess, complicated by a stroke occurring during the admission for SPKT.

Seven pancreas grafts and three kidney grafts were lost. The causes for pancreas loss were thrombosis (three patients); infection (three patients); or immunologic (one patient). Kidney loss was due to patient death with a functioning graft (one patient); immunologic (one patient); or thrombosis (one patient).

At their last visit, the mean serum creatinine values of the 39 patients with a functioning kidney was 0.98 ± 0.15 mg/dL

(range 0.6–1.6 mg/dL), and the creatinine clearance, 88.7 ± 22.3 mL/min (range 49.6–135 mL/min). The fasting blood glucose of the 35 insulin-free patients was 79 ± 16 mg/dL (range 42–93 mg/dL), and their HbA1c and C-peptide values were 4.7 ± 1.1% (range 3.4%–5.3%) and 3.5 ± 2.6 ng/mL (range 1.1–12.7 ng/mL), respectively.

Patient survivals at 1 and 5 years were 97.3% and 91.7%, respectively. Graft survivals, also at 1 and 5 years, were 94.6% and 89.2% for the kidney graft and 83.8% and 78.7% for the pancreas graft, respectively (Fig 1).

DISCUSSION

SPKT is the best treatment choice for type I diabetic patients with advanced nephropathy.[1] Not only is patient survival superior for SPKT when compared with KTA, but pancreatic and kidney grafts have a longer half-life in the SPKT group.[3,10] Because of the limited resource of organs, SPK is recommended to optimize allograft utilization.[10]

When this double transplant succeeds the long-term complications of diabetes, like retinopathy,[11,12] or nephropathy,[13] may be prevented or at least their progression slowed. Neuropathy is one of the complications in which the improvement is more convincing,[5,14–16] just as happens with quality of life.[1,3,17] Microvascular disease is positively affected by the transplant.[18] A recent report[19] analyzing the evolution of macrovascular disease in patients with grafts surviving 5 to 10 years concluded that there was a slower progression of macroangiopathy and related events in SPKT compared with KTA patients.

All these data were strong motivations for us to build our SPKT program, trying to offer a greater quality and quantity of life to type I diabetic patients. The balance of 5-year activity of our SPKT program is positive. Some aspects, however, may deserve further reflection.

We did not perform a positive selection of candidates. Among this group of 42 patients, there were some with coronary artery disease, with exhaustion of vascular access for dialysis, many of them blind and with a large evolution time of diabetes. They were not a low morbidity group, so this cannot explain our good results.

The short cold ischemia time, below 12 hours in all but three patients, was one of the reasons for the 90.5% rate of immediate graft function. In fact, only three patients with longer cold ischemia time and another one needed dialysis after surgery.

An acute pancreas rejection episode is a difficult diagnosis to establish without a biopsy.[3] However, the necessity to avoid additional morbidity, possibly related to the biopsy technique, led to our decision to not perform it. The price to pay was a high number of acute rejection episodes, when another diagnosis was not apparent, in the face of a patient with fever, abdominal tenderness, and increased serum amylase and lipase levels. The kidney biopsy helped in some cases; but even with this protocol of immunosuppression, pancreas acute rejection could not be excluded in others, and those patients were treated as such. The real incidence of acute rejection episodes was certainly lower than 21.4%. In fact, in the last 2 years, this incidence has decreased to 9.1%. The added experience has allowed us to wait and search more before deciding to treat; sometimes the diagnosis of acute rejection could be ruled out.

The registered rate of postoperative complications requiring surgical repair was high, but others have published a similar incidence,[20] for example, in one of the arms of the Euro-SPK Study Group. As in other reports,[21–23] abdominal complications, mostly pancreas graft related, led to increased mortality, morbidity, graft loss, and hospital stay. Pancreas graft thrombosis remains the most important cause of graft loss.[21]

One of the two patients who died had a stroke as the immediate cause of death, in the presence of sepsis due to an abdominal abscess, so the death was transplant related. The other patient with sudden death probably died due to cardiovascular disease.

When compared to KTA, SPKT improves cardiac geometry and function[24] and reduces cardiovascular risk profile,[25] cardiovascular events, and mortality.[26] In spite of that, it is the major mortality cause of SPKT patients after the first year of follow-up.[27] Our patients with surviving grafts showed normal function for both grafts. The overall patient, kidney, and pancreas survival results of this SPKT

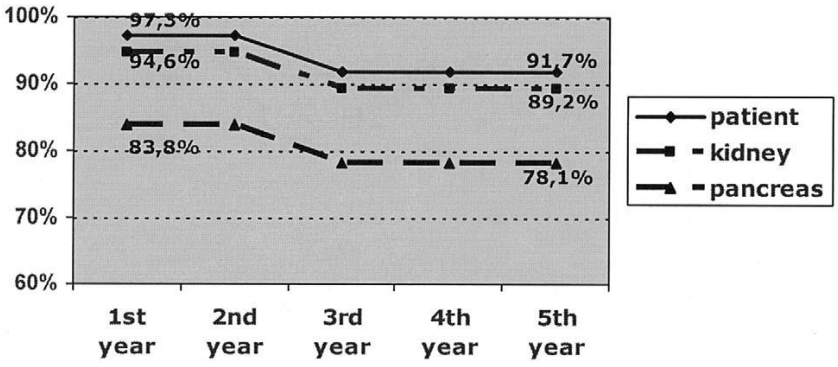

Fig 1. Patient and graft survival analysis.

group at 1 year of 97.3%, 94.6%, and 83.8%, were similar to the UNOS/IPTR data in their most recent report[28] for patients recruited from 2000 to 2004 for US cases (95%, 93%, and 85%) or non-US cases (94%, 92%, and 87%). And the 5-year results of SPKT in our unit (91.7%, 89.2%, and 78.7%) were similar to the results from other recognized centers throughout the world.[29,30]

In conclusion, our experience with SPKT has been stimulating, and we consider the 5-year results reported here as good. Although the short follow-up period may overestimate the results, these are encouraging factors to continue. We hope to be able to present a growing program in the near future.

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Resumo do artigo II.2

Os resultados do TRP foram melhorando progressivamente, parecendo ter atingido uma estabilização nos últimos anos. Depois de termos reportado a nossa experiência aos 5 anos, fomos neste trabalho analisar novamente os resultados, completados que estavam os 10 anos de atividade do programa de TRP.

Este estudo incluiu 111 TRP. A média etária, tempo de DM1 e tempos de diálise eram similares aos observados aos 5 anos. A percentagem de doentes com 6 *mismatches* HLA com o dador baixou significativamente (23,4%). Houve também uma descida na incidência de rejeição aguda (18%), encontrando-se então já disponível a possibilidade de realização de biópsia pancreática, nos casos de suspeita clínica ou de pancreatite de causa não óbvia.

A técnica cirúrgica também sofreu algumas alterações, com a anastomose direta do *patch* aórtico contendo a artéria mesentérica superior e a esplénica no recetor, evitando a necessidade de reconstruções arteriais. Como consequência, houve uma redução das complicações cirúrgicas.

De entre as causas de perda do enxerto pancreático, a infeção e a trombose permaneceram como as de maior relevo. Quanto à perda do rim, a causa imunológica emergiu como uma importante causa de falência do enxerto renal a longo-prazo.

A sobrevivência registada aos 1, 5 e 10 anos foi para o doente de 96%, 94% e 94%; para o enxerto renal de 96%, 91% e 62%; e para o enxerto pancreático de 83%, 75% e 69%, respetivamente. Estes resultados estão a par dos reportados por outros estudos envolvendo grande número de dontes e pelos registos internacionais (IPTR).

Assim, os dados globais do TRP na nossa experiência a 10 anos vieram confirmar os bons resultados apresentados no estudo prévio a 5 anos, contribuindo para consolidar e fomentar a atividade deste programa.

O artigo II.2.1. publicado no *Portuguese Journal of Nephrology and Hypertension*, que aqui se referencia (apesar de não indexado pela “ISI Web of Knowledge”, mas sim pela “SciELO”) - por se achar relevante no âmbito deste tema, da evolução clínica dos TRP - reporta os resultados globais obtidos numa amostra mais ampla, os primeiros 150 TRP da nossa Unidade, em linha com os resultados obtidos aos 10 anos.

One Hundred Eleven Simultaneous Pancreas-Kidney Transplantations: 10-Year Experience from a Single Center in Portugal

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ABSTRACT

From May 2000 to May 2010, we performed 111 simultaneous pancreas-kidney transplants (SPKT) from cadaveric donors, by using enteric drainage and systemic vascular anastomosis. In 26 cases they showed 6 HLA mismatches. Immunosuppression included antithymocyte globulin, tacrolimus, mycophenolate mofetil, and steroids. The patients' mean age was 34 ± 6 years, and mean time from diabetes diagnosis was 23 ± 6 years; 107 patients had been on dialysis for 32 ± 24 months, and 4 had a preemptive status. Acute rejection episodes were detected in 20 patients (18%): in 3 cases they affected both organs, in 9 only the kidney, and in 8 only the pancreas. The incidence of complications needing reoperation was 28.8%. They were mostly pancreas graft–related, including bleeding, thrombosis, and infection. In more recent years, after a slight modification of surgical technique, we noted a decreased rate of complications. Six patients died: 2 from cardiovascular or cerebrovascular disease, 3 from infection, and 1 from an unknown cause. Pancreas graft loss occurred in 26 and kidney graft loss in 12 patients. Four patients underwent a second pancreas and 5 a second kidney graft. Patients with surviving grafts showed good function: serum creatinine, 1.09 ± 0.23 mg/dL; fasting blood glucose, 79.7 ± 9.8 mg/dL; and HbA_{1c}, 4.88 ± 0.47%. Patient, kidney, and pancreas survival results were 96%, 96%, and 83% at 1; 94%, 91%, and 75% at 5; and 94%, 62%, and 69% at 10 years, respectively. These good results, compared with larger series and to recent pancreas transplant registry reports, are a strong motivation for the further development of this unique program in Portugal.

Simultaneous pancreas-kidney transplantation (SPKT) remains the best treatment for type 1 diabetes patients with chronic renal failure.[1] Its results can be surpassed regarding kidney graft survival in the short and medium term only by pancreas after a living kidney transplantation (PALK).[2] SPKT is still the modality with the best pancreas survivals. SPKT treats 2 severe pathologies simultaneously, diabetes and chronic renal insufficiency, releasing the patient from insulin injections and dialysis.

SPKT results have significantly improved over the past decades,[3] owing to technical advances, new immunosuppressive agents, and increased knowledge regarding management of complications. The last International Pancreas Transplant Registry (IPTR) reports[4,5] now show a stabilization in SPKT results, with 95%, 92%, and 85% 1-year survivals for patient, kidney, and pancreas, respectively.

In the present paper, we describe our SKPT program activity, evolution, and overall results since its inception 10 years ago.

PATIENTS AND METHODS

From the beginning of the program on May 2, 2000, until May 2, 2010, we performed 111 SPKT on type 1 diabetes patients with chronic renal failure. Except for 1 patient who had undergone a previous isolated kidney graft, this procedure was their fi transplantation. The graft source was always a cadaveric donor. Regarding surgical technique, all pancreas transplants were performed by using enteric drainage of exocrine secretions and vascular anastomosis to the iliac vessels; the kidney graft followed the usual technique.

The immunosuppressive protocol included antithymocyte globulin (ATG), tacrolimus, mycophenolate mofetil (MMF), and steroids. Infection prophylaxis included a third-generation cephalosporin, vancomycin, and fluconazole during the initial days followed

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by oral valgancyclovir and cotrimoxazole for 6 months. For thrombosis prophylaxis, we used oral aspirin before surgery, adding subcutaneous low-molecular weight heparin in the immediate postoperative period and during the hospitalization. After discharge, oral aspirin was continued with daily doses of 50 mg.

Pancreas graft biopsies were not performed in the initial years. The diagnosis of acute rejection episodes was inferred from clinical, laboratory, and imaging data. More recently, we began to perform guided pancreas biopsies to clarify the cause of pancreas graft dysfunction or infl A kidney graft biopsy has always been performed in the event of kidney and/or pancreas dysfunction.

The permanent need for dialysis or insulin, a retransplant, or patient death was considered to be graft loss.

After hospital discharge all patients were regularly followed at our outpatient clinic. Also during hospital admissions, SPKT patients were always treated in our inpatient unit.

Basic statistical results are expressed as mean values ± standard deviations. Survival curves for patient and renal and pancreas grafts, were calculated using the Kaplan-Meier method. We used SPSS statistical software, version 12.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

The 111 SPKT patients, including 63 women and 48 men, had a mean age of 34 ± 6 years and a mean time since diabetes diagnosis of 23 ± 6 years. In 4 cases, the procedure was a preemptive transplantation; the other 107 patients had been on dialysis for 32 ± 24 months, the majority (78.4%) of whom were on hemodialysis. In terms of glycemic control before the transplantation, their mean required dose of exogenous insulin was 39 ± 12 U/d. They had a mean HbA_{1c} of 8.5 ± 1.7%.

The mean donor age was 27 ± 11 years (range, 8–49 y). There were 6 HLA mismatches with the donor in 26 SPKT (23.4%).

The incidence of delayed renal graft function, namely, a transient need for dialysis during the first week after SPKT, was 17.1%. In the majority of patients with a successful pancreas transplantation, insulin administration was stopped during the first 24 hours after surgery (range, 0–18 days; median, 0 days).

Acute rejection incidence was 18% (20 patients). In 3 subjects, both grafts were affected, in 9 only the kidney, and in 8 only the pancreas. In 12 cases, the episode was successfully treated with steroids only, in 5 with ATG (or OKT3 in the first years), and in 3 (humoral rejection episodes) with plasmapheresis plus immunoglobulin (IV), with rituximab in 2 instances.

Thirty-two SPKT patients (28.8%) needed reoperations (1–12 reoperations/patient). The initial causes for surgical reintervention were bleeding (n = 11), infection (n = 10), thrombosis (n = 8: 5 pancreatic and 3 renal), intestinal occlusion (n = 2), and urinary leak (n = 1). We noted a decrease in the reoperation rate in recent years, possibly due to a modification in our vascular technique. During the first years, the arterial supply to the pancreas graft was provided by an arterial reconstruction, as described by Fernandez Cruz (University of Barcelona) in the published IPITA center survey[6] of pancreas transplantation techniques. Then, an anastomosis between the donor superior mesenteric and splenic arteries was made, resulting in an arterial arcade feeding the entire pancreas. Owing to difficulties or complications, and because the aortic segment containing the celiac trunk and the superior mesenteric artery was never used in our liver transplant program, our surgical team (Manuel Teixeira, MD, and Rui Almeida, MD, both of whom are also involved in the liver transplant program) decided to use it for the pancreas transplant. Since 2005, this aortic patch with the entire arterial supply to the pancreas graft is directly anastomosed to the recipient common iliac artery, with no further arterial reconstruction. Thereafter, we experienced a lower incidence of surgical complications related to the pancreas graft: 14/42 patients (33.3%) during the first 5 versus 12/69 (17.4%) during the last 5 years.

The median hospital stay for the SPKT procedure of the 111 patients was 21 days (range, 8–148 d), including (median) 2 days in the intensive care unit (range, 1–88 d).

Six patients died. One patient with known cardiovascular disease experienced sudden death at 34 months after transplantation. He had already been on dialysis for 5 months after loss of both grafts due to recurrent acute rejection. Another subject died due to a stroke, and 3 others had infections, including 2 in the postoperative period with abdominal sepsis and 1 with aspergillosis. In another case, the cause was unknown.

Twelve patients lost their kidney and 26 their pancreas graft. The causes of kidney loss were thrombosis (n = 3), infection (n = 1), immunologic (n = 5: 2 acute and 3 chronic rejection), and death with a functioning kidney (n = 3). Pancreas loss was due to thrombosis (n = 9), immunologic (n = 5; all acute rejections), graft or perigraft bleeding (n = 3), pancreas-related infection (n = 6), death with a functioning graft (n = 2); and in 1 instance possibly relapsed autoimmune pancreatic disease. As a note, among these patients, 5 received kidney retransplantations and 4 pancreas retransplantations. However, because the purpose of the present paper was the analysis of SPKT results, we did not include data from the retransplantations.

At the last visit, the mean serum creatinine among the 99 SPKT patients with functioning kidneys was 1.09 ± 0.23 mg/dL, with a creatinine clearance of 80.6 ± 26.4 mL/min and a urinary protein excretion of 0.29 ± 0.26 g/d. The 85 SPKT patients with a functioning pancreas showed a fasting blood glucose of 79.7 ± 9.8 mg/dL, HbA_{1c} of 4.88 ± 0.47%, and C-peptide of 3.29 ± 1.59 ng/mL.

Survival rates at 1, 5, and 10 years were, respectively, 96%, 94%, and 94% for patients; 96%, 91%, and 62% for kidneys (censored for patient death); and 83%, 75%, and 69% for pancreas (censored for patient death) (Table 1).

DISCUSSION

Recent analysis of living-donor kidney transplantation (LDKT) compared with SPKT for type 1 diabetes showed

Table 1. Simultaneous Pancreas-Kidney Transplantation: 10-Year Survival Results

	1 y	2 y	3 y	4 y	5 y	6 y	7 y	8 y	9 y	10 y
Patient	96%	96%	94%	94%	94%	94%	94%	94%	94%	94%
Kidney*	96%	96%	93%	93%	91%	91%	87%	87%	87%	62%
Pancreas*	83%	82%	81%	79%	75%	73%	69%	69%	69%	69%

*Data censored for patient death with a functioning graft.

good results in terms of kidney survival[2,7]; taking in account the organ shortage,[8] LDKT should therefore be considered whenever there is an available living donor.[7,8] However, data comparing LDKT and SPKT are not unanimous: short-term kidney results for LDKT have been reported to be better,[2,7] but this advantage disappears in the long term (10 y) when they are similar.[8] The benefits of LDKT for initial patient survival observed by some authors[2,7], have not been confirmed in the long term.[8,9] Moreover, pancreas survival after PALK is lower[2] than with SPKT. In fact, the other modalities of pancreas transplantation—pancreas alone or pancreas after kidney—consistently show a poorer prognosis than SPKT.[4,5,10]

The outcome for type 1 diabetes patients transplanted with kidneys from cadaveric donors is worse than with SPKT.[9] Therefore, from the perspective of rational administration of each available kidney and pancreas from a deceased donor, it is of greater benefit to perform SPKT.[11] This conclusion also derives from the recently developed scores of "life years from transplant" (LYFT scores), which estimate the years of life incremented by each transplantation.[12] For diabetics, LYFT scores are higher among subjects receiving an SPKT than those receiving a kidney alone.[12] In summary, and as stated again by Sollinger et al in 2009,[13] SPKT remains the treatment of choice for type 1 diabetes patients with advanced nephropathy.

Results of SPKT have improved over the past decades.[3] Not only advances in the immunosuppressive therapy, but also changes in surgical technique have contributed to this improvement. In our own experience, complications (bleeding, thrombosis) tend to be less frequent in recent years. The modification of the vascular technique described above, coincided with the reduced complication rate.

The major causes of pancreas graft loss are usually technical failures[10] that occur early after transplantation. We observed the same problem: 15/26 pancreas losses (57.7%) were secondary to bleeding or thrombosis. When the pancreas was functioning at 1 year, its long-term viability increases significantly.[14,15] All of the registered immunologic pancreatic losses in our unit were late losses (>1 year) with 3/5 due to medication noncompliance by the patient.

Chronic or acute kidney rejection leading to graft loss occurred in 4.5% of patients over these 10 years, which was a low rate. Our immunosuppressive protocol is the one most commonly used for SPKT around the world,[5] namely, antibody induction, tacrolimus, MMF, and steroids. Steroid withdrawal after the sixth month is our current practice. The acute rejection rate of 18% may be overestimated, because at the beginning of the program we did not perform pancreas biopsies. Based only on clinical, laboratory, and imaging data, we assumed and treated as rejection some of episodes without histologic confirmation.

The most frequent causes of death among SPKT patients after the first year are cardiovascular and cerebrovascular diseases.[13] In our population, 2 patients died from these causes, both with >1 year of follow-up. The other 4 deaths, included 3 from infection and 1 from unknown cause, all within a few months after transplantation. Early mortality in SPKT is mostly transplant related.

Patients with surviving grafts showed normal transplant function. The overall survival results of our SPKT population, at 1 year, (96% for patient, 96% for kidney, and 83% for pancreas) were not different from the data reported by the IPTR[4,5]: 95% for patient, 93% for kidney, and 85% for pancreas. The global outcome and 10-year survival results presented herein were consistent with those presented in our 5-year report.[16] The University of Wisconsin recently published long-term results for 1,000 SPKT,[13] showing 10-year survivals of 80% for the patient, 63% for the kidney, and 63% for the pancreas. The last report on long-term survival from the IPTR revealed a 51% 10-year pancreas survival for SPKT. Compared with larger series from other centers and from the IPTR, our 10-year survival rates—94% for patient, 62% for kidney, and 69% for pancreas—are good and encouraging results.

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ORIGINAL ARTICLE

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Pancreas-Kidney Transplantation: Analysis of 150 patients from one Centre in Portugal

Transplantação Reno-Pancreática: Análise de 150 doentes de um Centro em Portugal

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ABSTRACT

Introduction: Simultaneous pancreas-kidney transplantation (SPKT) outcomes are conditioned in the short-term mostly by post-operative complications. In the long-term, cardiovascular (CV) disease and immunological loss are the main limitations to transplant survival. **Aims:** To analyse retrospectively the results from 150 SPKT performed at our centre. **Patients and Methods:** The 81 females and 69 males had a mean age of 35±6 years; they were diabetic for 24±6 years and had been on dialysis for 30±21months (except 5 preemptive). Anti-lymphocyte globulin, tacrolimus, mycophenolate and steroids were used as immunosuppressive therapy. Deceased-donor mean age was 28±11 years. In 28.7% the transplant was performed with 6 HLA-mismatches. **Results**: Acute rejection's incidence was 16%. Ten SPKT patients died; infection was the leading cause of death (five cases), followed by Cardiovascular/cerebrovascular disease (three cases). In 21 patients the pancreas failed, mainly due to thrombosis or bleeding (11 cases), and infection (five cases); in two it was due to late acute rejection. In four patients only the kidney failed, due to chronic rejection. Five patients lost both grafts, from late acute rejection in four and thrombosis in one. We analyzed the 110 SPKT patients (73.3%) with both grafts functioning. Their mean serum creatinine was 1.2±0.4mg/dl; creatinine-clearance was 76±24 ml/min; fasting glycaemia was 81±10mg/dl; and HbA1c was 5.3±0.4%. Hypertension has been treated in 47.2% of patients, in the majority (28.2%) with only one drug. Hyperlipidaemia was observed in 19.1% and excessive weight (›25kg/m2) in 17.3%. **Conclusions**: From our cohort of SPKT, 93.3% of patients are alive, 73.3% have both grafts functioning. Rejection was the main cause of late pancreas loss. Early mortality was due to infection (3.3%). CV/cerebrovascular disease was the main cause of late mortality (2%). The prevalence of hyperlipidaemia and overweight was inferior to 20%. Hypertension was the most frequently found CV risk factor.

Key-words: graft loss; long-term results; pancreas-kidney transplantation; patient death

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RESUMO

Introdução: A sobrevivência do transplante de rim-pâncreas (TRP) é condicionada na fase precoce pelas complicações inerentes ao próprio acto cirúrgico. A sua perda tardia deve-se essencialmente a morte por doença cardiovascular (CV), ou imunológica. **Objectivos**: Analisámos retrospectivamente os resultados dos 150 TRP realizados no nosso centro. **Doentes e métodos**: Os 81 doentes do sexo F e 69 do sexo M, tinham uma idade média de 35±6 anos; eram diabéticos há 24±6 anos; e estavam em diálise há 30±21meses (excepto 5 *preemptive*). A terapêutica imunossupressora consistiu em globulina anti-linfocítica, tacrolimus, micofenolato e corticóides. A média de idades do dador (cadáver) foi 28±11 anos. Em 28.7% o transplante foi realizado com 6 incompatibilidades HLA. **Resultados**: A incidência de rejeição aguda foi de 16%. Faleceram 10 doentes: a causa mais frequente foi a infecciosa (5 casos), seguida da CV/cerebrovascular (3 casos). Em 21 casos houve falência isolada do pâncreas maioritariamente por trombose ou hemorragia (11 casos) e por infecção (5 casos), em 2 casos por rejeição aguda tardia. Em 4 TRP ocorreu falência isolada do rim, por rejeição crónica. Em 5 casos ambos os enxertos faliram: por rejeição aguda tardia 4 doentes; trombose 1 doente. Os 110 TRP (73.3%) que mantêm ambos os enxertos funcionantes, têm creatinina de 1.2±0.4mg/dl; clearance da creatinina de 76±24 ml/min; glicemia em jejum de 81±10mg/dl; e HbA1c de 5.3±0.4%. Apresentam hipertensão 47,2%, a maioria (28,2%) requerendo apenas 1 fármaco. Hiperlipidemia verificou-se em 19,1% e excesso de peso (>25kg/m2) em 17,3% dos doentes. **Conclusões**: Deste grupo de TRP estudado, estão vivos 93.3% e 73.3% têm ambos os enxertos funcionantes. Rejeição foi a principal causa de perda tardia do pâncreas. A mortalidade precoce deveu-se a infecção (3.3%). Doença CV/cerebrovascular foi a causa mais frequente de mortalidade tardia (2%). A prevalência de hiperlipidemia e excesso de peso foi inferior a 20%. Hipertensão foi o factor de risco CV mais frequentemente encontrado.

Palavras-chave: morte do doente; perda de enxerto; resultados a longo-prazo; transplantação reno-pancreática

INTRODUCTION

Simultaneous pancreas-kidney transplantation (SPKT) is the best treatment for type 1 diabetic patients with end-stage renal disease who have the conditions for this kind of transplant. The results of SPKT are better than those obtained from other modalities of pancreas transplantation, such as pancreas after kidney (PAK), or pancreas transplantation alone (PTA)[1]. A successful SPKT frees the patient from insulin and dialysis-dependence and avoids the life-threatening hypoglycaemic episodes. This may represent a significant improvement on their impaired quality of life. It is unquestionable that SPKT leads to a significant improvement in patient survival, when compared to those staying under dialysis and insulin, or even compared to those who underwent a cadaveric kidney alone transplant[2]. Moreover, the pancreas transplant may stop the progression or even ameliorate the various secondary diabetic complications[1,2].

Outcomes of SKPT have improved over time, in parallel with other organ transplants[1,2,3]. However, patient and graft loss after SPKT, namely in the early phase after surgery, is higher than in kidney transplantation alone. Thrombotic and infectious complications are the leading causes of graft failure or even patient death in the short-term[1]. Cardiovascular or cerebrovascular disease[2,3] and also infection[1,2] are the main limitations for long-term SPKT patient survival. Immunological loss[3] and death with a functioning graft[3] represent the main causes of graft failure in the long-term. It is well known for these patients the increase in mortality after graft loss: the relative risk of death increased more than 17-fold in recipients whose kidney failed and more than 3-fold in recipients whose pancreas failed[1].

The aim of this study was to analyze the outcome of the SPKT performed at our centre and to search for possible factors associated with the outcome.

PATIENTS AND METHODS

From May 2000 to October 2012, 150 type 1 diabetic patients underwent SKPT at the Transplantation Department of our Hospital. All the procedures were performed using grafts form deceased donors, both grafts from the same donor, and using systemic-enteric drainage (venous drainage to the iliac vein; exocrine drainage through an enteric anastomosis of the pancreatic-duodenal arch). Only patients with a minimum follow-up of 3 months were considered for this retrospective analysis.

Results are presented as mean ± standard deviation for continuous, normally distributed variables, and as percentages for categorical data. Patient survival was determined from the time of SPKT until death or end of follow-up. Death-censored kidney graft survival was determined from the time of kidney transplantation until kidney retransplantation, return to dialysis, or end of follow-up. Death-censored pancreas graft survival was determined from the time of SPKT until pancreas failure or end of follow-up. Survival analysis was performed using the Kaplan-Meier (product-limit) estimator of survival.

These 150 patients, 81 females and 69 males, had a mean age of 35±6 years at transplantation date. They were diabetic for 24±6 years; and had been on dialysis for 30±21months, excepting five patients who received a pre-emptive transplant. Anti-lymphocyte globulin, tacrolimus, mycophenolate and steroids were used as induction therapy. Deceased-donor mean age was 28±11 years. In 28.7% of the patients the transplant was performed with 6 HLA-mismatches.

RESULTS

The median admission time was 20 days. Global acute rejection incidence (for kidney, pancreas or both grafts) was 16%. Delayed kidney graft function, defined for dialysis need in the first week, occurred in 16%. In the vast majority of patients whose pancreas did not have complications, insulin administration could be stopped during the first hours after transplantation (median 0 days).

In 21 patients the pancreas failed, mainly (16 cases) in the very early period (<3 months) due to thrombosis (eight cases) or bleeding (three cases) and local infection leading to graft removal (five cases); late pancreas loss was due to late acute rejection (two cases), atheroembolism after coronary angiography using lower limb access (one case) and due to unknown cause in two cases.

In four patients only the kidney failed, due to chronic rejection, and these were late losses. In five others, both grafts failed: in one case it was an early loss due to thrombosis of both grafts; and in four it was due to late (> 6 months) acute rejection – in three cases we have confirmed patient non-compliance to medication. Tacrolimus trough levels were undetectable or markedly below the expected for this follow-up time (7-9 ng/ml).

Death occurred in 10 patients: in five during the early period, and later in five others. Analyzing the causes of patient death from the point of view of the different periods (< 12months vs. > 12 months): early deaths were due to post-operative sepsis (three cases), aspergillosis (one case), and unclear cause (one case); late deaths were caused by myocardial infarction (two cases), stroke (one case), CMV disease (one case) and digestive haemorrhage (one case). Globally, infection was the leading cause of death (five cases), followed by cardiovascular (CV) or cerebrovascular disease (three cases). Table 1 summarizes the causes of graft failure and patient death.

At the last visit, 110 SPKT patients (73.3%) maintained both grafts functioning. Their mean serum creatinine is 1.2±0.4mg/dl; creatinine clearance is 76±24 ml/min; fasting glycaemia is 81±10mg/dl; and HbA1c=5.3±0.4%. Hypertension has been treated in 52 SPKT (47.2%), in the majority (31SPKT – 28.2%) with a single drug, mostly a beta-blocking agent. Hyperlipidaemia was observed in 23 patients (20.9%) and excessive weight (> 25kg/m2) in 19 patients (17.3%).

Survival rates (death-censored) obtained for this cohort of SPKT at our centre for patient, kidney and pancreas, respectively, were: at 1 year 97%, 97% and 85%; at 5 years 94%, 93% and 78%; and at 10 years 91%, 79% and 69% (Graph 1).

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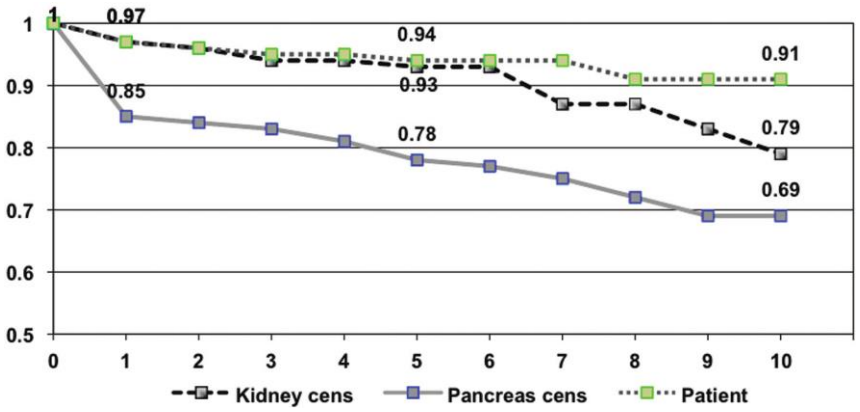
Table 1

Causes of graft failure and patient death.

(Total =150 SPK)	Timing of occurrence	Main cause	Specific cause
Pancreas loss (n=21)	Very early* loss (n=16) *(follow-up <3 months)	Thrombosis (n=8)	Pancreas graft thrombosis (n=8)
		Bleeding (n=3)	Peri-graft bleeding (n=3)
		Infection (n=5)	Pancreatic leak/ abdominal infection (n=5)
	Late loss (n=5)	Acute rejection (n=2)	Unsolved acute rejection (n=2)
		Atheroembolism (n=1)	(after coronariography)
		Unknown (n=2)	
Kidney loss (n=4)	Late* loss (n=4) *(>6 months)	Chronic rejection (n=4)	
Pancreas and kidney loss (n=5)	Early loss (n=1)	Thrombosis (n=1)	Pancreas and kidney graft thrombosis (n=1)
	Late* loss (n=4) *(>6 months)	Acute rejection (n=4)	Non-compliance confirmed (n=3)
			Non-compliance unconfirmed (n=1)
Patient death (n=10)	Early death (n=5)	Infection (n=4)	Post-operative sepsis (n=3)
			Aspergillosis (n=1)
		Unknown (n=1)	
	Late* death (n=5) *(follow-up >12 months)	Infection (n=1)	CMV disease (n=1)
		Cardiovascular / cerebrovascular disease (n=3)	Stroke (n=1)
			Myocardial infarction (n=2)
		Digestive haemorrhage (n=1)	



Graph 1

Patient and graft survival rates of the 150 SPKT (death-censored)

DISCUSSION

There are no longer any doubts that SPKT offers the best results to treat type 1 diabetic patients with end-stage renal disease. The last position statement of the American Diabetes Association[4] has confirmed, again, their previous recommendation[5] of pancreas transplantation for these patients, preferably done simultaneously to a kidney transplant, given the better pancreas survival of SPKT[4,5]. Also from a point of view of quality of life, SPKT can provide significant benefit[6,7]. The successive International Pancreas Transplant Registry (IPTR) reports have confirmed the progressive improvement in SPKT outcome over

the past decades[1,8,9]. However, it seems that a maximum and a plateau was reached in the survival curves, because no further significant improvements have been observed in the last 10 years[1].

Pancreas graft outcome is determined mostly by the success in early post-operative period. Thrombosis, bleeding, pancreatic leaks and infection after surgery are normally defined as technical failures. In the last IPTR[1], near 9% of the losses were reported as due to technical failure. It is 10.7% in our own experience (16/150 patients), not too different from the international results recorded on 25000 SPKT[1]. Thrombosis remained the main cause of early pancreas graft loss, as reported by large centers[2,3].

Pancreas losses due to acute rejection were observed in our study group in 6 SPKT (4%), but in half of them non-compliance of the patients to immunosuppression was verified. Blindness or very impaired vision may be a real problem for these patients to strictly follow the medication and this was the case in at least one patient. Thus, if we exclude the "expected" losses associated with confirmed/confessed non-compliance, we obtain a rate of 2% of "unexpected" acute rejection loss, similar to that observed in large series[1,8].

Kidney graft loss occurred in the late period in all but one of the 9 patients of our study group with kidney failure. Rejection was the main cause of these late losses and this has also been observed by others[2,3].

Systemic vascular disease, CV and cerebrovascular disease are strongly associated with an increased risk of patient death[2]. In our own experience, infection in the early period (3.3%) and CV/cerebrovascular disease (2%) in the late period were the leading causes of patient death. These same reasons for patient death were reported by the IPTR[1]; and the authors observed that death due to infection peaked between 3 and 12 months.

Hypertension was the most frequently found CV risk factor, although its prevalence was inferior to 50% and in only 28.2% requiring more than one drug. Beta-blocking agents were the most often prescribed drugs with anti-hypertensive properties, even when the goal is not necessarily to treat hypertension. In fact, in some of these patients high blood pressure was not recorded. However, it is our policy to maintain this medication, given its cardio-protective effects, especially if the patients had previously been under this drug. The prevalence of hyperlipidaemia and excessive weight was inferior to 20% in our SPKT patients.

Compared to kidney transplantation alone, SPKT is known to have more complications: a higher rate of readmissions[10] and early surgical complications that may lead to relaparotomy in more than 30% of cases[3,11]. Despite these feared complications and the initial increased risk (compared with those patients in the waiting list for transplant), transplanted patients perform better at 1 year[12]. Moreover, and against initial concerns, 10-year results do not confirm a higher mortality of diabetics after SPKT versus kidney transplantation alone[13]. The improvement in quality of life and the effect on long-term diabetic complications justify the option for surgery[12]. The developed LYFT scores (life years from transplant), which assess life enhancement achieved by each transplant, was greater for diabetics with a SPKT than with kidney transplantation alone[14]. Thus, it is currently stated that SPKT is the most cost-effective treatment for patients with type 1 diabetes and end-stage renal disease[15].

The authors of the latest IPTR reported a 10-year patient survival over 70%[16]. Considering only SPKT recipients who reached the 1-year mark with both grafts alive, they reported a 10-year survival rate around 66 % for the kidney and 62% for the pancreas grafts. In a more recent analysis, the 10-year pancreas graft function for SPKT was 68%[16]. We have published the 10-year results of SPKT at our centre two years ago[17] and they were not inferior to the international results. Those good outcomes and survival rates are substantiated by the results obtained in this cohort of patients with extended follow-up.

Conflict of interest statement: none declared

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Resumo II.3

Os corticóides fazem comummente parte da terapêutica imunossupressora após transplante de órgãos sólidos. Porém, são conhecidos os seus indesejáveis efeitos laterais: HTA, insulino-resistência, dislipidemia, osteoporose, fraturas ósseas, cataratas, entre outras. Em doentes que durante décadas foram diabéticos e que sofreram de insuficiência renal, estes efeitos acrescentam-lhes riscos, nomeadamente CV, a sua principal causa de morte.

Por outro lado, a suspensão de corticóides após TRP pode representar um risco aumentado de rejeição aguda ou crónica, pelo que deve ser realizada com precaução.

Ainda assim, se exequível, a retirada de corticóides pode ser benéfica em vários Aspetos (ósseo e CV, por exemplo). Segundo o protocolo adotado, que inclui a indução com globulina anti-timocítica, a retirada dos corticóides inicia-se findos os primeiros 6 meses do TRP, em doentes que não sejam de alto risco imunológico e que mantenham terapêutica combinada com tacrolimus e micofenolato ou sirolimus.

Numa análise retrospetiva de 77 doentes, a suspensão de corticóides foi concluída até aos 12 meses em 77,8%, sem registo de episódios de rejeição aguda. As causas mais frequentes de decisão de não suspensão completa foram episódio prévio de rejeição, ou infeção importante que implicou a suspensão de micofenolato ou sirolimus.

Quando comparados os 2 subgrupos, com e sem corticóides, não houve diferenças na sobrevivência do doente ou dos enxertos, nem na sua função. As sobrevivências obtidas enquadram-se nos *standards* internacionais. Embora os valores médios dos lípidos séricos analisados, do IMC e a percentagem de doentes com necessidade de terapêutica anti-hipertensora pareçam ligeiramente superiores no grupo com corticóides, estas diferenças não atingem significado estatístico. De realçar, todavia, que a prevalência de HTA, dislipidemia, ou excesso de peso foi globalmente baixa, quando comparada com a reportada para o TR isolado, sob esta mesma imunossupressão.

Demonstrámos neste estudo que a minimização da exposição aos corticóides é exequível e segura, em doentes selecionados. Com os resultados obtidos - inexistência de rejeições e estabilidade na função dos enxertos - a suspensão de corticóides com o propósito de reduzir o risco CV a médio e longo prazo, manter-se-á como protocolo da Unidade. Contudo, esta é uma estratégia que deve ser individualizada.

Steroid Withdrawal in Simultaneous Pancreas-Kidney Transplantation: A 7-Year Report

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ABSTRACT

Simultaneous pancreas-kidney transplantation (SPK) is the treatment of choice for selected diabetic patients with end-stage renal disease. Maintenance steroid therapy is associated with significant morbidity and mortality among SPK transplant recipients. Steroid withdrawal regimens are becoming more common, albeit with reservations regarding its safety and efficacy. We performed a retrospective review of 77 SPK transplant recipients from May 2000 to December 2007. The subjects received induction therapy with thymoglobulin followed by maintenance immunosuppression with tacrolimus and mycophenolate mofetil. A late steroid withdrawal protocol was adopted. The rates of acute rejection, graft and patient survival, and side effects were analyzed. One-year patient, kidney, and pancreas survivals were 93%, 91%, and 86%, respectively. Eleven patients experienced acute rejection. Mean follow-up time was 1155.5 ± 776.1 days. Prednisolone withdrawal was carried out between 6 and 12 months posttransplantation in 42 patients (77.8%) with at least 1 year follow-up; no case of acute rejection occurred. At present, 72 patients have a functioning kidney graft, and 65 patients also have a functioning pancreas graft. The mean serum creatinine is 1.12 ± 0.49 mg/dL and the mean HbA1c concentration is 4.5% ± 0.4%. The patients have a low prevalence of hypertension, hyperlipidemia, and obesity. Steroid withdrawal was successful and safe in the majority of in-study patients and safe without an increase of immune events. Our patient and graft outcomes are within other international SPK transplant units standards.

SIMULTANEOUS PANCREAS-KIDNEY (SPK) transplantation has been developed for patients suffering from type 1 diabetes mellitus and end-stage renal disease. Patients are generally given conventional immunosuppressive treatment after transplantation, using steroids, calcineurin inhibitors, and antagonists of purine metabolism.[1,2] Most centers also use induction with antilymphocyte preparations.[3,4]

The use of steroids for maintenance therapy in solid organ transplant recipients is common, despite their detrimental effects on osteoporosis, bone fractures, arterial hypertension, insulin resistance, and cataracts.[5,6] These side effects among patients undergoing SPK transplantation are particularly troublesome, given their long course of type 1 diabetes and their increased cardiovascular risk. The minimization or withdrawal of steroids in these patients is, therefore, an objective. Nevertheless, late steroid withdrawal has been reported to be associated with a significant risk of acute rejection episodes and/or functional adrenal impairment.[7–9] Herein we have presented our experience with the safety and feasibility of corticosteroid withdrawal among SKP transplant recipients.

PATIENTS AND METHODS

From May 2000 to December 2007, 77 C-peptide-negative patients with type 1 insulin-dependent diabetes mellitus underwent SPK transplantation. All subjects received induction therapy with antithymocyte globulin (Fresenius) administered at 3 mg/kg for 7 to 10

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doses; tacrolimus at a dose of 0.1 mg/kg twice daily; mycophenolate mofetil (MMF) 2 g/d; and intravenous methylprednisolone for 4 days (day 1: 500 mg; day 2: 250 mg; day 3: 250 mg; and day 4: 125 mg). The maintenance immunosuppression included tacrolimus (target trough level- 8–14 ng/mL), MMF adjusted according to tolerability, and oral prednisolone started (day 5) at 1 mg/kg/d and tapered to 10 mg/d by the end of the third month after transplantation. A further taper of the prednisolone dose was made after 6 months posttransplant when clinically feasible. Full steroid withdrawal was evaluated after 6-month follow-up, considering the exclusion criteria of a previous acute rejection episode, gastrointestinal intolerance to MMF, and clinical context to avoid MMF namely, previous severe infectious episodes and the presence of BK virus nephritis. It was started at 6 months with the purpose of complete steroid suspension by the end of the first year.

We performed a retrospective review of recipient demographic and clinical data, SPK transplantation details, patient and allograft outcomes, as well as serial laboratory measurements. Delayed graft function was defined as the need for dialysis during the first week after transplantation. Biopsy-proven rejection was diagnosed using the Banff criteria. No pancreatic biopsy was made.

Statistical Analysis

Descriptive statistics were expressed as mean values ± standard deviations or median (interquartile range). Differences between patients with versus without steroids were tested by the nonparametric Mann-Whitney *U* test due to the small number of patients with steroids. Categorical variables were analyzed with the chi-square or Fisher exact probability test. Patient and graft survivals were estimated using a life table statistical technique. A *P* value less than .05 was considered significant. Data were analyzed using the SPSS 15.0 statistical package.

RESULTS

We reviewed the clinical records of the 77 SPK transplants performed in our unit between May 2000 and December 2007. Average age at the time of transplantation was 33.7 ± 6.3 years. Time on dialysis was 34 ± 26 months. Four patients underwent premptive transplantation. Forty-eight patients were women. Mean diabetes mellitus duration had been 22.7 ± 5 years. Mean body mass index was 22.5 ± 2.8 kg/m^2. Nineteen patients had no HLA match with the donor. Average donor age was 22.3 ± 11.1 years.

Sixteen patients (20.8%) experienced delayed graft function. Acute rejection within the first month posttransplant occurred in 11 patients (14.3%); the diagnosis was clinically presumed or biopsy-proven; nine were corticosteroid-sensitive; one was a humoral rejection treated with plasmapheresis and human immunoglobulin; and one a late, refractory acute rejection treated with OKT3. Except for this case, no other acute rejection episode was documented after the first month. Median hospitalization time was 23 days, with 2-day stay in the intensive care unit. Early failure of pancreas and kidney grafts (<3 months) occurred in 12 and 2 patients, respectively.

Mean follow-up time is 1155.5 ± 776.1 days (range = 60–2779 days). Seventy-two patients have a functioning kidney graft, and 65 also a functioning pancreas graft. Among the 72 patients with at least one functioning graft, 27 had to change their maintenance immunosuppression from MMF to sirolimus (n = 23) or mycophenolic acid sodium (n = 4), because of digestive side effects. In four cases, both MMF and sirolimus were suspended: three patients had a severe infection event and one, thrombotic microangiopathy. These patients were maintained on tacrolimus and prednisolone.

The objective of full steroid withdrawal at 1 year was accomplished in 42/54 patients (77.8%) with more than 1-year follow-up. No acute rejection episode was registered, nor any case of adrenal failure. Hence, 12 patients maintained steroids beyond the first year posttransplant. The most common cause for failure to withdraw was a previous acute rejections episode (n = 6); a significant infectious event that determined the suspension of MMF or sirolimus (n = 4); BK-driven nephritis with the need for a low-dose or even suspension of MMF therapy (n = 2). Patient and graft survivals among this subset of patients at 2 and 5 years are presented in Table 1.

The patients with a functioning pancreas graft (n = 65) showed normal fasting glycemia and an average HgA1c of 4.5% ± 0.4%. Patients with a functioning kidney graft (n = 72) displayed a mean serum creatinine of 1.12 ± 0.49 mg/dL, a mean 24-hour measured creatinine clearance of 75.1 ± 25.8 mL/min, and a mean 24-hours proteinuria of 0.3 g. Only two patients have a serum creatinine higher than 2 mg/dL. No significant differences were observed concerning renal and pancreas graft function between patients with versus without steroids (Table 2).

Considering all patients in the follow-up, the average weight gain was 1.5 kg since the transplant. At the last visit, the mean total cholesterol was 166 ± 34 mg/dL; the mean triglyceride level, 97 ± 47 mg/dL, and the mean high-density lipoprotein cholesterol, 58 ± 17 mg/dL. Only 12 patients need statins and 15, some antihypertensive drug. Serum lipids compared between groups with versus without steroids showed no significant differences (Table 2). Patient and graft survivals at 1 and 5 years are shown in Table 3.

DISCUSSION

The use of steroids as a maintenance immunosuppressive drug in solid organ transplantation is still common, al-

Table 1. One-Year Follow-up Patients: Patient and Graft Survivals at 2 and 5 Years, According to Steroid Strategy

	2 Years (%)	5 Years (%)
Steroid-free (n = 42)		
Patient	100	98
Kidney graft	98	98
Censored kidney graft*	98	98
Pancreas graft	95	90
Steroid-based (n = 12)		
Patient	92	83
Kidney graft	92	83
Censored kidney graft*	100	100
Pancreas graft	83	75

*Death-censored kidney allograft survival.

Table 2. Graft Function and Metabolic Data Comparison Between Steroid-Based and Steroid-Free Groups

	Steroid-Based (n = 12), median (IQ)	Steroid-Free (n = 42), median (IQ)	P
Serum creatinine (mg/dL)	1.15 (0.83–1.60)	1.10 (0.88–1.30)	.41*
Urea (mg/dL)	54 (45–72)	49.0 (43–62)	.37*
Fasting glycemia (mg/dL)	78 (70–85)	81 (74–87)	.49*
HbA1c (%)	4,8 (3.8–4.9)	4.5 (4.2–4.8)	.92*
Total cholesterol (mg/dL)	172 (151–186)	165 (139–194)	.61*
Triglycerides (mg/dL)	82 (75–98)	81 (64–117)	.68*
HDL cholesterol (mg/dL)	61 (40–74)	54 (42–61)	.33*
Weight (kg)	59 (49–65)	58 (52–66)	.73*
BMI (kg/m^2)	23.8 (22.0–26.1)	22.6 (21.1–25.1)	.57*
Antihypertensive, n (%)	4 (33.3)	10 (23.8)	0.49†
Statin, n (%)	1 (8.3)	10 (23.8)	0.42†

HDL, high-density lipoprotein; BMI, body mass index; IQ, interquartile range.
*Mann-Whitney U test; †Fisher exact probability test.

though it is associated with significant morbidity and mortality. Death is the leading cause of failure in renal transplantation, accounting for 46% of graft failures occurring after 3 years posttransplantation.[10] The major causes are cardiovascular disease and infection, both of which may be triggered or favored by the use of steroids.

Several studies have tried to address the feasibility of steroid withdrawal in SPK transplant recipients. Two routine approaches to limit steroid-related side effects are systematic steroid withdrawal in stable allograft recipients and complete steroid avoidance or their rapid elimination. A 2004 meta-analysis of late steroid withdrawal in kidney transplantation showed an increased risk of acute rejection episodes, but no increased risk of early graft failure.[9]

However, the development of new immunosuppressive drugs has decreased the immediate immunologic risks, allowing greater emphasis on improving long-term wellness in SPK transplant recipients. The initial cyclosporine-microemulsion-based trials showed a 30% risk of acute and chronic rejection following late corticosteroid withdrawal, imposing the need to maintain virtually all renal transplant patients on low-dose steroids.[11,12] The addition of MMF to cyclosporine-microemulsion enabled prednisolone withdrawal at 6 months posttransplant without an increase in acute rejection episodes.[13] Likewise, with the association of cyclosporine-microemulsion and sirolimus, steroid tapering (initiated at a mean of 415 days posttransplant) was maintained for 3 years in 78% of kidney recipients.[14] Maintenance immunosuppression with tacrolimus and MMF showed a 94% likelihood of patients being maintained off steroids following late steroid withdrawal with good graft function.[15] A recent report showed a steroid-free regimen with thymoglobulin induction followed by tacrolimus and MMF for maintenance in SPK transplantation to be safe and effective in preventing rejection episodes, with a reduced incidence of cytomegalovirus infections and better-preserved kidney function.[3]

Recently approaches have been numerous. Several units have reported early steroid withdrawal at the end of the 1 week posttransplant with favorable short-term results in terms of graft function and recipient survival, without jeopardizing the risk of acute rejection episodes.[3,16–19] Similar results were reported by other SPK centers, although using a late schedule for the withdrawal: namely, the end of 3 months[20] or between 6 months and 1 year posttransplant.[21,22]

Cantarovich et al compared the benefits of early versus late (>3 months) steroid withdrawal.[20] Despite a small number of patients in this study, they observed that the rate of acute rejection episodes was similar in both groups, but that the serum creatinine concentration tended to be lower in the group with late steroid withdrawal. Notwithstanding, few studies have investigated the long-term effects of steroid withdrawal on graft and patient survivals. Opelz in the Collaborative Transplant Study showed that among a group of patients who received steroids for a minimum of 6 months after transplantation and who were clinically stable at the time of steroid elimination,[23] 90% of grafts functioned without steroids.

The pursuit of a steroid-free or reduced exposure among SPK transplant recipients has the purpose of controlling cardiovascular risk factors (obesity, hyperlipidemia, hypertension, and glucose intolerance) in this high-risk population. The published data about the long-term effects of steroid withdrawal to reduce vascular risk/events in this setting are scarce, although better control of these risk factors would allow us to expect such an outcome. Jaber et al published a report showing that an early steroid withdrawal protocol was associated with a reduced incidence of obesity, posttransplant diabetes, and hyperlipidemia among renal transplant recipients. No difference was acknowledged in the need for antihypertension drugs.[18] Noteworthy, hyperlipidemia is a known side effect not only of steroids but also of tacrolimus and sirolimus.[18,24]

Control of cardiovascular risk factors is possible in these patients as demonstrated by our data. Nevertheless, the comparison of the lipid profiles and the need for antihypertensive drugs did not show a significant difference between the steroid-treated and steroid-free patients. The need for statins was more frequent in the steroid-free group. The hyperlipidemic effects of sirolimus (more patients under sirolimus in this group) cannot be disregarded as a contributing factor. However, the rate of patients needing these treatments can be considered to be low, compared with results in kidney transplantation alone. We acknowledge

Table 3. All SKP Transplant Patients: Patient and Grafts Survivals

	1 Year (%)	5 Years (%)
Patient	93	90
Kidney graft	91	88
Pancreas graft	86	81

SPK, simultaneous pancreas-kidney.

the limitations of this report, as it is observational, retrospective, single center without randomization. Moreover, the criteria for selection of patients between the two groups, although clinically based, showed some degree of heterogeneity.

However, the previous studies allow us to put forward our late steroid withdrawal protocol. Hence, steroid withdrawal is usually undertaken in situations where there is a good long-term graft prognosis, evidence of steroid-induced morbidity and tolerance to the remaining maintenance drugs (MMF, tacrolimus).

Complete steroid withdrawal by the end of the first year was accomplished in 77.8% of our patients with more than 1 year follow-up ($n = 54$) and no subsequent acute rejection episodes. Our data showed good, stable graft function. No significant differences in graft function were noted between the steroid-based and steroid-free groups. The overall patient and graft survivals were similar to other SPK transplant units.[20] More precise clinical criteria are necessary for full implementation of this immunosuppressive strategy.

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III - Complicações mais frequentes após TRP

- **III.1 - Martins L**, Henriques AC, Dias L, Almeida M, Pedroso S, Freitas C, Pereira S, Fructuoso M, Dores J, Oliveira F, Almeida R, Cabrita A, Teixeira M. **Pancreas-Kidney Transplantation: complications and readmissions in 9-years of follow-up**. *Transplant Proc* 2010; 42(2): 552-554.

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- **III.2 -** França M, Certo M, **Martins L**, Varzim P, Teixeira M, Henriques AC, Ribeiro AM, Alves FC. **Pictorial review - Imaging of pancreas transplantation and its complications.** *Insights Imaging.* 2010 Nov;1(5-6): 329-338 [PMID: 22347926. DOI 10.1007/s13244-010-0041-8]

- **III.3- Martins LS**, Malheiro J, Pedroso S, Almeida M, Dias L, Henriques AC, Silva D, Davide J, Cabrita A, Noronha IL, Rodrigues AS. **Pancreas-Kidney transplantation: Impact of dialysis modality on the outcome**. *(Submetido*)

Resumo III.1

Quando comparado com o TR isolado, o TRP apresenta maior morbilidade pós-operatória, maior tempo de internamento e maiores custos. A complexidade cirúrgica, nomeadamente a cirurgia do pâncreas, intra-celômico e com anastomoses entéricas, confere-lhe um maior risco de complicações, que tratando-se de doentes imunossuprimidos são potencialmente graves.

Fomos analisar em 93 doentes submetidos a TRP num período de 9 anos, as complicações durante o primeiro internamento e as readmissões subsequentes. A mediana do primeiro internamento foi de 22 dias. A incidência de rejeição aguda foi de 11,8% e a de não função imediata do rim de 19%. Em 30 doentes (32%) houve lugar a reintervenções cirúrgicas após o transplante. A hemorragia, a infeção e a trombose representaram 90% dessas reintervenções.

Depois da alta e ao longo dum seguimento médio de 3,7anos, 74,2% dos doentes tiveram readmissões, vários deles múltiplas readmissões. A maioria destas (57,4%) no 1º ano. A infeção foi a causa mais frequente (121 episódios), o foco urinário liderando a lista das etiologias. De entre as causas infeciosas salientam-se 7 casos de infeções víricas - 6 por doença a CMV e 1 por doença a EBV - e 3 casos de infeções fúngicas sistémicas – 2 por aspergilose e 1 por candidemia. A infeção por EBV e os 3 casos de infeção fúngica implicaram os tempos de internamento mais prolongados, tendo-se registado 1 óbito num dos casos de aspergilose.

Dez doentes perderam o enxerto renal, 3 por rejeição e 2 por morte do doente com enxerto funcionante. A trombose (3 casos) e sepsis (2 casos) conduziram às outras perdas. Destes doentes, 4 tinham já feito retransplante renal. Houve 21 casos deperda do pâncreas, a trombose representou a causa major (9 casos), seguida da infeção / deiscência da anastomose do pâncreas (5 casos). Foi realizado retransplante pancreático em 4 casos, 3 dos quais mantinham-se funcionantes. Faleceram 6 doentes, metade de causa infeciosa e 2 de causa CV.

A sobrevivência deste grupo aos 9 anos foi de 93%, 90% e 79%, para doente, rim e pâncreas respetivamente.

A nossa experiência, suportada pelos dados internacionais, mostra que este é um grupo de doentes de elevada complexidade e risco acrescido de complicações, não só no curto prazo mas ao longo do seguimento. Contudo, a experiência cumulativa, uma avaliação atenta e uma abordagem exaustiva pelas várias especialidades envolvidas, pode conduzir a resultados muito satisfatórios no TRP.

Pancreas-Kidney Transplantation: Complications and Readmissions in 9-Years of Follow-up

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ABSTRACT

Over 9 years, we have performed 93 simultaneous pancreas-kidney transplants (SPKT). The morbidity of this procedure is high compared with kidney transplantation alone; readmissions are frequent and costs are higher. Herein we have presented the complications during follow-up of these 93 patients. Their mean age was 34 ± 6 years and prior dialysis time was 32 ± 25 months. The median hospital stay on the first admission for the transplant procedure was 22 days, including 2 days in the intensive care unit. Bleeding, thrombosis, and infection were the most frequent reasons for prolonged hospitalization. Thirty patients underwent >1 surgical reinterventions. Incidence of acute rejection episodes was 11.8%. After discharge, 74.2% of the patients had 197 readmission episodes with infection being the main cause, urinary tract infections, the most frequent; however, systemic viral and fungal infections required the longest readmission periods. The need for surgical interventions, graft dysfunction, and vascular problems were the remaining causes of readmission. At the end of follow-up, 87 patients were alive, 86 with well-functioning kidneys and 74 with normal functioning pancreata. Global survival rates for patient, kidney, and pancreas were 96%, 95%, and 81% at 1-year; 93%, 90%, and 79% at 5-years; and 93%, 90% and 79% at 9-years. Although pancreas–kidney transplant patients are complex, presenting many management difficulties, our overall results represent a positive stimulus for diabetic patients.

Simultaneous pancreas–kidney transplantation (SPKT) display greater morbidity, length of admission, and number of complications when compared to kidney transplantation alone (KTA), leading to higher costs. However, SPKT remains the best treatment option for good long-term results among type 1 diabetic patients with chronic kidney failure. A recently published review of 1000 SPKT from a single center[1] reinforced this position statement of the American Diabetes Association. We have presented herein the results of our SPKT program, including the rate and type of complications, causes of surgical reinterventions, and readmission episodes after the initial discharge.

PATIENTS AND METHODS

From May 2000 to May 2009, we performed 93 SPKT always using enteric diversion and vascular anastomoses to the systemic circulation. Immunosuppression included antithymocyte globulin (ATG) tacrolimus, mycophenolate mofetil (MMF), and steroids. After the 6th month, it was our policy to progressively taper steroids to complete withdrawal whenever possible. Infection prophylaxis comprised vancomycin, fluconazole, and a third-generation cephalosporin in the first days, with contrimoxazole and valgancyclovir thereafter. We also prescribed aspirin and low-molecular-weight heparin as soon as possible after surgery, if there were no bleeding complications.

RESULTS

The mean age of the 93 patients was 34 ± 6 years with a 23 ± 5.5 years, duration of diabetes. They had been on dialysis for 32 ± 25 months excepting 4 who were grafted preemptively. Twenty patients had no HLA match with the donor. Their

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mean insulin daily dose and HbA1c before transplantation were 39 ± 12 U and 8.5 ± 1.7%, respectively. Insulin administration was stopped at 1.3 ± 2.8 days after transplantation. A subset of 19% of subjects needed transient dialysis. An acute rejection episode was detected in 11 patients (11.8%). It involved both grafts in 3 cases; only the kidney in 6; and only the pancreas in 2. In all but 1, who had repeated episodes, the initial rejection episode was efficiently treated. The median hospital stay on the first admission for transplant surgery was 22 days (range, 9–46), including 2 days in the intensive care unit (range, 1–88). In 13 cases, residence in the intensive care was >4 days. The initial reasons for the prolonged stay were bleeding (n = 9), pancreatic leak/infection (n = 3), or thrombosis (n = 1). Eleven of 13 patients needed >1 reoperations, 2 resulting in pancreas loss, 1 in kidney loss, and 2 in death.

When we examined all patients who needed >1 surgical reinterventions (even with shorter stay in the intensive care unit) we noted 30 patients (32.2%), namely the 11 above mentioned and 19 other subjects who mostly experienced infections or bleeding (Table 1).

After the initial discharge, 197 readmissions occurred among 69/93 patients (74.2%), who required 1–11 episodes/patient during the 3.7 ± 2.5 years follow-up period (range, 0.2–9.1). The incidence of readmissions was greater in the first year (57.4%). Infection was the main reason for hospitalization, followed by surgical interventions, graft dysfunction, and vascular problems (Table 2). The etiology of infectious episodes (n = 121), was most frequently urinary tract origin (55.4%; n = 67 episodes); followed by digestive (10.7%, n = 13 episodes); respiratory (6.6%; n = 8 episodes, 2 tuberculosis); viral (5.8%; n = 7) namely cytomegalovirus disease (n = 6) or Epstcin-Barr virus (EBV) (n = 1); graft related; intra-abdominal infection (5.8%; n = 7); and systemic fungal (3.3%; n = 3 including, 2 aspergillosis and 1 candidemia. The longest admissions (>30 days) were noted in the 3 cases of fungal infections and in the EBV case.

At the end of follow-up, 10 kidney grafts had failed due to thrombosis (n = 3), rejection (n = 3; 1 repeated acute 1 late acute, and 1 chronic rejection), sepsis (n = 2), and death with functioning grafts (n = 2). The losses due to rejection occurred from 2.5 to 7 years after transplantation. Four of these 10 patients were retransplanted; 3 are still functioning and 1 lost due to patient death. At our last evaluation, the 86 patients with functioning kidneys displayed a mean serum creatinine value of 1.12 ± 0.30 mg/dL; creatinine clearance of 79.6 ± 26.4 mL/min and urinary protein excretion = 0.3 ± 0.27 g/da. Steroids were totally withdrawn in >80% of patients.

Table 1. Complications Leading to Relaparatomy: Causes for the First Surgical Reintervention (n = 30 Patients)

Infection	9*
Bleeding	10*
Thrombosis - Px	5
Thrombosis - Kx	3
Intestinal occlusion	2
Urinary fistula	1

One to 12 reinterventions/patient.
*Four of these evolved to pancreas thrombosis.
Px, pancreas; Kx, kidney.

Twenty-one patients lost their pancreas grafts due to late rejection (n = 2, >2 years after the transplant); thrombosis (n = 9); bleeding (n = 3); pancreatic leak/infection (n = 5) or patient death (n = 2). Four were retransplanted with a solitary pancreas, 3 of which are functioning, whereas 1 was lost due to noncompliance with immunosuppression and subsequent pancreas rejection. The 74 patients with functioning pancreas grafts show mean fasting glucose values of 80 ± 9.4 mg/dL HbA1c of 4.8 ± 0.4%, and C-peptide of 3.06 ± 1.46 ng/mL.

Six patients succumbed: due to sepsis in the first 3 months (n=2); invasive aspergillosis (n=1); stroke (n=1); myocardial infarction (n=1); or unknown cause (n=1). The actuarial survival rates at 1, 5, and 9 years were 81%, 79%, and 79% for the pancreas graft; 95%, 90%, and 90% for the kidney graft, and 96%, 93%, and 93% for the patients, respectively.

DISCUSSION

Our results reflect the complexity of SPKT. We observed the same causes for surgical reinterventions—infection, bleeding, and enzyme leak—as previously reported for enteric-drained patients.[1] The total length of hospital stay and intensive care residence were directly related to the presence of important complications: patients reoperated due to thrombosis, bleeding, pancreatic leak, or infection; or even patients without surgical reinterventions but who displayed relevant bleeding or infection. These patients also had an increased rate of graft loss and death. Intra-abdominal infection is a well-known risk factor for graft and patient loss;[2] pancreas thrombosis, for pancreas loss.[2]

The registered reoperation rate of 32.2% SPKT (n = 30) was similar to that observed in other series.[3] It eventuated in graft pancreatectomy in approximately half of these patients (17/30 patients) with an 18% incidence among all patients, which is similar to the experience of other centers.[3] Relaparotomy had been considered to be a risk factor for pancreas graft survival.[3] Not only the pancreas graft may be at risk when surgical complications occur, but also patient survival, and has a significant financial impact.[4]

The literature shows a reduction in patient survival during the first 3 months after a pancreas transplant compared with subjects a waiting a transplant. However, thereafter it reverses with transplanted patients performing better at 1 year.[5]

The EUROSPK study[6] reported a much higher 44.8% rejection rate than that in our study (11.8%), but we continued steroids for >6 months after transplantation, which may in part explain the difference. Four of our patients lost their grafts due to rejection: 1 pancreas and kidney loss, 2 kidney losses, and 1 sole pancreas loss. In all

Table 2. Causes of Readmission After First Discharge: Readmission Causes: 197 Episodes (in 69 from the Global 93 Patients, 74.2%)

Vascular (6.1%): 12 episodes in 8/93 patients (8.6%)	Cardiovascular	3	1 death	
	Cerebrovascular	1	1 death	
	Peripheral vascular	4		
Dysfunction Tx (8.1%): 16 episodes in 13/93 patients (14%)	With rejection	7	4 pancreas	3 kidney
	Without rejection	6		
Surgical (13.2%): 26 episodes in 22/93 patients (23.7%)	Ocular	4		
	Abdominal	8		
	Urinary	4		
	Others	10		
Infecction (61.4%): 121 episodes in 52/93 patients (55.9%)	Urinary (n = 67), digestive (n = 13), respiratory (n = 8), systemic viral (n = 7), systemic fungal (n = 3), or others			

4, it was a late occurrence (>2 years after the transplant); early rejection episodes were all controlled. Only 1 of these subjects died due to a myocardial infarction at 6 months after failure of both grafts while on dialysis. However, pancreas rejection and consequent loss have been reported to decrease patient survival.[6] Also kidney rejection has been associated with reduced patient survival among SPKT patients.[2] This was not our experience, possibly due to the low rejection rate.

After the initial discharge, three quarters of our SPKT had to be hospitalized at >1 times, namely 197 episodes or 2 episodes/patient, which is similar to other published data.[7] Infection was the main cause. Not surprisingly, the urinary tract was a frequent site, as in other series.[7,8] After the initial transplantation admission, infection remains an important morbidity factor during patients follow-up. Surgical procedures were the second cause for readmission, as we included all cases: namely ocular, orthopedic, urinary (ureteral stenosis principally), and abdominal surgeries (wound related, intestinal occlusion, peripancreatic collections, etc). Graft dysfunction was the third cause for readmission (8.1%; n = 13 episodes), among half of which we established a diagnosis of an acute rejection episode (n = 7). Late rejections were more difficult to resolve: 4 patients lost their grafts. Vascular complications were not common; the 8 affected patients included 4 who suffered cardiovascular or cerebrovascular episodes leading to death in 2 cases.

We considered our survival rates to be good and not inferior to those presented by other similar[2] or larger series[1] at 1 year[2] and in the longer term.[1] In fact, our 9-year survival data - 93% patient, 90% kidney, and 79% pancreas - were good when compared with other published 10-year results.[1]

Two important recently published papers have concluded that SPKT provides a significant extension to patient life,[1] namely 10 years longer than that of KTA from deceased donors.[5] Our perception is that SPKT patients are really difficult to manage not only in the early period, but also in follow-up. Complications are frequent; costs are high and directly related to the complication rate. However it is satisfactory to observe good results among these patients.

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Resumo III.2

O reconhecimento precoce das complicações do TRP é fulcral para o desfecho das mesmas e para a sobrevivência do doente e dos enxertos. A imagiologia é muitas vezes basilar para o estabelecimento do diagnóstico e do plano de abordagem. Neste trabalho, realizado em colaboração com o Serviço de Radiologia do HSA, revimos os aspecos imagiológicos deste transplante em si e das complicações pós-operatórias passíveis de interpretar por imagem.

São inicialmente apresentadas imagens, pelas diferentes técnicas, dos 2 enxertos com aspeto normal, as suas anastomoses vasculares e entéricas ou urológicas. Seguem-se Aspetos de algumas complicações registadas, como pancreatite; pseudocistos; rejeição pancreática com biópsia guida por ecografia; fístulas pancreáticas; abcesso intra-abdominal; Aspetos de peritonite; trombose vascular; pseudoaneurismas arteriais; e casos de hemorragia ativa.

Este trabalho demonstra a importância da familiarização da Radiologia com os Aspetos da transplantação pancreática e das suas complicações, e a sua contribuição para a deteção e até resolução de alguns destes problemas. Vem dar relevo à necessidade de abordagem e empenho multidisciplinar para o bom resultado do TRP.

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PICTORIAL REVIEW

Imaging of pancreas transplantation and its complications

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Abstract Pancreas transplantation is an effective treatment for type 1 diabetes mellitus and is being increasingly performed worldwide. Early recognition of graft-related complications is fundamental for graft survival; thus, radiologists must be aware of the transplantation technique, pancreas-graft imaging and postoperative complications. We present normal pancreas-graft imaging appearances and the imaging features of postoperative complications.

Keywords Diabetes mellitus · Type 1 · Pancreas transplantation · Pancreas-kidney · Complications

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Introduction

Pancreatic transplantation is currently the only effective treatment for type 1 *diabetes mellitus*, allowing long-term glycaemic control without exogenous insulin injections. In most cases, it is performed as a simultaneous pancreas-kidney (SPK) transplantation from the same donor but it can be performed after kidney transplantation or, rarely, as an isolated transplantation [1]. The first pancreatic transplantation was performed in 1966 [2] and, since then, different procedures and immunosuppressive regimens have been developed in order to improve graft survival rates. At our institution, one of the first referral transplant centres in Portugal, more than 100 pancreas transplants have been performed since 2000. Most of these (96%) were SPK transplants from the same donor. The 9-year mean pancreatic graft survival rate was 78% at our institution.

Early recognition of graft-related complications is fundamental for graft survival, and radiologists must be aware of the transplantation technique, pancreas-graft imaging and postoperative complications. In this article, we present our 10-year experience with the procedure, describing the normal postoperative imaging findings and complications in a large series of 104 patients.

Transplantation procedure

At our centre, transplantation of the whole pancreatic graft is performed with a duodenal segment, with systemic endocrine drainage via the grafted portal vein into the recipient's inferior vena cava or common iliac vein, and enteric exocrine drainage via the anastomosis of the donor's duodenal segment to the recipient's small bowel (Fig. 1). Pancreatic endocrine drainage may also be performed to the

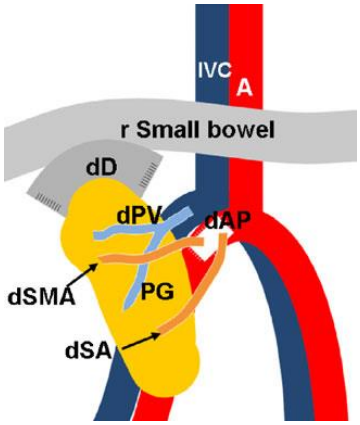

Fig. 1 Illustration of the pancreas transplantation technique performed at our institution, using duodeno-enterostomy for exocrine drainage and systemic endocrine drainage. The donor's aortic patch (*dAP*) with the origin of the superior mesenteric artery (*dSMA*) and the splenic artery (*dSA*) is anastomosed to the recipient's common iliac artery; the donor's portal vein (*dPV*) is anastomosed to the recipient's common iliac vein. *PG* pancreatic graft, *dD* donor's duodenum, *rSmall bowel* recipient small bowel, *IVC* inferior vena cava, *A* aorta

recipient's portal venous system [3, 4] and/or the exocrine drainage may be derived to the bladder, but these surgical techniques have never been performed at our institution. Arterial supply to the pancreatic graft is performed through a donor's aortic patch, containing the splenic artery and the superior mesenteric artery (SMA), which is anastomosed to the recipient's common or external iliac artery. The pancreatic graft is placed intraperitoneally laterally in the pelvis (preferably on the right side), with the duodenal segment facing cephalad. The donor's duodenal segment is anastomosed side-to-side to the recipient's small bowel (duodeno-enterostomy). The recipient's native pancreas in the upper abdomen is left untouched. When simultaneous kidney-pancreas transplantation is performed, the donor's kidney is preferably placed in the contralateral side of the pelvis, most frequently at the left iliac fossa.

Imaging of the transplanted pancreas

Imaging evaluation of the pancreas transplant grafts is commonly performed by a multi-technique approach. *Ultrasound* is usually the first technique to be used to search for early complications [4], as it is routinely performed in the postoperative period (in the first 24 h). In grey-scale B-mode, the normal pancreatic graft presents homogeneous echotexture, lower than the native pancreas and the surrounding mesenteric or epiploic fatty tissue. Doppler imaging provides vascular assessment (Fig. 2), with allograft vein velocities ranging between 10 and 60 cm/s. In the immediate postoperative period, arterial velocities may be as high as 400 cm/s at the anastomotic site, due to kinking or oedema of the anastomosis, but are usually reduced at follow-up examinations. The resistive index (RI) may be as high as 0.9 and be variable throughout the gland, with even higher values at the tail segment. This variability makes it of limited value for the diagnosis of graft rejection [4–6].

Contrast-enhanced computed tomography (CT) allows excellent evaluation of the graft's parenchyma, vascular and enteric anastomosis, and detects several postoperative complications, such as ascites, fluid collections, pneumoperitoneum or vascular thrombosis [7, 8]. CT is generally required after an abnormal ultrasound or whenever the patient presents unexplained fever, abdominal pain or when abnormal laboratory data are found. Multidetector (MD) CT allows multiplanar imaging and three-dimensional (3D) reconstructions of the graft's vascular anatomy to better advantage (Figs. 3 and 4). As with imaging the normal pancreas, the graft should display homogeneous enhancement, with the main pancreatic duct (MPD) being unrecognisable or showing a minor degree of dilatation.

Magnetic resonance imaging (MRI) has the advantage of allowing exploitation of the native signal intensity of the graft besides the vascular information provided by Gd-enhanced dynamic study (Fig. 5). Also, magnetic resonance cholangiopancreatography (MRCP) helps to depict ductal abnormalities [8]. At our institution, MRI is rarely used for examination of pancreatic graft-related complications because of its lower spatial resolution, creating difficulties in the assessment of the enteric anastomosis, and, also, because of technical constraints involved in imaging acutely ill and intensively monitored patients. Normal pancreas should be isointense to the renal graft parenchyma on T1-weighted images, with an intermediate signal on T2-weighted images and homogeneous enhancement after intravenous contrast medium administration [5, 9].

The role of *digital subtraction angiography* is reserved for cases where vascular abnormalities need to be confirmed or when endovascular therapy is sought [8].

Complications of pancreatic transplantation

In the immediate postoperative period, one should expect to find small peri-graft fluid collections, donor's duodenal wall thickening, mild pancreatic duct dilation, slight stranding of peri-pancreatic fat or oedematous swelling of the donor's remaining mesenteric fat, surrounding the mesenteric artery (Fig. 6) [5]. These imaging findings are usually self-limited, normally seen to be resolving spontaneously on follow-up examinations. Post-transplantation complications of the allograft may be classified as

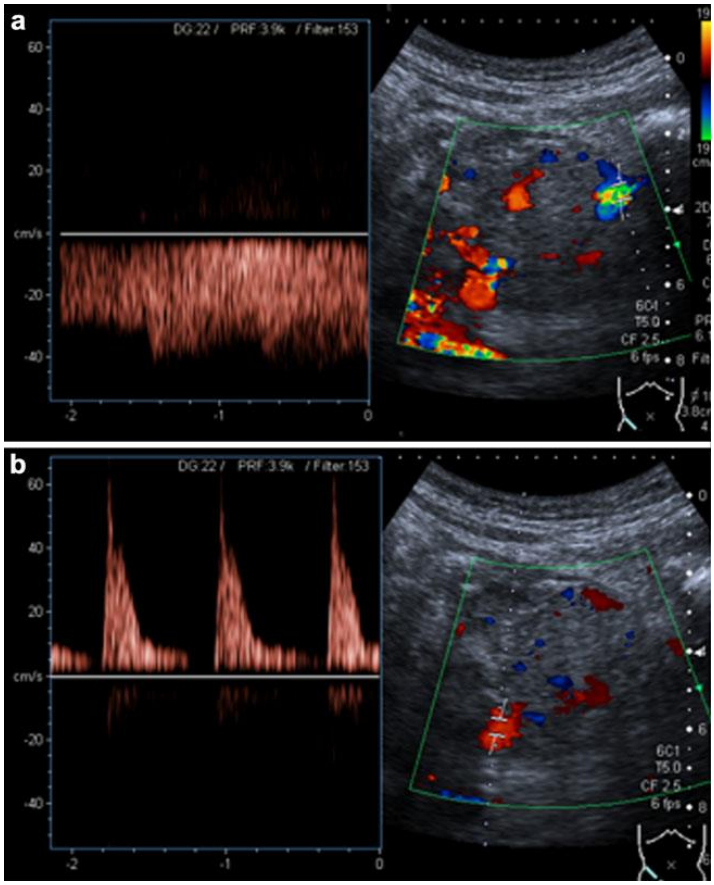

Fig. 2 Normal findings at colour and spectral Doppler ultrasound examination of pancreatic graft veins (**a**) and arteries (**b**) displaying good organ perfusion and clear arterial spectra with normal resistive index

parenchymal, infectious, enteric or vascular. In our series, the most frequent complications, by order of relevance, were graft pancreatitis, infection, and necrosis secondary to arterial or venous thrombosis. Less common complications included pancreatic fistula, bleeding, duodenal anastomosis dehiscence and small bowel obstruction (Table 1).

Allograft parenchymal complications

Acute pancreatitis

Graft acute pancreatitis, mild and self-limited, is frequently seen in the early postoperative period and is due to reperfusion injury [8]. Severe pancreatitis is uncommon,

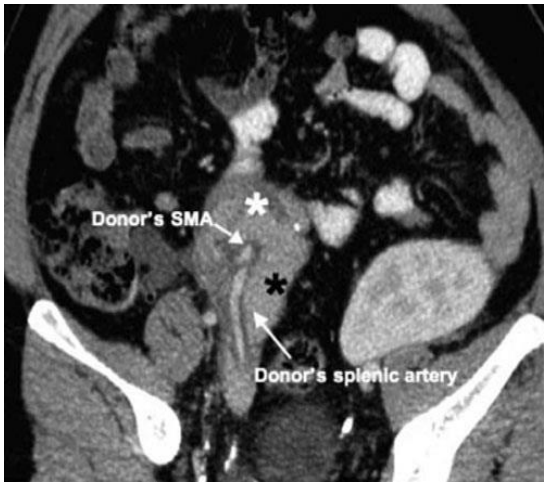

Fig. 3 MPR coronal oblique contrast-enhanced MDCT images showing a normal pancreatic graft arterial supply after SPK transplantation. The donor's superior mesenteric artery (*SMA*) supplies the pancreatic graft head (*white asterisk*), and the donor's splenic artery irrigates the graft body and tail (*black asterisk*)

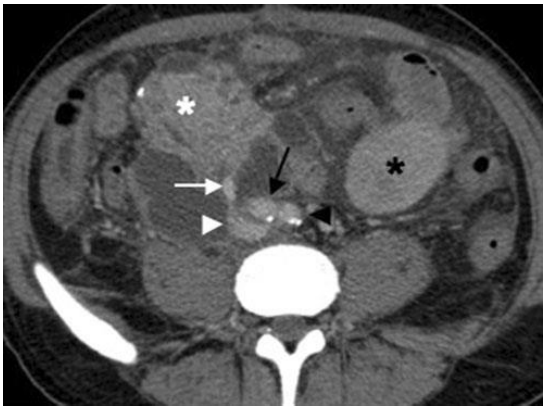

Fig. 4 Axial contrast-enhanced MDCT after SPK transplantation. End-to-side anastomosis is used to connect the donor's portal vein (*white arrow*) to the recipient's right common iliac vein (*white arrowhead*). Homogeneous fluid collections adjacent to the pancreatic graft are observed. *Black arrow* right common iliac artery; *black arrowhead* left common iliac artery; *white asterisk* pancreatic graft; *black asterisk* renal graft

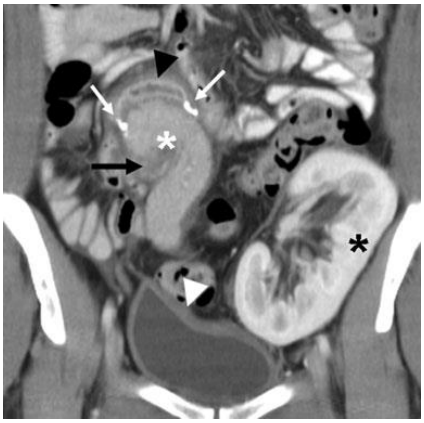

Fig. 6 Coronal reformatted MDCT image shows homogeneous enhancement of the pancreatic (*white asterisk*) and renal grafts (*black asterisk*) after SPK transplantation. The pancreas is placed laterally in the pelvis, on the right side, with the attached donor's duodenal segment facing cephalad (*black arrowhead*), which anastomoses to the recipient's jejunum. Surgical staples are present at the extremities of the duodenal segment (*white arrows*). Note the oedematous swelling of the donor's remaining mesenteric fat (*black arrow*) surrounding the pancreatic graft vessels. These findings are to be expected in the early postoperative period

occurring in about 10% of allografts [5]. Imaging findings are non-specific consisting of normal or enlarged pancreatic allograft, showing heterogeneous contrast enhancement, adjacent fat stranding and fluid collections (Fig. 7). Necrotising pancreatitis may occur in about 2–4% of the allografts [5] and is the most severe form of acute pancreatitis. Necrosis can result either from pancreatitis itself or from direct vascular occlusion. On ultrasound, necrosis manifests as hypoechoic areas within the graft parenchyma, sometimes with hyperechoic foci suggesting gas formation. Doppler interrogation assists in confirming absent arterial or venous flow within the affected segments of the pancreatic graft [9]. Contrast-enhanced CT or MRI is exquisitely sensitive to diagnosing and determining the extent of parenchymal necrosis due to lack of enhancement and possible gas formation (Fig. 8) [8].

Pseudocyst formation

Pseudocysts typically develop in the severe form of pancreatitis, usually being located within (Fig. 9) or adjacent to the graft (Fig. 10) [5, 8]. Imaging reveals thin-walled fluid collections. Wall thickening showing contrast

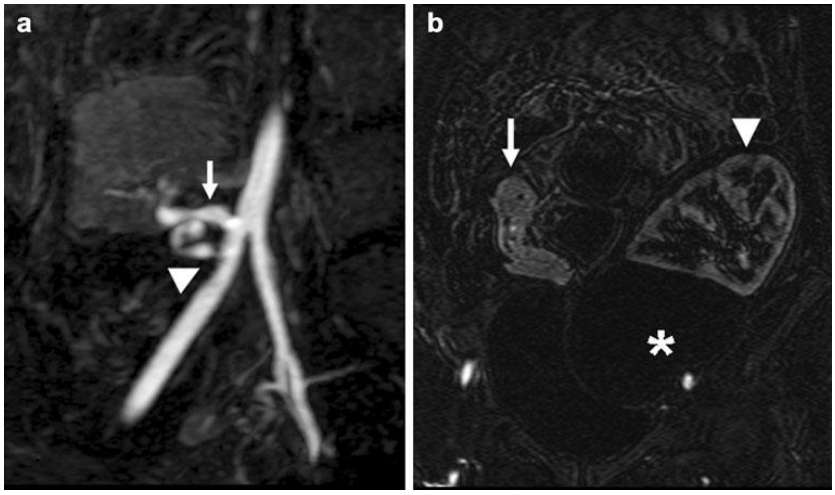

Fig. 5 A 32-year-old female patient after SPK transplantation. **a** Coronal MIP image in the arterial phase of an MRA study demonstrates the arterial vessel anatomy of the pancreatic graft. Note that the donor's SMA (*arrow*) presents normal calibre and the donor's splenic artery (*arrowhead*) shows a stenosis with mild post-stenotic dilation. **b** MR angiography in the arterial phase shows normal enhancement of the pancreas (*arrow*) and kidney (*arrowhead*) transplants. At the lower pole of the kidney graft a large lymphocele is seen (*asterisk*)

Table 1 Complications of pancreatic transplantation

Early complications	
Allograft parenchymal complications	Acute pancreatitis
	Necrotising pancreatitis
	Fistulous tracts
Infection and abscesses	
Enteric complications	Anastomotic leakage at the duodeno-enterostomy
	Ileus
	Colonic infections
Vascular complications	Venous or arterial graft thrombosis
	Acute bleeding
Late complications	
Allograft parenchymal complications	Rejection
	Pseudocyst formation
	Post-transplant lymphoproliferative disease
Enteric complications	Small bowel obstruction
	Colonic infections
Vascular complications	Arterial or venous pseudoaneurysms

enhancement and heterogeneous content should raise the suspicion of super-seeded infection, which may be managed by percutaneous drainage.

Rejection

Rejection is less common in pancreatic transplantation than in renal transplantation but it is a common cause of pancreatic graft loss [5]. Imaging findings are non-specific and they may look similar to other complications, such as pancreatitis. Contrast-enhanced CT or MRI may show

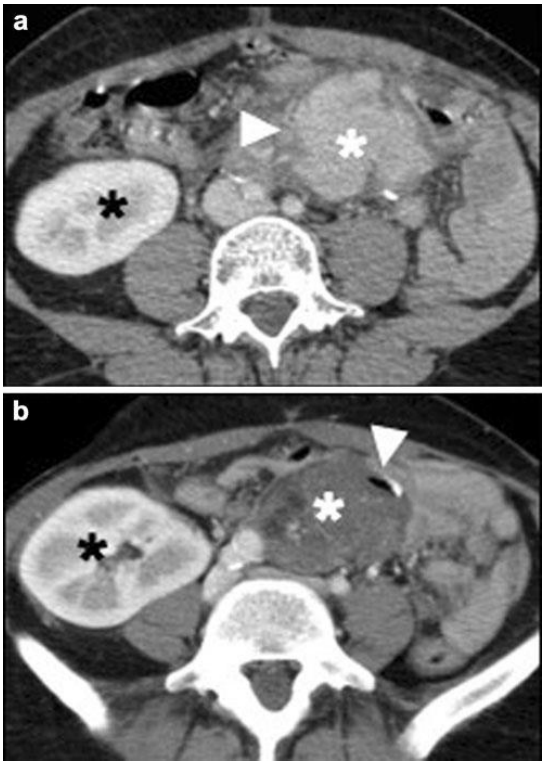

Fig. 8 A 40-year-old female patient after SPK transplantation. Axial contrast-enhanced MDCT (**a**) shows an enlarged pancreatic graft (*white asterisk*), with homogeneous parenchymal enhancement and peri-pancreatic fat stranding (*arrowhead*). The patient presented with interstitial oedematous pancreatitis. Two months later, axial contrast-enhanced MDCT (**b**) shows absent parenchymal enhancement of the pancreatic graft (*white asterisk*) and intra-pancreatic gas bubbles (*white arrowhead*). These findings were consistent with necrotising pancreatitis and graft pancreatectomy was performed. *Black asterisk* renal graft

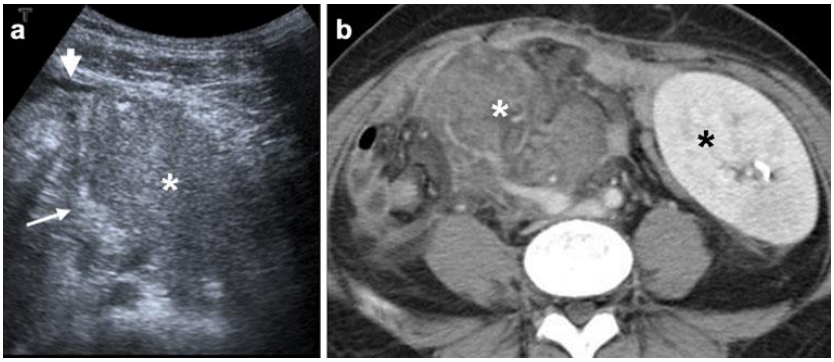

Fig. 7 A 36-year-old woman after SPK transplantation presenting graft pancreatitis. **a** Ultrasound shows enlarged pancreatic graft (*asterisk*) with adjacent hyperechoic fatty tissue (*thin arrow*) and a small amount of free intraperitoneal fluid (*large arrow*). **b** Axial contrast-enhanced helical CT shows an enlarged pancreatic graft with heterogeneous contrast enhancement (*white asterisk*), peri-pancreatic fat stranding and free peritoneal fluid. Normal renal graft is located on the left (*black asterisk*)

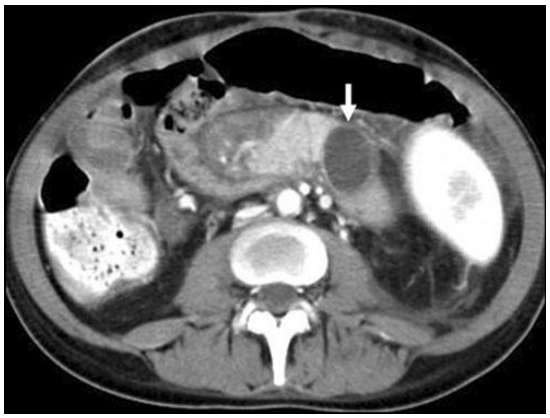

Fig. 9 A 36-year-old male patient after SPK transplantation with pancreatitis and pseudocyst formation. Axial contrast-enhanced CT shows heterogeneous enhancement of the pancreatic graft and a thin-walled pseudocyst (*arrow*)

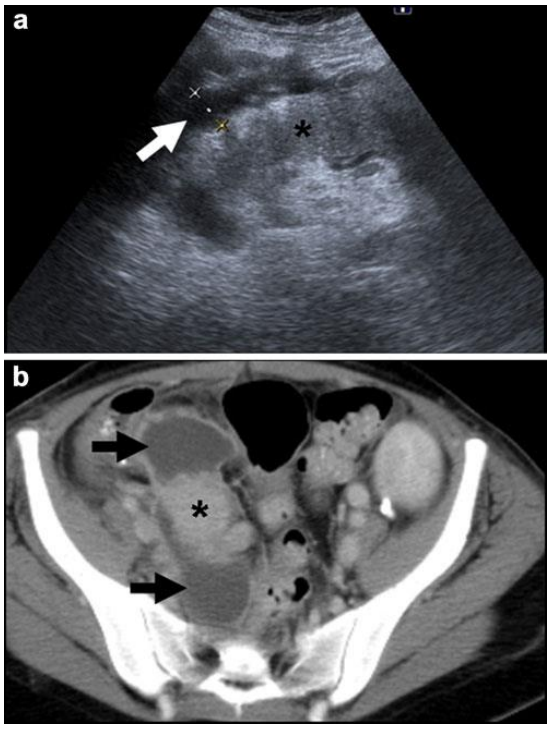

Fig. 10 A 30-year-old woman 5 weeks after SPK transplantation with an infected peri-pancreatic pseudocyst. Sagittal ultrasound of the right lower quadrant (**a**) demonstrates a peri-pancreatic fluid collection (*white arrow*). Axial contrast-enhanced MDCT (**b**) shows homogeneous contrast enhancement of the pancreatic graft (*asterisk*) surrounded by a thin, contrast-enhanced wall fluid collection (*black arrows*). Image-guided percutaneous drainage confirmed superimposed infection of the pseudocyst

heterogeneous parenchymal enhancement. As imaging and laboratory values are non-specific, graft biopsy is the only reliable test to diagnose graft rejection [10]. Graft biopsy can be performed under ultrasound or CT guidance (Fig. 11), and a low rate of complications has been previously reported [11].

Fistulous tracts

A pancreatic fistula usually appears as peri-pancreatic graft collection possessing high amylase levels when a puncture is performed. On MRCP, communication with the main duct may be identified. Although fistulas tend to resolve with conservative treatment, infection (abscess) or fistulous tract to the skin, peritoneal cavity, gut or uterine cavity may also develop; thus, follow-up imaging should be proposed (Fig. 12) [8].

Post-transplant lymphoproliferative disease

Lymphoproliferative disease is a rare late complication and has been reported with an incidence of 3–12% after pancreatic transplantation [5, 12]. In our institution, no cases have been diagnosed so far. Imaging may depict diffuse graft enlargement, indistinguishable from acute pancreatitis or rejection, but typically unresponsive to immunosuppressive therapy. Focal masses, inside or outside the graft, lymphadenopathy and/or organomegaly may also be seen [12].

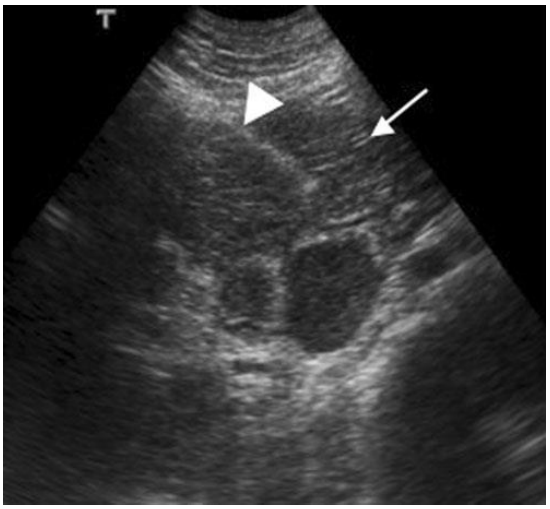

Fig. 11 A 29-year-old patient with rejection of the pancreatic graft after SPK transplantation. Ultrasound-guided biopsy of the pancreatic graft (*arrowhead* needle biopsy). Pancreatic graft (*arrow*) shows normal size but with heterogeneous texture of the parenchyma

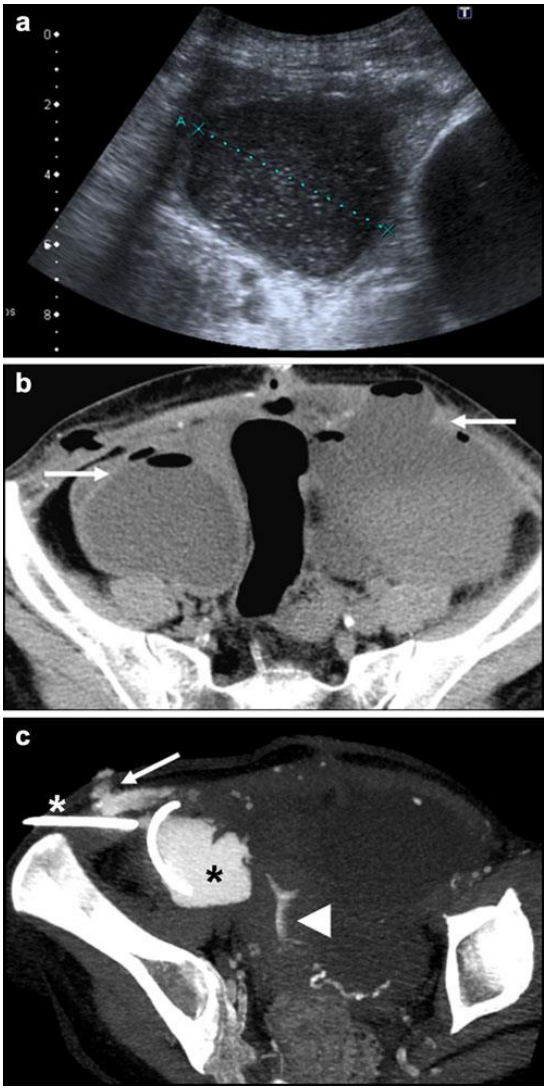

Fig. 12 A 30-year-old woman after SPK transplantation. **a** Abdominal ultrasound shows peri-pancreatic graft collections with a thick wall and echogenic content; **b** axial MDCT reveals multiple abscesses at the abdomen and pelvis, with extension to the abdominal wall. Ultrasound-percutaneous drainage was performed and chemical analysis of the fluid showed high amylase content, compatible with a pancreatic fistula. **c** A 3D-MPR MIP reconstructed from follow-up MDCT performed 3 days later, with introduction of iodinated contrast agent through the percutaneous drain (*white asterisk*), demonstrates the extension of intra-abdominal collection (*black asterisk*) and the fistulous tract to the abdominal wall (*arrow*). Contrast agent was also detected inside the uterine cavity (*arrowhead*) suggesting a fistula. Despite this complication, the pancreatic and renal grafts functioned normally

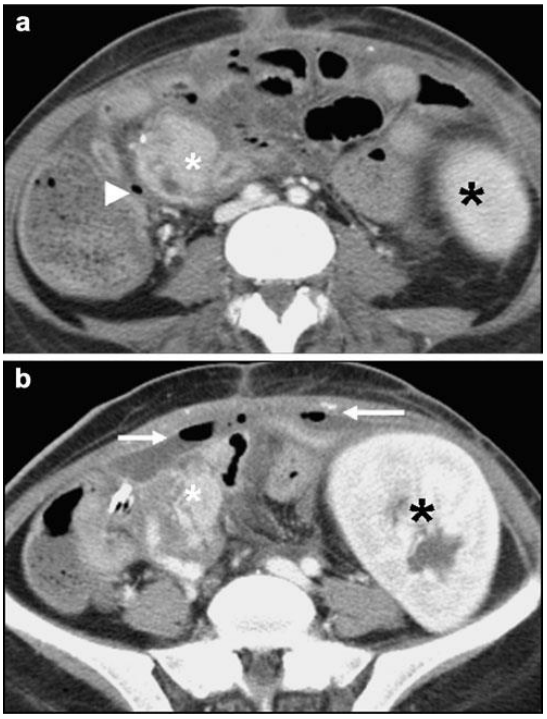

Fig. 13a, b A 30-year-old woman after SPK transplantation. Axial MDCT performed six weeks after transplantation shows intra-abdominal fluid collections with contrast-enhancing wall, with air-fluid levels (*arrows*), consistent with abscesses. Ascites and gas bubbles (*arrowhead*) are noted near the duodeno-enterostomy. The pancreatic (*white asterisk*) and renal grafts (*black asterisk*) enhance homogeneously. Duodenal dehiscence was suggested. The patient underwent surgery, which revealed a fistula at the donor's duodenal cuff

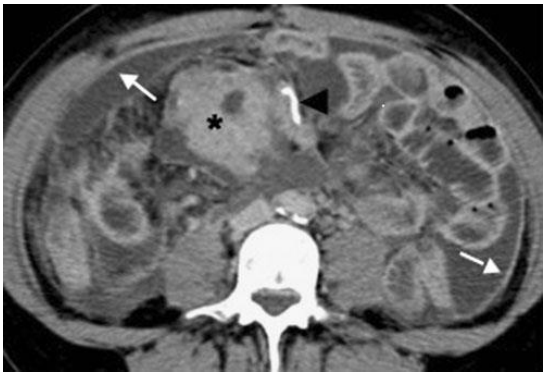

Fig. 14 A 37-year-old woman with peritonitis 5 weeks after SPK transplantation. Contrast-enhanced MDCT shows fluid collections surrounded by enhanced peritoneum (*arrows*). *Asterisk* pancreatic graft, *arrowhead* surgical staples at the graft's duodenal segment

Infection

Abscesses may result from infection of peri-pancreatic fluid collections, pseudocysts (Fig. 10), leakage of the enteric anastomosis (Fig. 13), or abdominal wall surgical wound infection [5]. They usually present as complex fluid collections, with a thick wall and possible intralesional gas. Either ultrasound or CT can be used to guide the percutaneous drainage of these collections.

Enteric complications

These are mainly represented by anastomotic leakage at the duodeno-enterostomy site and small bowel obstruction. CT is especially useful for evaluating these complications, directly demonstrating intra-abdominal abscesses, peritoneal inflammation (Figs. 13 and 14), or the enteric leakage observed as extravasation of orally administered contrast agent. The most common cause of small bowel obstruction after abdominal surgery is intestinal adhesions, but obstructions due to internal hernias or volvulus have also been reported [13]. Colonic infections, such as CMV infection, *Clostridium difficile* colitis or typhlitis, may occur, related to antibiotic therapy and to the immunocompromised state of the patient. These conditions may be suspected whenever colonic wall thickening with increased contrast enhancement is observed [7].

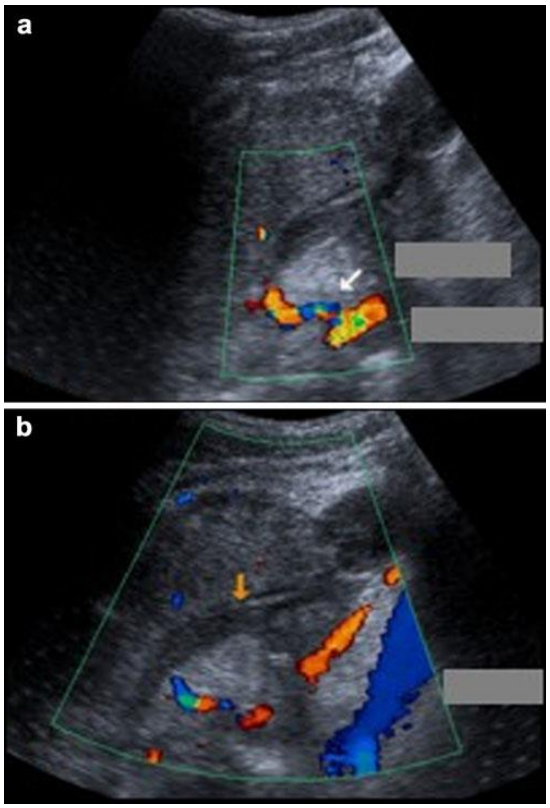

Fig. 15a, b A 37-year-old man after SPK transplantation, with persistent amylase elevation. Abdominal ultrasound-Doppler performed 2 weeks after transplantation shows the iliac arteries and the graft's arterial vessels (**a** *arrow*) permeable but with aliasing and high resistive index (RI). The iliac vein showed normal flow. However, no flow was detected at the graft's portal vein, suggesting thrombosis (**b** *arrow*). At surgery, thrombosis was confirmed and pancreatectomy was performed

Vascular complications

Venous or arterial graft thrombosis is a serious complication, being the second most common cause of transplant dysfunction after graft rejection [14]. Generally, it results in massive graft necrosis and requires pancreatectomy [8]. Doppler ultrasound depicts parenchymal heterogeneity with absent pulsatile or continuous flow of the pancreatic graft vessels (Fig. 15). In cases of venous thrombosis, pan-diastolic reversal of the arterial flow and a resistive index greater than 1.0 can be seen [9]. Intravenous contrast agent administration is especially useful for demonstrating intraluminal filling defects in the graft vessels and the lack of the parenchyma's enhancement (Fig. 16) [8, 15–17]. Emphysematous changes can occur with further progression to parenchymal necrosis. When superimposed infection is suspected, image-guided biopsy should be performed [8].

Early arterial occlusion of the pancreatic graft is usually due to technical surgical difficulties involved in performing the ligation of small pancreatic arterial vessels during organ harvesting. Late occlusion is generally related to the end point of graft rejection because of progression of the alloimmune response [8].

Arterial or venous pseudoaneurysms may develop after pseudocyst infection or after biopsy [8, 9, 18]. On Doppler ultrasound, they present as blood-filled lesions. Direct communication with the feeding vessel may also be identified and pulsed-wave Doppler ultrasound may show "to-and-fro" waveform at the pseudoaneurysm neck. Contrast-enhanced CT or MRI is better for demonstrating the focal loss of vessel wall integrity (Fig. 17).

When a simultaneous pancreas-kidney transplant is performed, with bilateral revascularisation to the respective iliac vessels, delayed opacification of the iliac vein ipsilateral to the pancreatic graft may be observed, compared with the contralateral iliac vein draining the kidney transplant (Fig. 18) [19]. This finding should not be interpreted as a real thrombosis of the iliac vein.

Although acute bleeding is rare, it may appear during the early postoperative period and is usually clinically suspected. Ultrasound demonstrates fluid collections but CT better identifies haemorrhage because of its spontaneous hyperdensity on non-enhanced images. After intravenous contrast

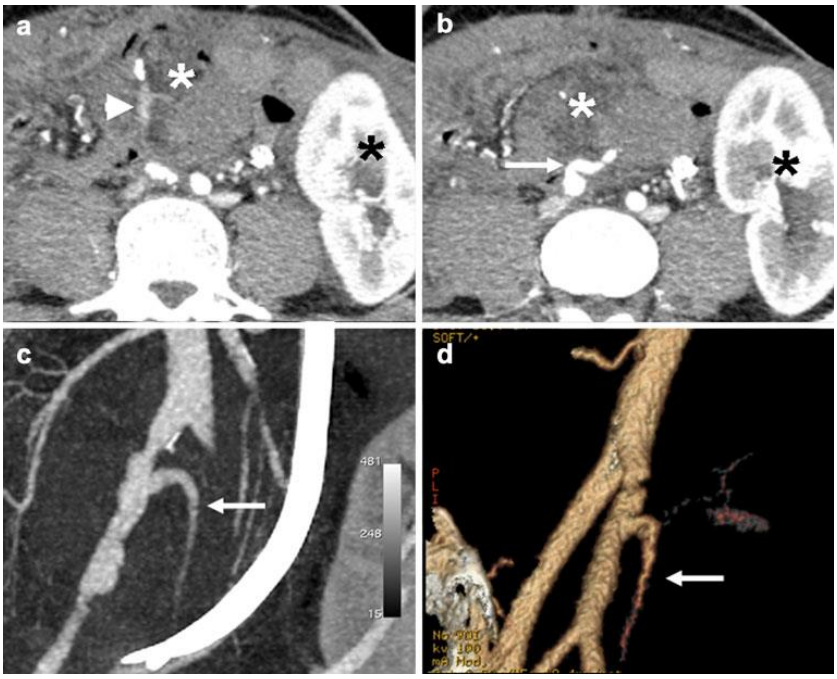

Fig. 16a–d A 37-year-old patient, the same patient as in Fig. 17, a day after a second pancreatic transplant. Axial MDCT (**a, b**) shows heterogeneous enhancement of the pancreatic head of the graft with normal, contrast-enhancing iliac arteries and the graft's splenic artery (*arrow*). Poor enhancement of the graft's SMA is noted (*arrowhead*), together with non-enhancing arterial segments, suggesting occlusion. There is homogeneous enhancement of the renal graft (*black asterisk*). Coronal reformatted MIP image (**c**) and VR reconstruction (**d**) illustrate the graft's splenic artery (*arrow*) but not the graft's SMA. The patient underwent pancreatectomy and the arterial occlusion was confirmed

medium administration, extravasation of the contrast medium may be seen and the bleeding point identified [8, 9].

Conclusion

Different imaging techniques can assess postoperative pancreatic graft. Although Doppler ultrasound is the first-line technique, CT and, to a minor degree, MRI, have been increasingly performed when ultrasound findings are equivocal.

Radiologists should know the imaging appearances of normal pancreatic grafts, be able to recognise early and late complications related to this complex surgical procedure, contribute to the clinical management and, ultimately, to the long-term survival of pancreatic grafts.

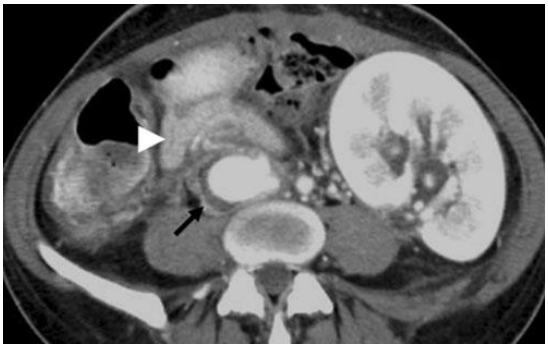

Fig. 17 A 36-year-old female patient after SPK transplantation. Contrast-enhanced CT performed 4 weeks after graft pancreatitis shows homogeneous pancreatic graft enhancement (*arrowhead*) and a partially thrombosed pseudoaneurysm of the right common iliac artery (*arrow*)

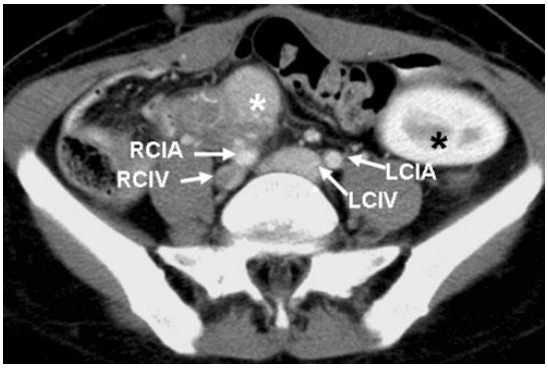

Fig. 18 A 44-year-old woman after SPK transplantation. Contrast-enhanced MDCT shows non-enhancement of the right common iliac vein compared with left side. This results from the longer transit time and the reduced blood flow of the right-sided pancreatic graft (*white asterisk*) compared with the left-sided renal graft (*black asterisk*)

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Resumo III.3

Permanece controverso se a modalidade de diálise pré-transplante, hemodiálise (HD) ou diálise peritoneal (DP), tem ou não impacto no resultado da transplantação de rim-pâncreas. Em alguns estudos foi reportado um desfecho mais adverso nos doentes que fizeram previamente DP, sem que exista consenso nesta matéria.

Realizámos um estudo em 158 TRP realizados na nossa Unidade até final de 2013, estudando os 39 que tinham realizado DP e os 119 que tinham realizado HD. Estes 2 grupos eram comparáveis em todas as variáveis pré-TRP (idade, tempo de diabetes, medicação com aspirina, Hb sérica, HbA1c, doença CV prévia, idade do dador, nº de compatibilidades HLA e tempo de isquemia frio), exceto quanto ao tempo em diálise, que era menor nos doentes em DP (21±15 vs 30±23 meses, p=0.003). A incidência de rejeição aguda e a duração média do internamento foi igual em ambos os grupos, mas o atraso na função do enxerto renal tendeu a ser menor nos doentes em DP.

A taxa global de relaparotomias foi equivalente nos doentes em HD ou DP (25.2% vs 28.2%), mas quanto motivada por trombose vascular, foi superior nos doentes em DP (12.8% vs 1.7%, p=0.014). A incidência de perda do enxerto pancreático de causa infeciosa foi mais alta nos doentes que fizeram DP (12.8% vs 3.4%, p=0.042); a perda do enxerto renal revelou-se também tendencialmente superior nos doentes em DP (20.5% vs 9.2%), sobretudo por trombose (5.1% vs 0%, p=0.058).

Sete dos 13 doentes que faleceram tinham feito DP, e esta frequência comparada com a dos doentes que faziam HD foi significativamente superior (17.9%, vs 5%, p=0.011). A causa predominante de morte nestes doentes foi a infeção (13.5%, vs 1.7% nos que faziam HD, p=0.010). A sobrevivência do doente aos 4 e 8 anos foi inferior nos doentes em DP. A análise multivariada, nesta amostra, mostrou que a DP, a doença CV e a falência de pelo menos um dos enxertos, foram preditores de morte do doente.

Na nossa experiência, a DP prévia ao TRP revelou-se um fator de pior prognóstico. Vários estudos apontaram um perfil pró-trombótico nos doentes em DP, sem que haja contudo consenso quanto às alterações encontradas. Fatores locais, como o dano do peritoneu com a DP crónica e a existência prévia do cateter peritoneal, podem ajudar a explicar a maior taxa de infeção abdominal e perda do pâncreas. Estudos nesta área, com mais doentes em DP, serão importantes para validar e clarificar as razões para este desfecho negativo. A profilaxia da trombose e da infeção, que os TRP realizam por rotina, poderão ter de ser ajustadas nestes doentes.

Pancreas-Kidney transplantation: Impact of dialysis modality on the outcome.

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Abbreviations: CVD (cardiovascular disease); DM1 (type 1 diabetes); Hb (hemoglobin); HbA1c (glycated hemoglobin); HD (hemodialysis); KTA (kidney transplantation alone); PD (peritoneal dialysis); SPKT (simultaneous pancreas-kidney transplantation/trasplants); VT (vascular thrombosis);

Running Title: Pancreas-Kidney transplants: dialysis modality and outcome.

Abstract:

Background: It remains controversial whether dialysis modality prior to SPKT (Simultaneous Pancreas-Kidney Transplantation) affects the outcome.

Aims and methods: We conducted this study to analyze the outcomes in type 1 diabetic patients undergoing SPKT, comparing peritoneal dialysis (PD) and hemodialysis (HD) groups. SPKT was preemptive in 7 patients; 119 had been on HD, 39 on PD. The single difference found at transplantation date was dialysis time, higher in HD patients (30±23 vs 21±15 months, p=0.003).

Results: Relaparotomy rate was similar in PD and HD patients (28.2% vs 25.2%), however, thrombosis as cause for reintervention was more frequent in PD patients (12.8% vs 1.7%, P=0.014). Pancreas loss due to infection was higher in PD patients (12.8% vs 3.4%, P=0.042). Kidney loss tended to be more frequent in PD patients, mainly caused by thrombosis (5.1%, vs 0% in HD patients, P=0.058).

Thirteen deaths occurred, more in the PD than in the HD group (17.9% vs 5%; P=0.011) and the leading cause of death was infection (13.5%, vs 1.7% in HD patients, P=0.010). Four and 8-year patient survival was inferior in PD patients. Besides PD, cardiovascular disease (CVD) and graft failure were independent predictors of patient death.

Conclusions: PD patients more frequently complicated with intraabominal infection leading to pancreatic loss and with renal thrombosis, with adverse impact on survival. Since a PD first strategy in end-stage renal disease patients is generally associated with good clinical outcomes, these gloomier results after SPKT urge for careful adjustment of transplant infection and thrombosis prophylactic protocols in PD patients.

Key words: graft survival; intraabdominal infection; peritoneal dialysis; simultaneous pancreas-kidney transplantation; type 1 diabetes; vascular thrombosis.

Word count: main document - 2584 words, abstract: 252 words

Introduction:

Several studies demonstrated that preemptive transplantation, defined as transplantation before chronic dialysis is required, improves patient and graft outcomes in renal transplant patients(1,2,3). However, most patients have to start renal replacement by dialysis because the kidney graft is not immediately available.

Regarding dialysis modality, peritoneal dialysis (PD) or hemodialysis (HD), and its impact on transplant outcomes, there is no consensus on which is associated with better results(1,3,4,5), meaning that outcomes might be generally similar after adjusting for patient characteristics and center experience.

The amount of time on dialysis may be the crucial factor with impact on outcome(1,6), along with patient comorbidities(7). Nevertheless, some studies reported an increment on renal graft loss in PD patients(8), namely due to renal vascular thrombosis (VT)(9,10,11), compared to those on HD. Other studies did not find such an increased risk(4,5). The underlying mechanisms predisposing to thrombotic events in PD patients are not totally known(12).

Increased incidence of sepsis in PD patients was also reported(13) but, again, there are conflicting results regarding this issue(14). Higher rates of early infection may probably be related to the length of hospitalization and also to a more intense immunosuppression, as in cases of acute rejection(15).

Data about the preferable dialysis modality prior to simultaneous pancreas-kidney transplantation (SPKT) are even more difficult to interpret. Option for PD or HD normally depends on patient condition, such as their own autonomy; comorbid situations; vascular and peritoneal conditions; dialysis-center factors; and patient convenience. Patient selection biases for each modality cannot be ruled out.

The purpose of this study was to analyze grafts and patient outcomes, in our type 1 diabetic (DM1) patients undergoing SPKT, comparing the subgroup that had been on PD with the other on HD.

Patients and methods:

We conducted a retrospective longitudinal cohort study in adult SPKT performed at our Unit. Among the 165 performed between May 2000 and December 2013 we studied 158 on dialysis prior to SPKT. Cases who received a preemptive transplant were not included in the analysis because of the small number of patients (n=7). PD was the dialysis modality in 39 patients, while 119 were on HD.

Systemic-enteric drainage was the technique used in all SPKT. Immunosuppression comprised anti-thymocyte globulin, tacrolimus, mycophenolate and steroids. Two abdominal drains are

left for some days in the transplanted patient: one draining the abdominal cavity; the second draining the renal graft fossae. Drains removal depends on volume and characteristics of the drainages. All the patients had one bladder catheter for at least 5 days; and one central venous catheter, usually for the 5 days of anti-thymocyte globulin administration. Antibiotic prophylaxis included vancomycin, flucanozole and second-generation cephalosporin preoperatively and during the first few days while catheter and drains persist; cotrimoxazole, nystatin and valgancyclovr were started after surgery. Thrombosis prophylaxis was made with aspirin (100mg/day) started before surgery; and enoxaparin (from 20 to 40mg/day, depending on patient weight and renal function recovery) started immediately after surgery, or when blood losses were considered to be not significant. Data were collected from patient file records, during the admission and after discharge, along with the outpatient follow-up.

Statistical Analysis

Continuous data were described using mean (±standard deviation) and categorical data were expressed as number (and percentages). Categorical data were compared using Pearson $\chi 2$ test or Fisher´s exact test and continuous variables were compared with Student t-test or Mann–Whitney U test, as appropriate. Patient survival was determined from the time of SPKT until death or end of follow-up. Death-censored kidney graft survival was determined from the time of SPKT until kidney failure (return to dialysis or retransplantation), or end of follow-up. Death-censored pancreas graft survival was determined from the time of SPKT until pancreas failure (permanent insulin requirement or retransplantation), or end of follow-up. Graft survival curves were done using Kaplan-Meier method and compared by log-rank test. Multivariable Cox proportional hazards analysis was applied to assess independent predictors of patient death, including clinically relevant variables and/or those presenting $P \leq 0.15$ in univariable analysis: recipient gender and age, dialysis technique (HD vs PD), time on dialysis, years of DM1, pretransplant glycated hemoglobin (HbA1c) < or ≥ 9%, concomitant cardiovascular disease and graft failure (kidney and/or pancreas).

A two-sided P-value <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

The relevant demographic and clinical data of the study population are presented in table 1. PD and HD patients were similar for the majority of their pretransplant characteristics: age; gender; duration of diabetes; HbA1c; percent of patients chronically taking aspirin; percent of patients with previously known cardiovascular disease (CVD); and value of hemoglobin (Hb) before surgery. Time on dialysis was the single distinguishable parameter, being inferior in the

PD group (20.7±14.6 months, vs 32.0±22.1 months in the HD group, P=0.003). Donor age; number of HLA-mismatches; cold ischemia time; acute rejection rate; and length of admission did not differ between both groups. There was a trend toward a lower rate of delayed renal graft function in PD patients.

The global relaparotomy rate was similar in PD and HD patients. However, when we analyzed the reasons for reintervention, thrombosis predominated (n=5) among all causes for reoperation in PD patients (n=11), being significantly higher when compared to HD patients (12.8% vs 1.7%, P=0.014). A nonsignificant higher proportion of HD patients (7.6%, vs 2.6% in PD patients) underwent bleeding-driven relaparotomy. To exclude a possible effect of era and cumulative experience on the rate of infection and thrombosis complications, the rate of such events were explored in each year of the SPKT program and it was seen that the events were distributed similarly along the time-course of the SPKT program.

Table 2 summarizes complications leading to each graft loss and patient death, in both groups. Death-censored pancreas graft loss was not significantly different in both groups (28.2% for PD, vs 19.3% for HD patients). However, we registered more pancreas losses due to infection among PD patients (12.8%, vs 3.4% among HD patients, P=0.042). There was a trend towards a higher rate of global pancreas failure (death-censored) at month 1 in PD patients (15.4%, vs 6.7% in HD patients, P=0.099), but thereafter this difference disappeared. Additionally, we verified that the permanence of the abdominal drain after surgery was almost double in PD patients (means of 4.7±1.5 days, vs 2.6±1.2 days in HD patients, P<0.001).

As to the renal graft, death-censored graft failure was also not different in both groups (12.8% for PD, vs 5.9% for HD patients). Analyzing the causes of renal loss, we noted a similar acute rejection rate in both groups, but a tendency to a higher rate of losses due to thrombosis in PD patients (5.1%, vs 0% in HD patients, P=0.058). There was a trend toward an increased first-month renal graft failure in PD patients (5.1%, vs 0% in HD patients, P=0.060); and we observed that this tendency persisted after this period, when accounting for the total number of renal losses during follow-up (20.5%, vs 9.2% among HD patients, P=0.060).

We also analyzed other factors prior to SPKT that might have influenced thrombosis rate, leading or not to graft loss: the rate of patients taking aspirin as chronic medication; the level of Hb; and preexistent CVD, were similar in patients with and without thrombosis. On the contrary, patients who complicated with thrombosis more often were under PD (6/39 vs 7/119, P=0.061). The same analysis was made for preexistent conditions predisposing to bleeding, needing surgery or even leading to graft loss. None of the studied factors (chronic medication with aspirin, Hb level, previous CVD and dialysis modality) were associated with a

higher incidence of bleeding. Among those who complicated with bleeding 11 out 12 were on HD, but this was not statistically significant (P=0.296).

Patient death was significantly higher in the PD group: 17.9% (n=7), compared to 5.0% (n=6) in the HD group, P=0.011. Infection, as a cause of death, predominated in PD patients (13.5%, vs 1.7% in HD patients, P=0.010). CVD-related death was registered only in HD group (4 cases, or 3.4%), although not statistically different from the PD group.

Four-year and 8-year survival rates are presented in table 3. Death-censored survival rates for pancreas graft were similar in HD and PD patients, irrespective of first-month losses inclusion or not. Death-censored survival rates for the renal graft were similar in HD and PD patients when first-month losses were excluded. If these early losses were also considered, then renal survival was inferior in PD patients. Lower patient survival was observed in the PD group (87.0% and 71.2%, vs 98.1% and 95.2% in HD group, at 4 and 8 years respectively, P=0.003).

On multivariate analysis, graft failure (one or both grafts), the modality of dialysis and concomitant CVD were confirmed as independent predictors of patient death (table 4). The likelihood of death was 8.76 times higher if at least one graft failed; 6.23 times higher if PD was the dialysis modality prior to SPKT; and 4.05 times higher when they have had clinically significant CVD. Figure 1 illustrates patient survival curves.

Discussion:

Results from published studies about the relationship between dialysis modality and the outcome of transplant are not concordant. PD has been associated with poorer transplant outcomes by some authors(8,9,10,11), but others did not report any detrimental effect of PD(4,5). However, most of these results came from kidney transplantation alone (KTA).

In SPKT, infection(16,17,18,19,20), thrombosis(21,22), and bleeding(21), leading to subsequent relaparotomy(20,21,23) are feared complications, since they have been associated with lower graft survival.

There are several classical and well-recognized risk factors for VT, such as multiple vessels, technical problems during anastomosis, very young pediatric donors or elderly donors, thrombocytosis, hemoconcentration, hypotension, and the existence of a previous transplant(9). Obviously, hypercoagulable states substantially increase the rate of thrombosis(9,21). Diabetes, itself, has been considered an additional risk factor for thrombosis(9) and prothrombotic disorders may be frequent in DM1 patients undergoing SPKT(24).

Though controversies still exist, PD may predispose to a thrombophilic state by several mechanisms(9) that are not completely clear. Enhanced plasmatic activity of procoagulant

factors(25) and hemoconcentration(26) were mentioned as being more likely to occur in PD than in HD patients. Robertson AJ et al(12), showed that the addition of low dose aspirin was beneficial in reducing the rate of VT in KTA patients. Additionally impaired fibrinolysis caused by increased plasma plasminogen activator inhibitor-1(PAI-1) levels is linked with insulin resistance that occurs with uremia and might be exacerbated in certain PD patients(27).

Concerning the prevention of pancreas graft thrombosis, one recent study reported potential beneficial effects with low-dose heparin started in the early postoperative period (in association with aspirin), at the expense of a higher number of relaparotomies(21). Others have found better results using lower-molecular-weight heparin and concluded that this prophylaxis strategy might not be inferior to the one using dose-adjusted intravenous unfractionated heparin(22).

PD population usually includes patients with vascular access problems, possibly due to a preexisting prothrombotic state in some of them – a selection bias that may help to explain higher rates of renal VT in PD patients(9). The transplant center volume and professional skill can additionally influence the results. Our transplant team for SPKT is restricted and has remained stable over the years, rendering a bias from different technical skill of multiple surgeons very unlikely.

In this study we observed a higher relaparotomy rate due to thrombosis and a near significant higher rate of renal graft loss secondary to thrombosis, in PD patients. Only 2 patients had a previous renal transplant and none complicated with VT, albeit repeated transplantation is an established risk factor(9); Hb level and chronic medication with aspirin were comparable in PD and HD patients. However, we have to be cautious in interpreting these results, given the small number of renal thrombosis (2 cases). Future results from larger series including PD patients, may bring more consistent data regarding the association between PD and thrombosis in SPKT patients who are normally under thrombosis prophylaxis.

A distinct approach, with more aggressive anticoagulation prophylaxis has been suggested in PD patients, but it is not definitely established. Yet, the bleeding risk must be weighed. More studies clarifying the predisposing factors to thrombotic events in PD patients are needed, in order to design an effective prophylaxis against thrombosis.

While some authors reported similar abdominal infection rates in PD and HD patients(16,17), there are several others reporting higher incidence of peritonitis in PD patients(18,19,28). Manipulation of the peritoneal catheter, communicating with the skin and the external environment, is the major cause for peritonitis in PD patients. Whether this catheter remains colonized by microbial agents due to biofilm formation, even without overt infection, is a real

possibility(16). However, the cultured agents from the drainage are frequently diverse (gram negative) from those more often cultured during peritonitis episodes (gram positive) in PD patients(16). In SPKT, there are other confounding factors, such as the surgery procedure itself and the opening of the small bowel to perform duodenal anastomosis.

Fluid collections, as well as vascular catheters or surgical drains, contribute to the risk of infection. Some degree of persistent ascites after PD catheter removal is frequently observed, representing chronic and remarkable changes in peritoneal membrane(29). Important volume drainage leads to the maintenance of the surgical drain for more days, as we observed in our PD group, again increasing the risk of infection. In our practice, PD catheter is always removed at the beginning of procedure of SPKT and residual ascites cultured. The abdominal drainage is repeatedly cultured while it remains important and the drain maintained, being removed as soon as possible when drainage amylase decreases and microbiological analysis is sterile. However prophylactic antibiotics may need to be extended in patients with more prolonged drain patency.

Fibrosis and peritoneal thickening following PD(30) may adversely affect peritoneal and intestinal healing after the surgery, contributing to increase the rate of leak and infection(16).Graft pancreatitis and duodenal leak are of paramount importance for abdominal cavity infection occurrence(31). Abdominal surgical reexploration, in immusuppressed patients, represents another risk factor for infectious complications and for adverse graft outcomes(23), leading to an enhanced rate of transplantectomy(20).

More intense immunosuppression used in SPKT, compared to KTA, also augments patient susceptibility for several types of infections. Our results demonstrated an increased rate of pancreas loss due to infection in PD patients. The length of admission and the acute rejection incidence, possible contributors to infection(15), cannot explain the higher infection rate of in this group, given that they were similar between HD and PD patients.

One cannot underappreciate that our transplantation results achieved in the PD group were inferior, compared to those in the HD group. Thrombosis-related reinterventions and infection-related pancreatic losses were more frequent within PD patients; and patient survival was lower. We cannot say that PD patients were in worse condition: both groups had similar age, time of diabetes, acute rejection and delayed graft function rates as well as comorbidities such as CVD. On the contrary, PD patients had a mean time on dialysis lower than HD patients, meaning they were early referred to SPKT, which is a positive aspect. It has been reported that PD patients are more likely to receive a renal transplant(8); and less likely to evolve with delayed kidney graft function(8), a tendency also observed in our study.

CVD and graft loss are unquestionable risk factors for patient death(32). In our study PD was a predictor of death, mainly due to infection and thrombosis. Focused investigation in broader sample of patients is needed since the risk of these complications after SPKT is potentially modifiable with adjusted per-operatory infection and thrombosis prophylactic protocols.

Authorship: L.S.M. designed the study, performed research, analyzed data, contributed to discussion and wrote the manuscript. A.C.H. and D.S. performed research and contributed to discussion. J.M. analyzed data and contributed to discussion. A.C., S.P., M.A, L.D. and J.D contributed to discussion and interpretation of data. A.S.R. and I.L.N. contributed to discussion and edited the manuscript. All the authors reviewed and approved the final version of the manuscript.

This work was approved by the appropriate ethics committee. Therefore it has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments; and in accordance with the Declaration of Istanbul.

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Table 1 – Demographic and clinical characteristics of the study population

	Total (n=165)	**Preemptive (n=7)**	**HD (n=119)**	**PD (n=39)**	**P value HD vs PD**
Age (R-Recipient)	34.8±6.0	33.6±7.5	35.2±6.3	33.9±4.8	0.167
Female gender (R)	87 (52.7%)	2 (28.6%)	57 (47.9%)	25 (64.1%)	0.079
Time of Diabetes (years)	23.87±5.98	24.4±9.2	23.6±6.2	24.7±4.5	0.285
Time on dialysis (months)	**29.2±21.0**	**-**	**32.0±22.1**	**20.7±14.6**	**0.003**
HbA1c pre-SPKT (%)	9.0±6.9	7.8±1.5	9.4±7.8	8.0±1.4	0.130
HbA1c pre-SPKT≥9%	52 (37.4%)	2 (33.3%)	42 (40%)	8 (28.6%)	0.267
Cardiovascular disease (n/%)	31 (18.8%)	2 (28.6%)	24 (20.2%)	5 (12.8%)	0.304
Taking Aspirin pre-SPKT	120 (72.7%)	5 (71.4%)	86 (72.3%)	29 (74.4%)	0.799
Hb pre-SPKT (g/dl)	10.9±1.2	11.1±0.9	10.9±1.3	10.9±0.7	0.943
Hospital stay (days)	25±19	16±6	25±17	27±26	0.718
Age (Donor)	28.2±10.6	28.7±15.3	28.3±10.4	28.0±10.7	0.248
HLA-mismatches (total)	4.53±1.08	4.86±1.07	4.55±1.04	4.42±1.20	0.566
Cold ischemia time (hours)	11.3±4.0	9.3±3.2	11.6±5.1	10.9±4.4	0.786
Delayed (renal) graft function	24 (14.5%)	1 (14.3%)	21 (17.6%)	2 (5.1%)	0.067
Acute Rejection (n/%)	26 (15.8%)	1 (14.3%)	21 (17.6%)	4 (10.3%)	0.272
SPKT with relaparotomy	42 (25.5%)	1 (14.3%)	30 (25.2%)	11 (28.2%)	0.711
Causes:					
- Infection	20 (12.1%)	0	16 (13.4%)	4 (10.3%)	1.0
- bleeding	10 (6.1%)	0	9 (7.6%)	1 (2.6%)	0.453
- **thrombosis**	8 (4.8%)	1 (14.3%)	2 (1.7%)	5 (12.8%)	**0.014**
- others	4 (2.4%)	0	3 (2.5%)	1 (2.6%)	1.0

Table 2 – Graft failure and patient death occurrence and its causes.

	Total (n=165)	**Preemptive (n=7)**	**HD (n=119)**	**PD (n=39)**	**P value (HD vs PD)**
Pancreas (Px) failure*	35 (21.2%)	1 (14.3%)	23 (19.3%)	11 (28.2%)	0.242
- Rejection	8 (4.8%)	1 (14.3%)	5 (4.2%)	2 (5.1%)	0.659
- Thrombosis	11 (6.7%)	0	7 (5.9%)	4 (10.3%)	0.290
- Bleeding	3 (1.8%)	0	3 (2.5%)	0	1.0
- **Infection**	9 (5.5%)	0	4 (3.4%)	5 (12.8%)	**0.042**
- Other causes	4 (2.4%)	0	4 (3.4%)	0	0.576
>1 month			15 (13.5%)	5 (15.2%)	0.811
Px Failure_global	41 (24.8%)	1 (14.3%)	28 (23.5%)	12 (30.8%)	0.367
Px Failure_1month*	14 (8.5%)	0	8 (6.7%)	6 (15.4%)	**0.099**
Kidney (Kx) failure*	13 (7.9%)	1 (14.3%)	7 (5.9%)	5 (12.8%)	0.156
- Rejection	9 (5.5%)	0	7 (5.9%)	2 (5.1%)	1.0
- **Thrombosis**	3 (1.8%)	1 (14.3%)	0	2 (5.1%)	**0.058**
- Infection	1 (0.6%)	0	0	1 (2.6%)	0.238
>1 month			7 (5.9%)	3 (8.1%)	0.629
Kx Failure_global	20 (12.1%)	1 (14.3%)	11 (9.2%)	8 (20.5%)	**0.060**
Kx Failure_1month*	3 (1.8%)	1 (14.3%)	0	2 (5.1%)	**0.060**
Patient death	**13 (7.9%)**	**0**	**6 (5.0%)**	**7 (17.9%)**	**0.011**
- Cardiovascular	4 (2.4%)	0	4 (3.4%)	0	0.578
- Infection	7 (4.2%)	0	2 (1.7%)	5 (13.5%)	**0.010**
- Other causes	2 (1.2%)	0	0	2 (5.9%)	0.052
Follow-up (years)	**5.87±3.64**	**5.89±3.77**	**6.30±3.48**	**4.55±3.88**	**0.015**

*** death-censored**

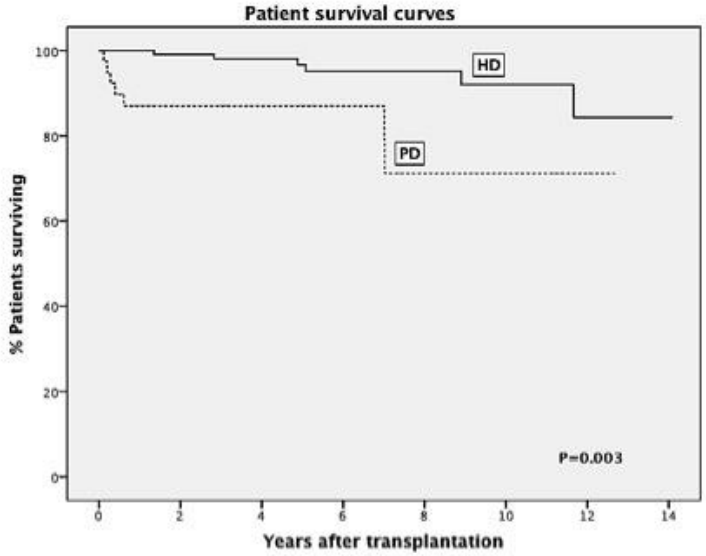
Table 3 – Four-year and 8-year survival rates (log-rank test) of HD and PD patients

	4-years	8-years	
Pancreas graft survival (death-censored)			
HD	82.3%	78.9%	
DP	79.5%	57.7%	P=0.128
Pancreas graft survival (death-censored) **– excluding 1st-month losses**			
HD	88.2%	84.5%	
DP	93.9%	68.1%	P=0.561
Kidney graft survival (death-censored)			
HD	98.1%	92.4%	
DP	88.1%	82.9%	**P=0.048**
Kidney graft survival (death-censored) **– excluding 1st-month losses**			
HD	98.1%	92.4%	
DP	92.5%	87.4%	P=0.333
Patient survival			
HD	98.1%	95.2%	
DP	87.0%	71.2%	**P=0.003**

Table 4 - Multivariable Cox proportional analysis of predictors of patient death

	HR	**IC 95%**	***P***
Concomitant cardiovascular disease	**4.051**	**1.091-15.041**	**0.037**
Graft failure (kidney and/or pancreas)	**8.764**	**2.198-34.950**	**0.002**
Dialysis modality (PD vs HD)	**6.231**	**1.460-26.591**	**0.013**
Recipient Age	1.055	0.939-1.186	0.367
Recipient gender (M vs F)	1.987	0.508-7.773	0.324
Months on dialysis	0.941	0.962-1.037	0.941
Years of diabetes evolution	1.089	0.946-1.253	0.235
Pretransplant HbA1c (< vs ≥9%)	0.243	0.053-1.126	0.071

Figure 1 – Patient survival curves (Kaplan-Meier method)



IV - Doença cardiovascular no TRP

- **Martins L**, Fonseca I, Dias L, Malheiro J, Rocha A, Azevedo P, Silva H, Almeida R, Henriques AC, Davide J, Cabrita A. **Cardiovascular risk factors and events in pancreas-kidney transplants.** *Transplant Proc*. 2013; 45(3): 1063-1065.

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Resumo IV

A doença cardiovascular (CV) e cerebrovascular representam as principais causas de morbilidade e mortalidade em doentes diabéticos. O controlo dos fatores de risco modificáveis para a aterosclerose é importante para reduzir a incidência de AVC, EAM e doença arterial periférica. Espera-se que o TRP possa melhorar estes riscos nos DM1 submetidos a este procedimento. Avaliámos em 103 TRP com ambos os enxertos funcionantes, os parâmetros de controlo metabólico, a HTA, o IMC e os eventos CV. Este grupo tinha um seguimento mínimo de 6 meses e máximo de 142 meses, HbA1c média de 5,3% e depuração da creatinina de 76ml/min. Em 67% tinha sido possível suspender os corticóides; todos estavam sob tacrolimus; 9,7% sob sirolimus em alternativa ao micofenolato.

Dos critérios para síndrome metabólica, tínhamos apenas 1 doente com HbA1c>5,6%; 4 com glicemia em jejum >100mg/dl; 38,5% com HTA; 19,4% com HDL-colesterol baixo; 7,8% com hipertrigliceridemia; 21,4% sob estatinas; 2 casos com IMC >30. Foram registados eventos CV em 6,8%.

Observámos que os doentes com corticóides tinham triglicerídeos mais altos (122±53 vs 90±36mg/dl, P=0,001) e tendiam a ter mais frequentemente HTA. Os doentes com HTA tinham IMC mais elevado (24,1±2,8 vs 22,3±2,9kg/m^2, P=0,002). IMC >25 associou-se a valores mais altos de colesterol total (195±47 vs 169±28mg/dl, P=0,015) e LDL-colesterol (116±40 vs 96±27mg/dl, P=0,003).

Neste grupo de TRP, a prevalência de eventos CV, síndrome metabólica e obesidade foi baixa, quando confrontada com resultados de outros estudos internacionais. A HTA foi o fator que isoladamente mais se observou, sendo que todos os doentes que faziam qualquer tipo de fármaco com ação anti-HTA (mesmo se faziam apenas beta-bloqueador pelo seu efeito cardio-protetor) foram contabilizados como hipertensos. Confirmámos que os doentes mantidos sob corticoterapia tinham trigliceridemia mais elevada e tendência a ser mais frequentemente hipertensos; e que os hipertensos tinham IMC mais alto e perfil lipídico mais adverso.

Estes dados permitem-nos concluir que a retirada de corticóides deve ser levada a cabo sempre que possível, tentando melhorar o perfil metabólico e minimizar o risco CV nos TRP.

Cardiovascular Risk Factors and Events in Pancreas-Kidney Transplants

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ABSTRACT

Cardiovascular and cerebrovascular disease (CCVD) are major causes of morbidity and mortality among patients with diabetes. Strict control of treatable risk factors that contribute to atherosclerosis is important to reduce the risk of stroke, myocardial infarction, and peripheral arterial disease. Simultaneous pancreas-kidney transplantation (SPKT) may significantly improve these risk factors in patients with type 1 diabetes. We studied 103 SPKT from our center with both organs functioning for metabolic and hypertensive control; body mass index (BMI); immunosuppression; and CCVD events. The 53 females/50 males showed a mean age of 35 ± 6 years, diabetes for 24 ± 6 years, and on dialysis for 31 ± 23 months. The follow-up ranged from 6–142 months. Mean value of last creatinine clearance was 76 ± 24 mL/min, all 103 SPKT were insulin-independent with mean glycemia = 81 ± 10 mg/dL and hemoglobin A_{1c} (HbA_{1c}) = 5.3% ± 0.4%. All of them were under tacrolimus treatment; 9.7% also with sirolimus but 67% steroid-free. According to the National Cholesterol Education Program Adult Treatment Panel 3 criteria, 4 patients showed a fasting glucose > 100 mg/dL; only one, HbA_{1c} > 5.6%. Hypertension was recorded in 38.5%; low high-density lipoprotein cholesterol in 19.4%; hypertriglyceridemia in 7.8%; BMI > 30 in only 2 patients; 21.4% were prescribed statins. We registered cardiovascular events in 7 patients (6.8%). Patients with steroid treatment showed higher triglycerides (122 ± 53 vs 90 ± 36 mg/dL; P = .001) and more often tended to be hypertensive (41.2% vs 37.7%, P = .073) compared with those free of these drugs. Hypertension was associated with an higher BMI (24.1 ± 2.8 vs 22.3 ± 2.9 kg/m^2, P = .002). BMI > 25 was associated with higher total cholesterol (195 ± 47 vs 169 ± 28 mg/dL, P = .015) and low-density lipoprotein cholesterol (116 ± 40 vs 96 ± 27 mg/dL, P = .003). Among our SPKT the prevalences of CCVD and metabolic syndrome were low. Hypertension was the most frequent single factor. Obesity was rare. In patients on steroids, hypertriglyceridemia was more prevalent and hypertension tended to be more frequent. Hypertensive patients showed a higher BMI, which correlated with a worse lipid profile. Steroid withdrawal, whenever possible, may be important to achieve metabolic goals and minimize cardiovascular risk.

PATIENTS WITH DIABETES and chronic renal disease are at an increased risk of adverse outcomes of cardiovascular and cerebrovascular disease (CCVD). Metabolic syndrome (MS) is a known independent cardiovascular (CV) risk factor. Simultaneous pancreas-kidney transplantation (SPKT) was established as the best treatment for selected patients with type 1 diabetes and end-stage renal disease. Compared with kidney transplantation alone (KTA), SPKT may prevent or diminish the progression of

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diabetic complications, not only in terms of the microvascular but also macrovascular disease.[1] In fact, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study has already reported a reduction in fatal and nonfatal CCVD events with long-term strict glycemic control,[2] which can be achieved by successful pancreas transplantation. MS prevalence among KTA patients is high: up to 33% at 1 year and 63% at 6 years after transplantation.[3,4] Our aim was to evaluate the prevalence of and associated factors with MS among our cohort of SPKT.

SUBJECTS AND METHODS

From May 1, 2000, to October 31, 2011, we performed 135 SPKT. During this period 6 patients died, 26 lost at least 1 graft (pancreas and/or kidney), and 103 maintained their first pancreas and kidney grafts. These, 103 patients with both grafts functioning were included in this cross-sectional study to study their metabolic and hypertensive, control body mass index (BMI), immunosuppressive regimen as well as occurrences of CCVD and nonfatal events. The diagnosis of MS was established according to the National Cholesterol Education Program Adult Treatment Panel 3 (NCEP/ATP3) criteria based upon BMI.

Statistical Analysis

Results are presented as mean values ± standard deviations for continuous, normally distributed variables and as percentages for categorical data. Normal distribution of variables was examined using the Kolmogorov-Smimov test. Continuous variables were analyzed using 2-tailed *t* tests for independent observations. Categorical data and proportions were analyzed using χ^2 or Fisher exact test when required. A *P* value less than .05 was considered statistically significant. Analyses were performed using SPSS software version 20.0 (SPSS, Chicago, Ill, USA).

RESULTS

We studied a cohort of 103 SPKT patients, including 53 females and 50 males, who maintained both grafts as functional. Their mean age at transplantation was 35 ± 6 years; the mean time of diabetes evolution was 24 ± 6 years; and they had been on dialysis for 31 ± 23 months (except 5, who underwent preemptive transplantation). The initial immunosuppressive protocol included antithymocyte globulin, tacrolimus, mycophenolate, and steroids. The follow-up after transplantation ranged from 6 to 142 months.

At last evaluation, all the patients were prescribed tacrolimus, the majority of whom (82.5%) were also taking mycophenolate and 9.7% sirolimus. Steroid tapering below the daily dose of 10 mg was normally initiated after month 6 with complete withdrawal achieved in 67% of patients at the end of the first year. No rejection episodes were registered in patients withdrawn from steroids. The 103 SPKT are insulin-free with a mean fasting glycemia of 81 ± 10 mg/dL hemoglobin A_{1c} (HbA_{1c}) of 5.3% ± 0.4% and C-peptide of 3.1 ± 1.9 ng/mL. In terms of kidney graft function, the mean serum creatinine concentration was 1.2 ± 0.4 mg/dL and creatinine clearance 76 ± 24 mL/min.

According to the NCEP/ATP3 criteria, 4 (3.9%) subjects displayed fasting glucose concentrations above 100 mg/dL, but only one an HbA_{1c} > 5.6%. Hypertension (>130/85 mm Hg) was recorded in 38.5%, the majority of whom were treated with only one drug, mostly a low-dose of a beta-blocking agent. Low high-density lipoprotein (HDL)-cholesterol (<40 mg/dL in men and <50 mg/dL in women) was observed in 19.4%; hypertriglyceridemia (>150 mg/dL) in 7.8%; BMI > 30% in only 2 patients (1.94%), and 21.4% were prescribed statins. Only 9 patients (8.7%) fulfilled the criteria for MS (>3 criteria). Table 1 summarizes these results.

We registered 7 (6.8%) nonfatal CV events among the whole cohort of 103 SKPT: angina pectoris (n = 2) and peripheral artery disease (PAD; n = 5). Among the initial group of 135 SPKT 6 patients died, including from CCVD 1 stroke and 1 myocardial infarction. Among the 26 patients who had at least one graft failed 5 had symptomatic CV disease 2 had PAD, 2 had myocardial infarction, and 1 had angina pectoris.

We compared patients with (*n* = 34) versus without (*n* = 69) steroid therapy. Patients with steroids showed higher levels of total cholesterol (182 ± 41 vs 171 ± 31 mg/dL, *P* = .128), lower levels of HDL cholesterol (54 ± 16 vs 56 ± 14 mg/dL, *P* = .571), and more frequent statin prescriptions (26.5% vs 18.8%, *P* = .374), but none of these differences were significant. However, among patients on steroids, the triglyceride levels were significantly higher (122 ± 53 vs 90 ± 36 mg/dL, *P* = .001) and there was a tendency to more frequently display hypertension (41.2% vs 37.7%, *P* = .073).

Hypertensive patients showed a higher mean BMI (24.1 ± 2.8 vs 22.3 ± 2.9 kg/m^2, *P* = .002) with a trend to higher low-density lipoprotein (LDL) cholesterol levels (107 ± 32 vs 96 ± 29 mg/dL, *P* = .071). Comparing patients with BMI >25 kg/m^2 (*n* = 23) with <25 kg/m^2 (*n* = 80), the overweight patients displayed unfavourable lipid profiles with higher levels of total cholesterol (195 ± 47 vs 169 ± 28

Table 1. Prevalence of Glucose and Lipid Abnormalities and MS*

Abnormalities	*n* (%)
Parameters of MS:	
Glucose (>100 mg/dL; or drug treatment)	4 (3.9%)
HDL cholesterol (<40 mg/dL men; <50 mg/dL women; or drug treatment)	20 (19.4%)
Triglycerides (>150 mg/dL; or drug treatment)	8 (7.8%)
Obesity (>30 kg/m^2)	2 (1.9%)
Hypertension (>130/85 mm Hg; or under treatment)	40 (38.5%)
With MS (>3 criteria)	9 (8.7%)
Other	
Overweight (25–29 kg/m^2)	21 (20.4%)
Use of statins	22 (21.4%)
HbA1c > 5.6%	1 (0.97%)

MS, metabolic syndrome; HDL, high-density lipoprotein; HbA_{1c}, hemoglobin A_{1c}.
*In a cohort of 103 simultaneous pancreas-kidney transplantation with functioning pancreas and kidney grafts.

mg/dL, $P = .015$) and LDL cholesterol (116 ± 40 vs 96 ± 27 mg/dL, $P = .003$). A significant positive correlation was observed between BMI and the length of follow-up after SPKT ($r = .32$; $P = .001$).

DISCUSSION

Among our patients with type 1 diabetes after SPKT, we observed a low prevalence of MS (8.7%) and nonfatal events (6.8%). These values were substantially better than those reported for patients with KTA,[3,4] although in the majority of published studies, the analysis included a mixed group of type 1 and type 2 diabetic subjects. Among a large cohort after KTA the myocardial infarction incidence has been reported to exceed 5% at 1 year and 11% at 3 years.[5] Isolated hypertension was the most frequent registered parameter of MS, however, 38.5% could be considered to be a low prevalence. Beta-blocking agents are widely used in diabetics based upon their cardioprotective effects in patients with coronary artery disease. In this study we counted as hypertensive all patients who were prescribed any kind of hypotensive medication, including a low doses of a beta-blocking drug.

We observed an association between hypertension and high BMI. Obesity, defined as >30 kg/m^2, was rare. BMI higher than 25 kg/m^2 was associated with higher levels of total cholesterol and LDL cholesterol. Nevertheless, the mean values of total cholesterol and triglycerides recorded among our study population were substantially better than those reported in earlier[6] or even more recent studies[7] among KTA recipients. Moreover, we verified a correlation between the length of the follow-up and the BMI. This shows a tendency of SPKT patients to gain weight after transplantation. Weight gain commonly described in KTA is due to several factors, including reversal of the uremic state with increased appetite and physical inactivity.[8]

CV disease is the most common cause of death among diabetic recipients of a kidney transplant.[9] Not only diabetics, but even the global population of KTA patients, independent of the cause of end-stage of renal disease, are considered to be among the highest risk group for CV disease due to their accelerated atherosclerosis.[10] Type 1 diabetic patients with end-stage renal failure, who have had long courses of diabetes, are definitely a high-risk population for CV disease and death.

Steroids are well-known contributors to atherosclerosis and represent an independent CV risk factors due to associated hypertension, hyperlipidemia, and glucose intolerance. Steroid avoidance or withdrawal among SPKT recipients seeks to reduce this risk. In a previous study, we have already reported the safety and feasibility of steroid withdrawal without an increase in immune events.[11] In the present analysis with an extended follow-up, we confirmed not only the feasibility of steroid discontinuation, but also the above benefits. Maintenance immunosuppression with tacrolimus and mycophenolate usually allows a high proportion of patients to be maintained without steroids with good outcomes.[12]

In conclusions, this study of SPKT patients with both grafts functioning showed a low incidence of MS and nonfatal events. We observed an association between steroid maintenance treatment and hypertriglyceridemia and a near significant association with hypertension. Hypertensive patients showed a higher BMI, which correlated with an adverse lipid profile. We discontinued steroids in the majority of cases without acute rejection episodes. We will pursue this strategy to minimize steroid exposition in SPKT, believing this may be an important step to achieve metabolic goals and to reduce CV risk.

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V - Evolução da doença mineral óssea após o TRP

- **V.1 -** Pereira S, Pedroso S, **Martins L**, Santos P, Almeida M, Freitas C, Dias L, Dores J, Almeida R, Castro Henriques A, Teixeira M. **Bone mineral density after Kidney-Pancreas Transplantation: four years follow-up in 57 patients**. *Transplant Proc* 2010; 42(2): 555-557.

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- **V.2 –** Ana Rocha A, **La Salete Martins**, Jorge Malheirro, Jorge Dores, Clara Santos, Rui Almeida, José Davide, Castro Henriques. **Changes in bone mineral density following long term simultaneous pancreas-kidney transplantation**. (*submetido*).

Resumo V.1

A doença óssea é um problema major após transplantação de órgãos, sobretudo nos doentes com longo tempo de diabetes e doença renal crónica, ambas as situações implicadas na perda de massa óssea. As complicações ósseas, como as fraturas, são frequentes nestes doentes. No TRP os estudos são muito escassos.

Apesar de ser lógico pensar que se obtém uma recuperação da densidade mineral óssea (DMO) após o transplante, várias publicações reportam uma perda de massa óssea significativa nos primeiros 6 meses, podendo observar-se alguma recuperação apenas posteriormente. A imunossupressão, nomeadamente os corticóides, são uma das causas apontadas para essa perda.

O objetivo deste estudo foi avaliar a evolução da DMO em 57 TRP, até 4 anos de evolução pós-transplante. Tinham em média 34 anos de idade e 23 anos de DM1, 65% eram mulheres. À data de transplante 28% tinham critérios de osteoporose e um T-score da coluna lombar de -1.75±1.05 e do colo do fémur de -1.95±0.73. Passado 1 ano, observou-se uma melhoria do T-score lombar em 76% dos casos, tendo passado a -1.33±0.94 (P=0.044), e estabilização do T-score femoral. Verificou-se uma melhoria gradual ao longo dos 4 anos, sendo significativa a nível lombar, para -1.04±0.67 (P=0.004) e menor a nível do fémur (-1.69±0.49, P=0.12).

De realçar que nesta avaliação final não havia casos com critérios de osteoporose, que 86,7% destes doentes estavam sem corticóides e os restantes com ≤5mg/dia de prednisolona. A utilização de bifosfonatos, cálcio e/ou análogos da vitamina D é prática comum nos nossos doentes TRP.

A incidência de fraturas ósseas foi baixa, <10%, nestes doentes. Consistentemente com outros estudos, verificou-se uma melhoria mais significativa da DMO na coluna lombar do que no fémur.

A estratégia de redução/retirada de corticóides na nossa Unidade, bem como o uso de terapêuticas com potencial na prevenção e/ou tratamento da perda óssea, podem estar relacionadas com estes resultados positivos da evolução da DMO nos TRP estudados.

Bone Mineral Density After Simultaneous Kidney–Pancreas Transplantation: Four Years Follow-up of 57 Recipients

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ABSTRACT

Bone disease and an high risk of fractures are major problems in transplantation. Among diabetic patients undergoing simultaneous kidney–pancreas (SKP) transplantation, there are few studies assessing long-term effects on bone mass. The aim of this study was to evaluate bone mineral density (BMD) over 4 years follow-up after SKP transplantation. Fifty-seven patients had 22.8 ± 5.3 years of prior diabetes, 65% were female, and the overall mean age was 34.3 ± 5.93 years. At the time of transplantation, the lumbar spine and femoral neck T-scores were -1.75 ± 1.05 and -1.95 ± 0.73, respectively; 28% of subjects had evidence of osteoporosis. One year after transplantation, 77.6% of patients displayed improved lumbar T-scores to -1.33 ± 0.94 ($P = .044$) with stable femoral neck T-scores. Bone densitometry enhanced gradually through the 4 years follow-up: lumbar T-score to -1.04 ± 0.67 ($P = .004$) and femoral neck T-score to -1.69 ± 0.49 ($P = .12$). At year 4, no osteoporosis cases were detected but 86.7% of patients did not receive steroids in the immunosuppressive regimen. The graft function remained stable (serum creatinine, 1.2 mg/dL; fasting glucose, 87.7 mg/dL). During the follow-up, BMD improved more significantly at lumbar sites. Our study reports a reduced prevalence of fractures (8.7%) compared with the literature, which could be related to a steroid-sparing protocol and/or aggressively treatment of osteoporosis.

SIMULTANEOUS kidney–pancreas (SPK) transplantation is the treatment of choice for patients with end-stage renal disease secondary to diabetic nephropathy. Despite advances in patient and graft survival after SKP transplantation, bone disease is one of the most common complications.[1] Bone loss and consequent fracture are prevalent.[2–4] The incidence of fractures in kidney transplant patients has been reported to be as high as 45% at 1 year posttransplantation.[5–7] Among SKP transplants the risk of fracture is even greater compared with kidney recipients. The increased prevalence of fractures may incapacitate some patients affecting quality of life.[8] In some solid organ transplants, there is a significant amount of evidence that steroids are the main cause of posttransplant bone loss, especially the rapid loss that occurs in the first 6–12 months. In SKP other factors may contribute such as the diabetic state and consequent nephropathy.[9–11] Data characterizing bone loss after SKP transplantation, particularly in long-term are scarce. Some studies have demonstrated a rapid loss of bone density over the first 6 months, with trabecular bone being the most affected. After this period it tends to stabilize or even recover.[4,10] The optimal strategy to prevent or improve management of bone loss in transplantation patients, particularly after SKP, is controversial. The aim of our study was to evaluate BMD over 4 years follow-up among 57 patients who underwent SKP transplantation.

METHODS

Eighty-eight patients with diabetic nephropathy underwent SKP transplantation between 2000 and 2008. A retrospective study was performed in a cohort of 57 subjects with functioning kidney and pancreas grafts excluding patients with recent procedures, insufficient x-ray densitometry data, or kidney or pancreas graft loss.

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Graft function was determined by measuring serum creatinine, fasting glycemia, and glycosyilated hemoglobin (HbA_{1C}). Bone mineral density determined by X-ray densitometry had results expressed as T-scores and BMD (g/cm^2). Patients underwent a BMD measurement at the time of transplantation and then annually. The normal references values of BMD were those defined by the World Health Organization.

The data were analysed with SPSS 12.0 (SPSS, Inc, Chicago, Ill). Results were expressed as mean values and standard deviations. The univariate analysis used Student's t- and chi-square tests. Statistical significance was considered when $P < .05$.

RESULTS

We analyzed 57 transplanted patients among whom 65% ($n = 37$) were women. At the time of transplantation, the overall mean age was 34.3 ± 5.93 years; the duration of diabetes, 22.8 ± 5.30 years; and renal replacement therapy (hemodialysis or peritoneal dialysis), 36.4 ± 29.4 months (Table 1). Induction immunosuppressive therapy included steroids, tacrolimus, mycophenolate mofetil, and antithymocyte globulin. At transplantation, the lumbar spine T-score was -1.75 ± 1.05 with a BMD of 0.87 ± 0.12 g/cm^2; the femoral neck T-score -1.95 ± 0.73 with a BMD of 0.71 ± 0.12 g/cm^2. The BMD revealed osteoporosis in 28% of patients and osteopenia in 36.8%. Table 2 summarizes laboratory data at the time of transplantation and during the follow-up period.

One year after transplantation, there was an improvement in lumbar spine T-score (-1.33 ± 0.94; $P = .044$) among 77.5% of the patients including 66% women. No significant differences were observed related to gender, age, time on dialysis, body mass index, parathormone (PTH), creatinine, dose of prednisone, dose of oral calcium carbonate and vitamin D analogs or biphosphonate therapy. The dose of oral calcium per day in this group was greater (1.25 g/d) then among the other patients (0.33 g/d), although the difference has not significant. No change in the femur T-score (-1.92 ± 0.73) occurred during the first year. At 1 year, the mean dose of prednisone was 4.3 mg/d.

BMD at the lumbar spine and femoral neck improved gradually over the 4 years of follow-up (Fig 1). In the fourth year the lumbar spine T-score improved to -1.04 ± 0.67 ($P = .004$) and the femoral neck T-score to -1.69 ± 0.49 ($P = .12$). At this time, no cases of osteoporosis were diagnosed. The patients maintained good kidney (serum creatinine = 1.2 mg/dL) and pancreatic graft function (fasting glucose = 87.7 mg/dL; HbA1C = 4.6 ± 0.3%). At the end of the fourth year, 86.7% of patients were not prescribed steroids and in the others it had been tapered to a minimum of 5 mg/d prednisone.

Table 1. Demographic Characteristics at Time of SKP Transplantation

	Mean (± SD)
Age (y)	34.3 (± 5.93)
Duration of diabetes mellitus (y)	22.8 (± 5.30)
Time on dialysis (mos)	36.4 (± 29.4)
Body mass index (kg/m^2)	22.3 (± 2.79)

SD, standard deviation.

Table 2. Laboratory Data During Follow-up

	Year 0	Year 1	Year 2	Year 3	Year 4
Body mass index (kg/m^2)	22.3	22.7	22.7	23.5	23.5
Creatinine (mg/dL)	1.2	1.1	1.2	1.3	1.2
Phosphorous (mmol/L)	1.04	1.03	1.05	1.01	0.98
Calcium (mmol/L)	2.31	2.34	2.36	2.34	2.32
Fasting glucose (mg/dL)	87.1	79.4	78.6	80.2	87.7
Prednisone (mg/d)	20	4.3	2.3	1.5	1.3
Calcium carbonate (g/d)	0.89	1.18	0.71	1.5	0.95
Biphosphonates (*n*)	7	17	12	15	11

During this period 5 symptomatic fractures occurred in 4 patients; all of which occurred in small peripheral bones. At the time of transplantation, the fracture patients, showed T-scores in the lumbar and femoral neck of -2.5 ± 0.9 and -2.1 ± 0.5, respectively.

DISCUSSION

SKP transplantation is the first option for patients with diabetes mellitus and end-stage nephropathy. Bone disease is one of the most common complications of transplantation.[1] Among SKP transplants data are scarce characterizating posttransplant bone loss, particularly after the first year.[4,8–10] Our results demonstrate a progressive improvement in BMD after transplantation. This recovery was more pronounced in the lumbar spine than the femoral neck cortical bone, results that were consistent with other studies,[4,8,10] which documented a predominant cortical osteopenia with consequent increased risk of peripheral fractures. Longitudinal studies have shown that the majority of bone loss occurs in the first 6–18 months.[4–8] In our study no data were collected during the first 6 months posttransplant.

Preventing long-term complications of SKP transplantation, such as bone disease, has become an essential part of posttransplant care. In kidney recipients the presence of renal osteodystrophy contributes to low BMD.[12] The diabetic condition adds to the risk of renal bone disease. Diabetes significantly decreases bone mass, particularly at cortical sites. Controversy exists regarding prevention and management of transplantation osteoporosis in the renal population.[11,13] The diabetic state and end-stage nephropa-

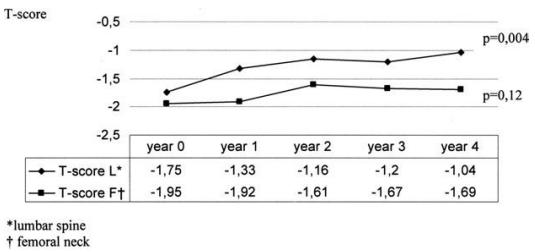

Figure 1. Evolution of BMD in the 4 years after SKP transplantation.

thy are corrected with successful SKP transplantation.[11,13–15] Numerous studies have demonstrated significant evidence that steroids are a major contributor to posttransplant bone loss, especially the rapid loss that occurs in the first 6–12 months.[9,16] A rapid reduction of steroids dose is a general practice in our transplant unit. This therapeutic approach did not increase acute rejection episodes. An average of 4.3 mg/d of prednisone, the dose at 1 year, was tapered over time. At the end of the first year, about 26% of patients had no steroids in the immunosuppressive therapy, which may be the main reason for the improved BMD. These results are consistent with those showing that steroid sparing protocols prevent bone loss.[16,17] Other therapies believed efficient for prevention and/or treatment of bone reduction include calcium carbonate, vitamin D analogs and bisphosphonates. Our patients were treated with these drugs during the 4 years an approach that was possible due to the good kidney graft function.[14,18–20]

Limitations of the study are related to the interpretation of BMD in end-stage renal disease, and the lack of evaluations during the first 6 months posttransplant, which is an essential period to treat and prevent bone loss.

Transplanted patients display an increased risk of fractures. The 6 to 45% prevalence among kidney transplant alone patients is increased 40%–50%, 5%–10% per year among diabetic patients who receive SKP transplantations.[6,7,18] Documented risk factors for fractures include age, body mass index, dialysis time, diabetes, steroids, and other immunosuppressive drugs. In SPK transplantation, diabetic blindness and neuropathy are associated risk factors for falls and fractures.[21] Only 5 patients (8,7%) experienced symptomatic fractures in our unit, a lower prevalence than that described in the literature. At the initial evaluation, osteoporosis was diagnosed in 28% and osteopenia in 37% of our patients. During the 4 years follow-up no patient displayed criteria of osteoporosis, an improvement related to the strategy of early steroid reduction and to intensive therapy with bisphosphonate vitamin D analog or calcium carbonate.

In conclusion, posttransplant bone disease is a common complication frequently associated with osteoporosis. Our patients experienced a lower prevalence of fractures than that in the literature. Using a steroid sparing immunosuppressive protocol and active treatment of osteoporosis may be a good approach to reduce bone loss. More randomized trials are needed to define the best option to manage bone disease after SKP transplantation.

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Resumo V.2

Os favoráveis resultados obtidos no estudo retrospetivo, de recuperação da massa óssea após TRP, levaram-nos a realizar um segundo estudo, desta vez prospetivo. Neste trabalho fomos estudar 48 doentes TRP, 28 do sexo feminino, que tinham em média 35 anos, 36 meses de diálise e 24 anos de DM1. Analisámos marcadores bioquímicos séricos de metabolismo ósseo e 3 densitometrias ósseas (DMO) consecutivas. A primeira avaliação foi realizada peri-transplante, as seguintes com mínimo de 2 anos de seguimento e intercaladas pelo menos 1 ano uma da outra.

Na 1ª avaliação, 35,4% dos doentes tinham critérios de osteoporose na coluna lombar e 39,6% no colo do fémur. Estes doentes tinham níveis de fosfatase alcalina (FA) e hormona paratiroideia (PTH) mais altos e IMC mais baixo. Não se observou correlação do T-score com o sexo, tempo de diálise ou de diabetes, nem com os níveis de cálcio e fósforo séricos. Ao longo do tempo verificou-se uma descida da PTH e subida do IMC. Relativamente à DMO, houve uma melhoria do T-score lombar ao longo das 3 medições; e do T-score femoral da 1ª para a 2ª medição. No final do estudo, menos de 10% tinham osteoporose. Tinham descontinuado corticóides 81,2%.

Verificou-se que os doentes com osteoporose tinham FA mais elevada. A análise multivariada identificou ainda o aumento do IMC como fator preditor de melhoria da DMO.

São raros os estudos sobre evolução da doença óssea após TRP. Na população geral e também no TR isolado, um IMC mais baixo já havia sido identificado como fator de risco para a osteoporose. Outros estudos, na doença renal crónica, apontaram a FA como tendo boa correlação com a histomorfometria óssea.

Este trabalho demonstra pela primeira vez a associação de piores índices na DMO com FA mais alta e IMC mais baixo, nos TRP. Enfatiza que a avaliação mais adequada da doença óssea nestes doentes deve englobar a medição de ambos os parâmetros, FA e DMO, no sentido de estabelecer o diagnóstico e o risco de forma mais precisa, e possivelmente conduzir a uma estratégia terapêutica mais eficaz.

CHANGES IN BONE MINERAL DENSITY FOLLOWING LONG TERM SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION

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On behalf of all authors, the corresponding author states that there is no conflict of interest.

ABSTRACT

Purpose: The symptoms of renal osteodystrophy improve significantly in patients after successful simultaneous pancreas-kidney transplantation (SPKT); however bone pathology is still present even after many post-transplant years. The aim of this study was to analyze the bone densitometry in different periods after SPKT.

Methods: Three-point densitometry was performed with dual-energy x-ray absorptiometry (DXA) technique. Serum levels of alkaline phosphatase (ALP), total serum calcium, phosphate and parathyroid hormone were analyzed as markers of mineral metabolism.

Results: Study population consisted of 48 patients of mean age 35 ± 6 years (28F, 20M) and mean 24 ± 6 years of prior diabetes. Mean period of maintenance dialysis was 36 ± 26 months. The median time from SPKT and DXA measurement was 0.53, 26.2 and 41.9 months, respectively. Based on DXA technique, 35.4% of patients were categorized as having osteoporosis at lumbar spine and 39.6% at femoral neck. Patients with diagnosed osteoporosis had significantly higher level of ALP (OR=1.5; 95% CI=1.1-2.2; $p<0.05$ at lumbar spine; OR=1.4; 95% CI=1.0-1.9; $p<0.05$ at femoral neck). In addition, subjects with lumbar osteoporosis were characterized by significantly lower body mass index (BMI) (OR=0.5; 95% CI=0.3-0.9; $p<0.05$). In long-term follow-up, BMD increased significantly at lumbar spine (T-score -1.86

± 1.07 to -1.08 ± 0.89) and femoral neck (T-score -2.12 ± 0.78 to -1.63 ± 0.65). Multivariate linear model identified BMI increase as a significant factor associated with improvement in BMD.
Conclusions: Results of our study led us to conclude that BMD in three-point densitometry among patients with functioning kidney and pancreas graft improved. Increased serum levels of ALP associated significantly with a decrease of BMD suggesting a higher risk of osteoporosis. BMI gain was predictive of BMD improve.

Keywords: simultaneous pancreas-kidney transplantation, osteoporosis, alkaline phosphatase, bone mineral density, body mass index

INTRODUCTION

Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for selected patients with type 1 diabetes and end-stage renal disease (ESRD).
Despite the ability of reversing many complications following transplantation, namely on renal osteodystrophy, bone mineral loss with subsequent development of osteopenia and osteoporosis remains a frequent and serious complication. Although it has been recognized, the routine application of adequate diagnostic tools and preventive or treatment strategies to correct bone loss or mineral disarrays may often be suboptimal [1].
The guidelines published by Kidney Disease Improving Global Outcomes (KDIGO) in 2009 recommends in patients with an estimated glomerular filtration rate (GFR) greater than approximately 30 ml/min per 1.73m^2, measuring bone mineral density (BMD) in the first 3 months after kidney transplant if they receive corticosteroids or have risk factors for osteoporosis as in the general population [2].

The magnitude of pre-existing renal osteodystrophy, hypophosphatemia and disturbances in the fibroblastic growth factor 23-parathyroid-vitamin D axis before transplant, the degree of kidney function recovery, and the effects of immunosuppressive and other therapies create a heterogeneous patient population in the first year after transplantation. So, the same guidelines define that is probably useful to distinguish the time period immediately after kidney transplant, with rapidly changing GFR and concomitantly given therapies, from the subsequent time period when a more stable graft function has been achieved [2].

Data characterizing bone loss after SPKT, particularly in long-term are scarce. The aim of our study was to evaluate serum markers of mineral metabolism, grafts function and lumbar and femoral BMD determined by dual-energy x-ray absorptiometry (DXA) over at least 3 years of follow up among 48 patients who underwent SPKT.

METHODS

From May 2000 to December 2009, 107 type 1 diabetic patients underwent SPKT at the Transplantation Department of our Unit.

All the procedures were performed using grafts from deceased donors, both grafts from the same donor, and using systemic-enteric drainage (venous drainage to the iliac vein; exocrine drainage through an enteric anastomosis of the pancreatic-duodenal arch).

The BMD was determined by DXA using the same Hologic QDR® 4500 X-ray Bone Densitometer over the study period. Instrument quality control on the DXA scanner was performed by daily scanning of a spine phantom. The coefficients of variation for BMD measurements were 1% (spine) and 2% (femoral neck). The results were expressed as T-scores (the number of standard deviations a person's BMD is below the mean BMD for the young healthy population). The osteoporotic label cutoff was of – 2,5 or lower, in accordance with World Health Organization.

A prospective, single center study was performed with three serial DXA examinations.

Forthy eight patients fulfilled the following criteria: a first DXA measurement in the first 3 months after SPKT, and two other DXA measurements performed at least 2 years after transplant and one year apart, respectively.

Biological markers of mineral metabolism (total serum calcium, phosphate, intact- parathyroid hormone (PTH), alkaline phosphatase) were regularly measured during follow-up. Blood was drawn after an overnight fast. Serum calcium, phosphorus and alkaline phosphatase were determined by colorimetric methods. The binding reagent was o-cresolphtalein, acid molybdate and para-nitrophenylphosphate, respectively. Serum PTH levels were measured using electrochemiluminescence immunoassay (ECLIA) performed on the fully automated immunoanalyzer Elecsys® 2010. Hyperparathyroidism was defined as an intact PTH level higher than 70 pg/ml.

Body weight was recorded at each examination.

Kidney graft function was determined by measuring serum creatinine and estimated GFR using the Modification of Diet in Renal Disease (MDRD) formula. Pancreas graft function was determined by measuring fasting glycemia, glycosylated hemoglobin (HbA1C) and C-peptide.

Because of small number of patients initiating bone active medications, which included vitamin D with or without calcium supplement, we were unable to assess whether thay had effects on the changes reported in this investigation.

The data were analysed with SPSS 18.0 (Statistical Package for the Social Sciences, Evanston, IL, USA). Results were expressed as mean values and standard deviations for continuous, normally distributed variables, and as percentages for categorical data. Paired and unpaired t-tests, chi-square tests and Kruskal–Wallis one-way analysis of variance were performed to assess the significance of differences between groups by using continuous and categorical variables. For significant risk factors in univariate analysis, multivariate logistic models were builts using the stepwise forward elimination of nonsignificant factors.

To determine clinical predictors of changes in bone densitometry – Δ BMD – a multivariate linear regression model was used. Statistical significance was considered when $P<.05$.

RESULTS

Baseline evaluation

Forthy eight patients had 24 ± 6 years (range 11-38) of prior diabetes, 58.3% were female and the overall mean age was 35 ± 6 (range 20-47) years at transplantation date. The dialysis vintage (hemodialysis or peritoneal dialysis) was 36 ± 26 months (range 5 to 98) but 2 patients underwent a preemptive procedure.

Induction immunossupressive theraphy included glucocorticoids, tacrolimus, mycophenolate mofetil and antithymocyte globulin.

At the time of transplantation, the average BMD was low. The lumbar spine and femoral neck T-scores were -1.86 ± 1.07 and -2.12 ± 0.78, respectively. Osteoporosis was reported in 35.4% recipients in the lumbar spine and in 39.6% recipients in femoral neck. Genders did not differ regarding T-scores.

In univariable analysis, higher values of alkaline phosphatase were associated with osteoporosis at both regions, while low BMI and high PTH were associated, respectively, with lumbar spine and femoral neck osteoporosis (table 1).

No correlation was found between BMD and diabetes and dialysis duration, calcium and phosphate values, kidney and pancreas function.

In multivariable analysis, high alkaline phosphatase levels was found to significantly increase the risk of low BMD. Another risk factor for lumbar spine osteoporosis was low BMI (table 2).

Sequencial changes post-transplantation

Glucocorticoid tapering bellow the daily dose of 10 mg was normally initiated after month 6 with complete withdrawal achived in 60.4% of patients at the end of second year. No rejection episodes were registered. At the end of follow-up, 81.2% of patients did not receive glucocorticoids in the immunossupressive regimen.

Their mean serum creatinine and creatinine clearance remained stable. Al were insulin-independent.

After SPKT, a decrease in serum PTH levels occurred (table 3). Serum phosphate, alkaline phosphatase and calcium levels did not change significantly after transplantation.

Bone densitometry enhanced gradually through the follow-up (figure 1 and 2). Kruskal-Wallis test revealed a significant difference between evaluations. There was a good correlation between femoral neck and lumbar spine BMD at three point densitometry ($r=0.673$, $P<0.001$; $r=0.394$, $P=0.006$; $r=0.439$; $P=0.002$, respectively).

After two years of SPKT, 10.4% and 18.8% of patients remained osteoporotic in the lumbar and femoral regions, respectively. At the end of follow-up, only 2 and 4 osteoporosis cases at lumbar and femoral regions, respectively, were verified.

In a model adjusted for gender, age, diabetes duration, Δ BMI and cumulative glucocorticoid dose, higher values of Δ BMI predicted a significant improvement in Δ BMD. Each 10% increase in Δ BMI was associated with an improvement of 3.62 and 3.53% in Δ BMD at lumbar spine [beta=3.620 (95%CI 0.155-7.086) P=0.041] and femoral neck [beta=3.533 (95%CI 0.430-6.636) P=0.027], respectively.

DISCUSSION

Combined kidney-pancreas transplantation is a recognised risk factor for osteoporosis and consequently fractures [3,4]. Beyond the mechanisms associated with transplantation, type I diabetes per se is an independent risk factor. The anabolic effects of insulin on bone, alteration of bone metabolism by advanced glycation end products or vascular complications of diabetes such as neuropathy, visual impairment and amputation are possible explanations [5].

A study in SPKT demonstrated a prevalence of vertebral and nonvertebral fracture of 45% and a high incidence of osteoporosis 1 year-following transplantation [4]. In a population of 31 patients, 23% had a significant decrease in bone mass (T score < -2.5) at the predominantly trabecular lumbar spine sites and 58% demonstrated a similarly low bone mass at the femoral neck, where cortical bone is prevalent [4].

A group recently evaluated the rate of hospitalization for fracture among 6212 patients with type I diabetes that received a kidney transplant alone compared to 4933 patients that received a SPKT and came to an interesting conclusion. They found a significant difference between both, 5.9% versus 4.7% rate of hospitalization, respectively, persisted after adjustment for many fracture risk factors [5], challenging the higher risk of osteoporosis in SPKT patients.

In most published scientific research studies, follow up period may have been to short, therefore the results should be interpreted carefully. In the first year following transplantation, is likely that at least two major factors influence BMD: hyperparathyroidism and use of corticosteroids. Normalization of PTH secretion and, consequently, of bone remodeling could be responsible for prevention of further deterioration of the BMD over time post-transplantation [6]. Furthermore, persistent hyperparathyroidism is an independent risk factor for fractures [7]. Moreover, glucocorticoids withdrawal in transplant recipients results in an increase in BMD [8].

Previous prospective studies evaluating BMD in consecutive SPKT recipients before and at 3,6 and 12 months after establishment of graft function [9] revealed a significant bone loss within 6 months of transplantation at both trabecular and cortical sites, mainly due to glucocorticoid therapy [10].

In our study, in the baseline evaluation, low BMI, a traditional risk factor for low BMD in general population, was a risk factor for osteoporosis of lumbar spine in univariate and multivariate analysis. This correlation between loss of BMD and low BMI values was identified in kidney transplant recipients [11, 12]. The putative mechanisms are several such as effect of adipokines in bone remodeling and conversion of androgen to estrogen in the adipose tissue [13]. The World Health Organization fracture risk assessment tool (FRAX) includes BMI on evaluation [14].

The patients who remained osteoporotic at lumbar level after third evaluation, in our survey, had a BMI lower than 20.5 Kg/m^2, being in one of them lower than 18.5 Kg/m^2. At long-term, the only clinical predictor of BMD improvement was the increase in BMI.

A risk factor of accelerated BMD losses at femoral region following transplantation was hyperparathyroidism, as revealed by other evaluations [15-17]. A novel and interesting finding was the significant association between the decrease in T-score in both sites of lumbar spine and femoral neck and

an increase of alkaline phosphatase value from 10 units the upper limit of the reference interval.

A rise in serum alkaline phosphatase, known as hyperphosphatasemia or hyperphosphatasia, in patients with otherwise intact liver and biliary systems usually result from excess of the bone isoforms of the enzyme [18]. Maruyama *et al.* examined the baseline data in 185 277 prevalent hemodialysis patients in Japan and related them to 1-year mortality and incident hip fracture events through calendar year 2010. They found that patients in the highest quartile of serum alkaline phosphatase had 46% and 25% higher all-cause and cardiovascular death risk, respectively, as well as a 71% higher incidence of hip fracture events, than those within the lowest quartile [18].

Data from the Scientific Registry of Transplant Recipients relative to 11 776 patients also concluded that recipients with pretransplant serum alkaline phosphatase of 120-160 and ≥160 U/L had 49% and 64% higher graft failure censored all-cause mortality in multivariable adjusted models [19]. Several studies revealed that alkaline phosphatase level appears to be at least as tightly linked to bone histomorphometry as PTH concentration and to be more closely linked than PTH concentration to clinical outcomes [20].

Osteoporosis results from complex interactions between genetic and environmental factors, and was demonstrated the association of single nucleotide polymorphism in alkaline phosphatase gene with BMD [21]. Studies, in general population and patients with chronic renal disease, recognized elevation of alkaline phosphatase levels as a possible more efficient method to detect patients with fast bone turnover rates, helpful to predict osteoporosis [22, 23]. In our study, bone loss occurred in patients with higher levels of alkaline phosphatase.

As expected [6, 24], we verified a higher incidence of osteoporosis in the femoral neck, wich contains more cortical bone than the spine. It's well known, that the most of fragility fractures occurs at non-vertebral sites where bone is composed mainly by compact (or cortical) tissue, since it accounts for 80% of the total bone mass of an adult skeleton [25, 26].

The development and implementation of a comprehensive bone health protocol in posttransplant SPK patients is important for screening but certainly also for prevention of osteopenia and osteoporosis. In a protocol applied in 76 posttransplant kidney and SPK patients, DXA scan was performed within 2 weeks of transplant. Prevention of bone disease was considered appropriate if the patient was educated on reduction of modifiable risk factors and received at least 1000 mg of elemental calcium and 400 IU of vitamin D daily. Treatment of bone disease was considered appropriate if the patient was receiving either an oral or intravenous bisphophonate, oral raloxifene, or nasal or subcutaneous calcitonin for documented osteoporosis. According to the T-score the DXA scan was repeated in 6 months, 1 years ou every 2 years [27], with beneficial results in prevention and treatment.

Although the present study has limitations due to a relatively small and heteregeneous sample, it leads to the reasonable conclusion that bone density enhances following SPKT.

CONCLUSION

From this survey we obtained several relevant data: first we found an improvement in BMD present in long term follow-up of SPKT recipients. Another important aspect of this study was the identifications of

alkaline phosphatase as an independent risk factor of BMD in these patients. A multivariate clinical linear model determined that the change in BMD was positively associated with increase in BMI.

This sudy supports that evaluation of SPK recipient bone disease demands use of BMD measurements together with alkaline phosphatase and BMI to make the diagnosis, risk evaluation, and probably therapy of osteoporosis more effective.

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Table 1: Risk factors for lumbar spine and femoral neck osteoporosis in univariate analysis

	Osteoporotic Lumbar Spine			Osteoporotic Femoral Neck		
Variable	No (n=31)	Yes (n=17)	P	No (n=29)	Yes(n=19)	P
Age (years)	36.0 ± 5.8	33.5 ± 5.8	.164	34.9 ± 6.0	35.4 ± 5.8	.786
Female gender (%)	64.5	47.1	.241	58.6	57.9	.960
Diabetes duration (years)	24.3 ± 5.6	23.8 ± 6.8	.789	23.1 ± 5.8	25.6 ± 6.2	.165
Dialysis vintage (months)	37.2 ± 25.2	34.2 ± 29.4	.723	35.4 ± 27.2	37.3 ± 26.0	.811
Body mass index (kg/m^2)	22.1 ± 2.0	20.6 ± 2.1	**.019**	21.7 ± 2.2	21.4 ± 2.2	.684
eGFR by MDRD* (ml/min)	66.6 ± 25.1	73.0 ± 14.2	.263	68.7 ± 22.9	69.2 ± 20.9	.937
PO4 (mmol/L) (0.87-1.45)	1.09 ± 0.45	1.01 ± 0.29	.452	1.02 ± 0.30	1.12 ± 0.52	.448
Ca (mmol/L) (2.09-2.42)	2.31 ± 0.15	2.38 ± 0.19	.189	2.33 ± 0.13	2.35 ± 0.21	.720
AP (U/L) (32-104)	68.5 ± 23.0	106.1±51.0	**.003**	71.6 ± 23.3	96.6 ± 52.6	**.047**
PTHi (pg/ml) (15-65)	128.7±82.8	148.5 ± 7.8	.488	111.4±79.6	175.2±77.4	**.014**
Fasting glucose (mg/dl)	170 ± 113	194 ± 161	.586	168 ± 110	195 ± 159	.516
HbA1C (%)	7.7 ± 1.6	8.4 ± 2.4	.324	7.6 ± 1.6	8.5±2.3	.179
C-peptide (ng/ml) (1.1-4.4)	5.6 ± 6.4	4.5 ± 2.2	.497	5.37 ±6.3	5.0 ± 2.8	.824
Amilase (U/L) (0-100)	86.7 ± 71.4	84.4 ± 27.8	.875	80.6±56.2	94.0 ± 64.5	.464
Lipase (U/L) (30-190)	56.2 ± 50.3	41.1 ± 25.2	.173	47.4±32.0	56.2 ± 57.3	.550
Lumbar T-score	-1.22±0.71	-3.02±0.42	**< .001**	-1.41±0.99	-2.54±0.81	**< .001**
Femoral T-score	-1.83±0.74	-2.65±0.54	**< .001**	-1.62±0.53	-2.89±0.34	**< .001**

eGFR by MDRD* (ml/min/1.73 m^2); AP= alkaline phosphatase; Ca=Calcium; PO4=Phosphorus

Table 2: Logistic Regression Analysis for significant risk factors of osteoporosis in multivariate analysis

Variable	Odds Ratio	95% Confidence Interval	P
Lumbar spine osteoporosis			
BMI	0.511	0.277 – 0.943	.032
Alkaline phosphatase *	1.541	1.078-2.203	.018
Femoral neck osteoporosis			
Alkaline phosphatase *	1.411	1.034 – 1.925	.030

*Variations of 10 units

Table 3: Prednisolone, kidney, pancreatic function and biochemical markers of bone metabolism during study period

	1st DXA	2nd DXA	3th DXA
Time from transplant to DXA (months)	0.53 (-1.6-3.3)	26.2 (18.8-39.2)	41.9 (30.7-67.4)
Body mass index (kg/m^2)	21.6 ± 2.2	22.3 ± 2.3 [b]	22.6 ± 2.4
Prednisolone (%)	100	39.6	18.8
eGFR by MDRD (ml/min/1.73 m^2)	68.9 ± 21.9	69.5 ± 17.3	67.1 ± 19.9
Phosphorus (mmol/L)	1.06 ± 0.41	1.05 ± 0.24	1.08 ± 0.26
Calcium (mmol/L)	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
Alkaline phosphatase (U/L)	81.2 ± 41.5	71.7 ± 31.6	76.5 ± 38.5
Parathyroid hormone (pg/ml)	129.5 ± 77.9	77.3 ± 42.2 [a]	64.8 ± 27.7
Fasting glucose (mg/dl)	179 ± 130	80 ± 10 [a]	86 ± 42
HbA1C (%)	8.0 ± 2.1	4.7 ± 0.5 [a]	5.1 ± 0.9 [b]
C-peptide (ng/ml)	5.8 ± 5.7	2.7 ± 1.0 [c]	2.2 ± 0.7 [b]
Amilase (U/L)	86 ± 60	79 ± 36	90 ± 39 [b]
Lipase (U/L)	50 ± 43	32 ± 17 [c]	41 ± 41
Lumbar T-score	-1.86 ± 1.07	-1.37 ± 1.03 [c]	-1.08 ± 0.89 [c]
Femoral T-score	-2.12 ± 0.78	-1.65 ± 1.07 [c]	-1.63 ± 0.65

a $P < 0.001$ versus previous evaluation

b $P<0.05$ versus previous evaluation

c $P<0.01$ versus previous evaluation

GFR – glomerular filtration rate; MDRD - modification of diet in renal disease study euqation

Figure 1: Development of lumbar bone mineral density (T score) after SPKT at three different moments.

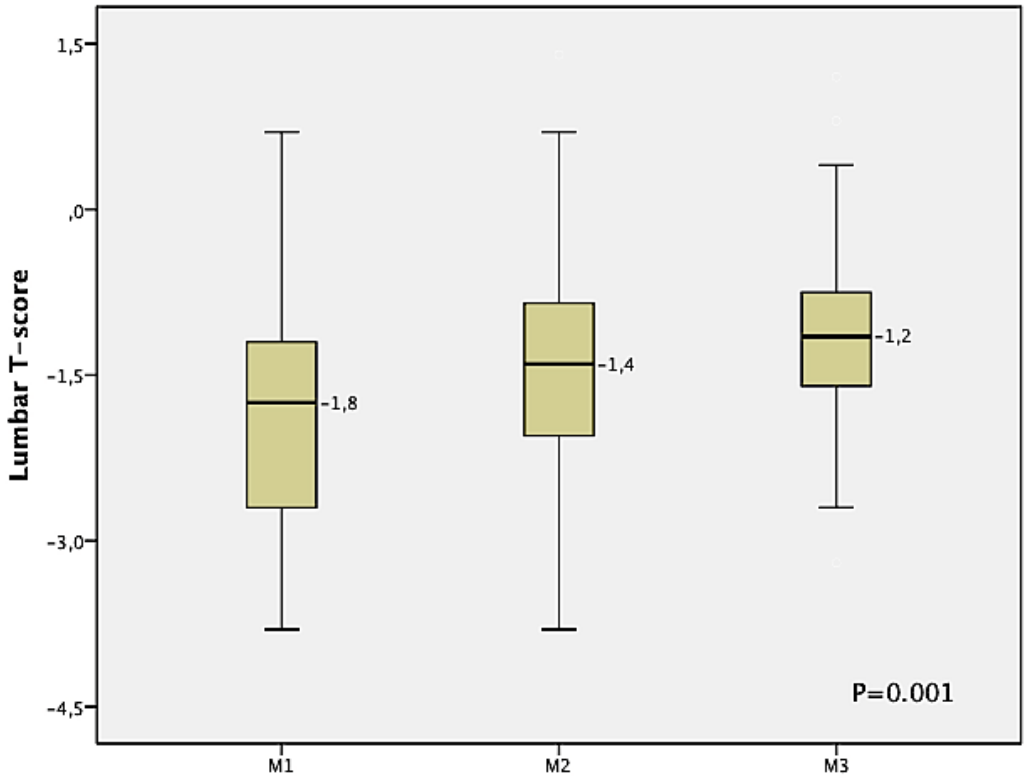
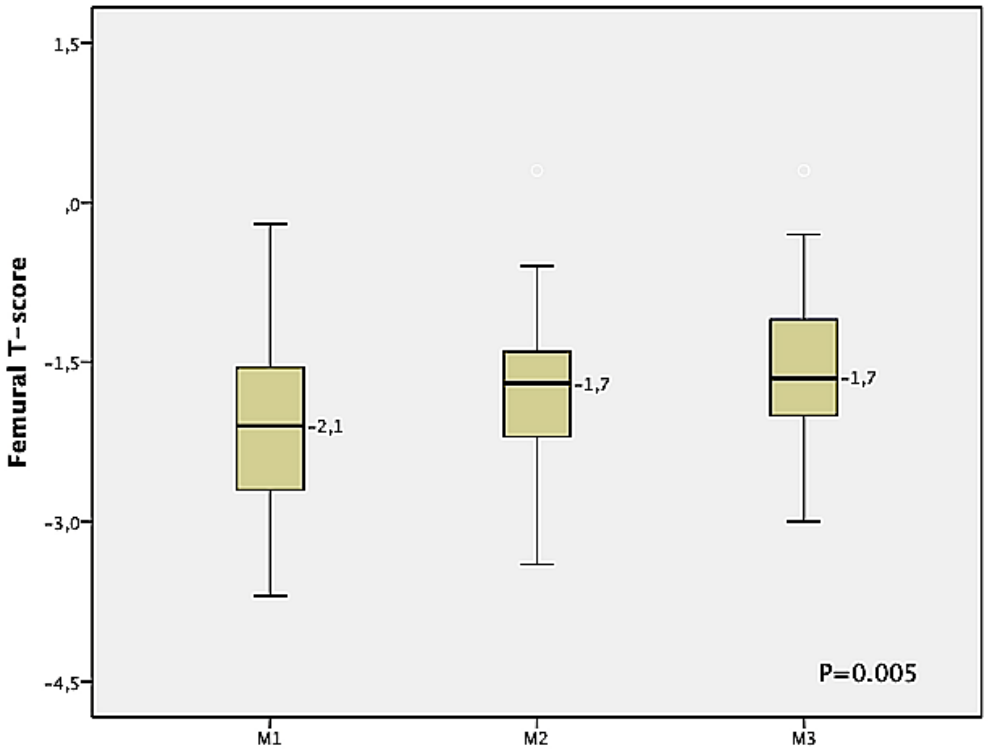


Figure 2: Development of femoral bone mineral density (T score) after SPKT at three different moments



VI – Aspetos imunológicos no TRP: recidiva da autoimunidade; aloimunidade

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- **VI.1 - Martins L**, Malheiro J, Henriques AC, Dias L, Dores J, Oliveira F, Seca R, Almeida R, Sarmento AM, Cabrita A, Teixeira M. **Pancreas-kidney transplantation and the evolution of pancreatic autoantibodies**. *Transplant Proc* 2009; 41(3): 913-915.

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- **VI.2** – **Martins LS**. Capítulo: **Recidiva da Diabetes Mellitus pós-Transplante de Pâncreas",** do Livro **" Manual de Transplante de Pâncreas" -** Editores: Irene L Noronha; Adriano M Gonzalez; Roberto F Meireles Jr. Editora: Segmento Farma. Edição: Manuais Ano: Set/2011. Páginas: 269-275. **ISBN 978-85-7900-023-2** (M294 / CDD 617.556). (*obtida permissão dos editores*)

- **VI.3** – **Martins LS**, Henriques AC, Fonseca IM, Rodrigues AS, Oliverira JC, Dores JM, Dias LS, Cabrita AM, Silva JD, Noronha IL. **Pancreatic autoantibodies after pancreas-kidney transplantation – do they matter?** *Clin Transplant* 2014; 28(4): 462-469.

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- **VI.4** – **Martins LS**. **Autoimmune diabetes recurrence should be routinely monitored after pancreas transplantation (mini-riview)".** *World J Transplant* 2014, Sept 24; 4(3): 183-187. DOI: 10.5500/wjt.v4.i3.183. PMID: 25346891 [PubMed].

- **VI.5** – Jorge Malheiro; **La Salete Martins**; Sandra Tafulo; Leonídio Dias; Isabel Fonseca; Idalina Beirão; António Castro-Henriques; António Cabrita **Detrimental effect of de novo donor-specific HLA antibodies on grafts survival in simultaneous pancreas-kidney transplantation: does it go beyond acute rejection?** (*submetido*)

Resumo VI.1

A recorrência ou persistência da autoimunidade após TRP, com imunossupressão mantida, é um facto que se tem observado e que causa alguma inquietude. Desconhece-se com clareza quais as suas implicações na função e sobrevivência do enxerto pancreático. Quando os primeiros casos foram descritos, aventou-se a hipótese da minimização da imunossupressão ou o maior grau de semelhança HLA ser um fator de risco para a autoimunidade.

Analisámos prospetivamente 77 TRP, tentando perceber quais os aspetos associados a esta autoimunidade e o seu impacto na função endócrina pancreática. Em 24,7% dos casos não havia qualquer compatibilidade HLA com o dador. Tiveram rejeição aguda 14,3%. A imunossupressão incluiu globulina anti-timócito, tacrolimus, micofenolato e corticóides. Desses doentes, 65 tinham ambos os enxertos funcionantes e mais de 6 meses pós-TRP. Em 11 deles persistiam (n=8) ou tinham recidivado (n=3) os anti-GAD, com títulos em crescendo em 4 casos. Dois doentes tinham anti-GAD e ICA positivos. Havia 9 casos com ICA positivo, 2 deles de novo. O subgrupo de 22 casos com 1 ou ambos os anticorpos detetáveis, designámos por grupo “positivo”. Quando comparados estes com os restantes, não encontrámos diferenças em termos de rejeições agudas, compatibilidades HLA ou suspensão de corticóides. As doses diárias de micofenolato e os níveis de tacrolimus também não diferiam. De entre os positivos, 2 apresentavam valores glicémicos no limiar superior e HbA1c >5,6%, e um deles o valor mais baixo de Pept-C registado, embora ainda considerado dentro do normal. Contudo, este subgrupo positivo no seu todo não apresentava valores significativamente mais altos de glicemia em jejum, HbA1c ou Pept-C que a globalidade do grupo.

Em conclusão, confirmámos que os autoanticorpos pancreáticos podem persistir ou recidivar após o TRP, apesar de imunossupressão aparentemente adequada. O seu impacto na função pancreática a longo prazo permanece incerto, no entanto o nosso estudo identifica doentes em que essa autoimunidade tem tradução funcional, com valores mais elevados de glicemia e HbA1c, e mais baixos de Pept-C. Dos parâmetros estudados, não identificámos possíveis fatores de risco. O seguimento mais prolongado destes doentes e o estudo de outros potenciais fatores de risco, poderão no futuro trazer algumas respostas a estas questões que permanecem em aberto.

Pancreas-Kidney Transplantation and the Evolution of Pancreatic Autoantibodies

L. Martins, J. Malheiro, A.C. Henriques, L. Dias, J. Dores, F. Oliveira, R. Seca, R. Almeida, A.M. Sarmento, A. Cabrita, and M. Teixeira

ABSTRACT

The recurrence or persistence of pancreatic autoantibodies after pancreas-kidney transplantation (PKT) is an intriguing finding. We prospectively analyzed 77 PKTs, searching for risk factors for the expression of these autoimmune markers and their impact on pancreas graft function. Among the 77 PKTs, 24.7% had 0 HLA matches, 20.8% displayed delayed graft function, and 14.3% had acute rejection episodes. Immunosuppression included antithymocyte globulin (ATG), tacrolimus, mycophenolate mofetil (MMF), and steroids. Sixty-five patients had both grafts functioning as a follow-up of more than 6 months. In 11 patients anti–glutamic acid decarboxylase (GAD) positivity persists (n = 8) or has recurred (n = 3), 4 of whom show increasing titers. Two patients maintain positive islet cell antibodies (ICA) and anti-GAD antibodies. The 9 patients positive for ICA included 2 who were negative before PKT and 7 who remain positive. The "positive" group (22 patients with positive ICA and/or anti-GAD) did not differ from the global group of 65 functioning PKT in terms of acute rejection episodes, HLA match, and steroid withdrawal. Among the positive patients, there were 2 with borderline glucose levels; however, among the entire "positive" group, the mean fasting glucose, HbA1c, and C-peptide measurements were not significantly different, when compared with the other 65 PKTs. In conclusion, pancreatic autoantibodies may be persistently positive or recur after PKT, despite appropriate immunosuppression. Its impact on long-term pancreas graft survival is unknown. We could not identify risk factors for their expression. An extended follow-up with monitoring and search for other risk factors may be necessary to increase our knowledge in this field.

THERE is substantial evidence for an autoimmune etiology of type 1 diabetes mellitus.[1] Pancreatic inflammatory infiltrates and circulating autoantibodies, such as anti–glutamic acid decarboxylase (GAD), islet cell antibodies (ICA), anti-tyrosine phosphatase (anti-IA2), and anti-insulin antibodies, have been documented at the onset of the disease. Several years after total endocrine pancreas loss, these autoantibodies persist or progressively fall to become undetectable. Measurement of autoantibodies is an easy, feasible technique that may be used to follow their evolution.

Pancreas-kidney transplantation (PKT) persists as the best treatment for type 1 diabetic patients with chronic renal failure.[2] After a successful PKT, graft loss due to acute rejection has become infrequent in recent years using current immunosuppressive protocols. Long-term graft loss may occur due to many factors, including chronic alloimmune responses (chronic rejection) and possibly also recurrence of autoimmunity.[3] Some authors have observed an association between the recurrence of pancreatic autoantibodies and poor pancreas survival,[4–9] or islet cell transplant survival.[10,11]

Many PKT patients have undetectable titers of pancreatic autoantibodies; others show a progressive decrease in positive titers becoming negative, or maintaining stable level; some others, who were previously negative, become

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positive, despite maintained immunosuppression. Prospective monitoring of these serological markers may be useful; however, significance and impact on long-term pancreas graft survival are still unclear.

In this study we have presented our findings from a prospective monitoring of anti-GAD and ICA before and after PKT, searching for risk factors for the reappearance or maintenance of these autoantibodies, and looking for their possible consequences on pancreas graft function.

PATIENTS AND METHODS

From May 2000 to December 2007, we performed 77 PKTs from cadaveric donors in type 1 diabetic patients with chronic renal failure. The PKT technique consisted of a duodenopancreatic graft with enteric drainage, and venous anastamosis to the systemic circulation (common iliac vessels); the kidney graft was performed using the same technique as for a kidney alone transplantation.

The immunosuppressive protocol included induction with antithymocyte globulin (ATG-Fresenius-S) with tacrolimus (Tac), mycophenolate mofetil (MMF), and steroids as maintenance immunosuppression. Beside infection prophylaxis, all patients also received thrombosis prophylaxis with oral aspirin and subcutaneous low-weight heparin during the hospitalization; after discharge only oral aspirin was prescribed.

The diagnosis of acute pancreas rejection was inferred based on clinical, laboratory, and imaging signs, as well as kidney graft biopsy, which was always performed where there was pancreas and/or kidney dysfunction, after having ruled out other etiologies. We did not perform pancreas graft biopsies.

The permanent need for dialysis or insulin use was considered an index of graft loss. All patients were regularly followed at our outpatient care unit for 7 to 97 months.

RESULTS

The study group of 77 PKT patients included 50 women and 27 men of overall mean age at the time of transplantation of 33.5 ± 6.1 years (range, 23–48). Four of them were preemptive PKT, the others had pretransplantation dialysis times of 34 ± 26 months. The mean duration of type 1 diabetes was 22 ± 5 years. The mean HbA1c before transplantation was 8.5 ± 1.6%. PKT was performed in the absence of any HLA match with the donor in 19 patients (24.7%).

The incidence of delayed renal graft function (transient need for dialysis during the first week after transplantation) was 20.8%. Insulin administration was discontinued at 1.5 ± 3.0 days after PKT. The median time from admission to discharge was 23 days, comprising a median stay in the intensive care unit of 2 days. The rate of postoperative surgical complications requiring surgical reinterventions was 33.8%, mostly due to bleeding, infection, or thrombosis.

Acute rejection was diagnosed in 11 patients (14.3%): in 3 patients both grafts were affected; in 6 patients only the kidney; and in 2 patients the diagnosis was presumed to affect only the pancreas graft. In 9 patients, the rejection episodes were efficiently treated with steroids only; in 1 patient with late, refractory, and recurrent rejection, OKT3 was used; in another patient it was a humoral rejection, which reversed with plasmapheresis and intravenous immunoglobulin. Seventy-two PKT patients maintain functioning kidney grafts, with a mean serum creatinine level at the last visit of 1.12 ± 0.49 mg/dL (all but 2 were <1.6 mg/dL). The mean creatinine clearance was 75.1 ± 25.8 mL/min, without significant proteinuria (<0.5 g/d) except for 2 subjects. The 5 kidney graft losses were due to rejection (n = 1), infection (n = 1), and patient death (n = 3).

Sixty-five PKT patients have the pancreas and kidney functioning. At the last visit the fasting blood glucose of the 65 insulin-free patients was 81 ± 12 mg/dL; their HbA1c and C-peptide were 4.5 ± 0.4% and 3.4 ± 1.7 ng/mL, respectively. Twelve pancreas grafts were lost. The causes were infection (n = 5); thrombosis (n = 4); rejection (n = 1); bleeding (n = 1); and patient death (n = 1).

Twenty-five patients were converted from MMF to sirolimus (SRL), due to digestive intolerance in 24 and polyomavirus in 1 patient. In 49 patients (68.1%) it was possible to stop steroids. Five PKT patients died. Three patients died due to infection; 1 patient died due to cardiovascular disease at 34 months after transplantation and 5 months after he became dialysis and insulin-dependent, as a consequence of late, recurrent acute rejection. In another subject the cause of death is unknown. Patient survival rates at 1 and 5 years were 93% and 90%, respectively. Graft survival rates, also at 1 and 5 years, were for the kidney 91% and 88% and for the pancreas 86% and 81%, respectively. Among the 65 PKT patients with both organs functioning, 22 are now ICA-positive and/or anti-GAD–positive; thereafter denoted as the "positive" group, who were compared with the "global" group. Eleven patients showed positive anti-GAD: 3 were negative before PKT and 8 maintained positivity. Seven subjects show stable or decreasing titers, and 4 show increasing titers. Nine patients were positive for ICA (in 2 it was negative before PKT, 7 remain positive). Two subjects are positive for both autoantibodies. Six PKT patients who were anti-GAD–positive and 2 who were ICA-positive before transplantation are now negative. None of the 22 positive subjects needs insulin and, at the moment, they have no signs of pancreatic graft dysfunction or rejection. Only 2 of these (9.1%) have had early acute pancreas rejection with good responses to therapy.

Nine of the 22 (36.4%) had no HLA match, an incidence slightly higher than in the global group, but without statistical significance (P = not significant [NS]). Steroid withdrawal in the positive group was possible in 68.2% (15/22), which was similar to the global group. Seven of the 22 (31.8%) were converted from MMF to SRL, almost the same as among the global group. Also in terms of Tac levels (7.3 ng/mL in the "positive" vs 7.6 ng/mL in the "global"), SRL levels (5.4 ng/mL in the "positive" vs 5.2 ng/mL in the "global"), or MMF daily dose (1000 mg in the "positive" vs 1250 mg in the "global"), the 2 groups did not differ.

Two positive patients show "normal high" glucose levels and HbA1c >5%, 1 of whom has the lowest C-peptide (0.7 ng/mL), which is still within the normal range. However, the mean fasting glucose and HbA1c values of all of the positive

group were not different from the values of the global group.

DISCUSSION

Pancreatic autoantibodies may in fact remain positive among PKT patients, as we have observed. Their evolution is uncertain. The conditions that may contribute to persistent positivity after transplantation under apparently sufficient immunosuppression are not known. Possible risk factors for the activation of the immune (at least alloimmune) response were analyzed in this study. Our results did not show any differences in terms of acute rejection episodes, type and dosage of immunosuppressants, and number of HLA matches between the positive and the other patients. Also, among the positive patients we have not observed a negative impact on pancreas graft function or poor glycemic control. Some authors have reported a correlation between reduction of immunosuppression and pancreatic autoantibody relapse and graft loss.[12,13] We did not observe this association; like other investigators[14] we could not correlate its positivity and pancreas graft loss. Without evident signs of acute rejection or uncontrolled alloimmune activity, there seems to be no reason to increase immunosuppression in these patients.

Long-term consequences of pancreatic autoimmune markers for pancreas graft function and survival are still unclear. We think that it is advisable to expand the follow-up and maintain careful monitoring of autoantibodies and pancreas function, meanwhile searching for other factors that may infl autoimmune expression.

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Resumo VI.2

Este capítulo do Manual de Transplante de Pâncreas sobre recidiva da diabetes mellitus pós-transplante de pâncreas foi escrito a convite dos editores.

Aborda inicialmente a génese autoimune da DM1 no pâncreas nativo, processo que se comprovou poder recorrer no enxerto pancreático.

Nele se descrevem os critérios para o diagnóstico da recidiva anti-ilhota pancreática, e os aspetos comprovativos da histologia.

Discute-se a relevância de alguns fatores na recorrência da autoimunidade. O papel dos autoanticorpos pancreáticos, que se podem documentar na circulação sanguínea na maioria dos casos, permanece controverso. Se têm uma ação patogénica direta ou se resultam da lesão, sendo apenas marcadores da existência dessa lesão, continua a não ser claro. Todavia, como método não invasivo e acessível na maioria dos centros, podem ser de grande utilidade na deteção da diabetes autoimune recidivada.

Assim, sugere-se o rastreio desta entidade, muitas vezes esquecida após transplantação pancreática, através da monitorização dos anticorpos anti-ilhota pancreática.

A importância dos imunossupressores na recorrência da autoimunidade é também revista: desde a associação observada nos primeiros casos entre redução da imunossupressão e a recidiva; à fraca eficácia dos esquemas imunossupressores que foram tentados para controlar uma recidiva já documentada.

CAPÍTULO 28

RECIDIVA DO *DIABETES MELLITUS* PÓS-TRANSPLANTE DE PÂNCREAS

La Salete Martins

INTRODUÇÃO

O transplante de pâncreas (TP) ou de ilhotas é o único método capaz de restabelecer um estado euglicêmico e insulino-independente em diabéticos tipo 1.

A falência técnica é a principal causa de perda do pâncreas. Após um transplante de órgão completo realizado com sucesso, são as complicações infecciosas e o controle da resposta aloimune (rejeição aguda ou crônica) os problemas maiores que impedem seu sucesso. A recidiva da doença autoimune no pâncreas, responsável pela destruição do pâncreas nativo, não é habitualmente considerada relevante. Acredita-se que o tratamento imunossupressor que o doente mantém após o transplante, tal como conseguirá controlar a resposta aloimune, deverá também ser capaz de bloquear a resposta autoimune.

ETIOLOGIA AUTOIMUNE DO DIABETES TIPO 1

O *diabetes mellitus* tipo 1 (DM1) é uma doença de etiologia autoimune[1,2]. Na maioria dos doentes existem, à data de diagnóstico, anticorpos positivos contra autoantigênios das células betapancreáticas, como anti-ilhota (ICA), antidescarboxilase do ácido glutâmico (anti-GAD), anti-insulina (IAA) ou antitirosina fosfatase (IA-2). Apenas uma pequena porcentagem dos doentes

com DM1 não tem essa expressão autoimune humoral e é classificada como tendo diabetes tipo 1-B ou idiopática.

Em avaliações histológicas, foi possível demonstrar um infiltrado linfocitário envolvendo as ilhotas secretoras de insulina e que pode desaparecer após a perda completa das células beta, em doentes com DM1. Assim, há também um papel da imunidade celular, mediada por linfócitos T, nesse processo.

Se os autoanticorpos pancreáticos têm um papel direto na patogênese ou se são apenas um testemunho da lesão, é uma questão que permanece em aberto. Sabe-se que para o desenvolvimento da DM1, há suscetibilidade genética, nomeadamente associada aos alelos HLA DR3 e DR4 – risco particularmente maior se ambos presentes – e a polimorfismos do gene da insulina, entre outros. Essa suscetibilidade será certamente poligênica, uma vez que a presença de um só desses genes não confere risco de 100% de desenvolvimento da doença. Também agentes ambientais e infecções víricas foram apontados como fatores despoletadores da DM1.

RECIDIVA DA DOENÇA AUTOIMUNE NO ENXERTO PANCREÁTICO

Ainda que não muito frequente, a recidiva do processo autoimune no pâncreas transplantado pode constituir uma ameaça real a sua sobrevivência. Existem, há mais de duas décadas, dados que provam ser este um problema real. Depois de ter descrito o primeiro caso em 1984, Sutherland publicou posteriormente uma série de oito casos[3], demonstrando a perda da secreção de insulina e a destruição seletiva das células beta das ilhotas de Langherans, após transplante segmentar de pâncreas entre irmãos, gêmeos idênticos ou haploidênticos, sob imunossupressão mínima ou nula. Esse fato levou-o a questionar o benefício da minimização da imunossupressão, nos casos de grande compatibilidade HLA, quando há patologia autoimune subjacente que poderá, nessa situação, ficar subcontrolada e a teorizar sobre a possibilidade de uma fraca compatibilidade HLA poder reduzir o risco de recidiva da autoimunidade do diabetes.

Esses dados documentam casos de recidiva da autoimunidade da DM1, mesmo sob imunossupressão aparentemente suficiente para controlar a resposta aloimune. Esse processo ocorre, aliás, à semelhança do que já se tinha observado com outras doenças de etiologia autoimune, como o lúpus eritematoso sistêmico ou as vasculites, que podem recidivar em transplantados renais, sem evidência de rejeição e com imunossupressão mantida.

DIAGNÓSTICO DA RECIDIVA DA AUTOIMUNIDADE

Para o diagnóstico desta patologia, a recidiva de DM1 no enxerto pancreático, é essencial o seu reconhecimento tão precoce quanto possível. A diminuição da secreção da insulina e a hiperglicemia traduzem uma disfunção já demasiada avançada do enxerto, em que pouco se poderá fazer para preservar a sua função.

O diagnóstico diferencial das causas de disfunção é um desafio. Entre elas, salientam-se a rejeição aguda ou crônica, a toxicidade dos ini-

bidores da calcineurina e agressões isquêmicas ou infecciosas que podem conduzir à lesão do pâncreas. O doente transplantado de pâncreas pode também desenvolver hiperglicemia por resistência periférica à ação da insulina e diabetes tipo 2, sobretudo se associado à evolução com obesidade e síndrome metabólica. Na rejeição ou infecções, coexiste acometimento do pâncreas endócrino e exócrino, traduzido normalmente também por pancreatite com elevação de amilase e lipase séricas.

IMPORTÂNCIA DA HISTOLOGIA

Na recidiva autoimune, há agressão seletiva das ilhotas que contêm células beta[4] produtoras de insulina, sendo as células alfa e delta e a produção de glucagon e cromogranina A poupadas. Na análise de biópsias[3] ou de enxertos pancreáticos removidos[4], utilizando marcação para insulina, verificou-se existir infiltrado inflamatório apenas nas áreas que marcavam positivamente para insulina, aspecto histológico designado por insulite, e ainda não havia evidência desse infiltrado nas áreas com marcação positiva para somatostatina ou glucagon[5] e em que não havia nenhuma função residual das células beta[3,4,6] (ilhotas com marcação negativa para insulina). Pode-se dizer que não havia evidência de rejeição aguda[4,6] que se manifesta por ilheíte difusa, não restrita às células beta e sem poupar pâncreas exócrino, nem havia vasculite; também não havia proliferação fibrosa da íntima das artérias ou outros sinais de rejeição crônica[4].

A persistência de amilasúria em alguns casos estudados, realizados com anastomose do pâncreas à bexiga[6], comprova a preservação da função exócrina. Em doentes em que foram repetidas biópsias ao longo do seu seguimento[3], nos casos que evoluíram com destruição completa das células beta e em alguns que responderam à intensificação da imunossupressão, verificou-se a resolução da insulite.

As biópsias do tecido pancreático são essenciais ao diagnóstico seguro da recidiva da DM1. Contudo, são difíceis de obter no caso do transplante de ilhotas, implantadas nos espaços porta do fígado, e mesmo no transplante de órgão completo podem não ser de fácil execução, sobretudo com a anastomose entérica do pâncreas. A opção preferencial pela derivação entérica, apesar das vantagens na morbilidade pós-operatória, veio, por outro lado, dificultar o acesso ao pâncreas por biópsia.

AUTOANTICORPOS PANCREÁTICOS – QUAL A SUA RELEVÂNCIA?

A determinação dos títulos dos autoanticorpos contra antigênios pancreáticos, IAA, IA-2, anti-GAD e ICA, pode contribuir para o diagnóstico, antes mesmo da queda da produção de insulina e do peptídeo C. Num estudo realizado com 882 familiares de doentes com DM1[7], observou-se que, entre aqueles que vieram a desenvolver DM1, 98% eram positivos para pelo menos um dos autoanticorpos e 80% eram positivos para dois ou mais desses anticorpos. Estimou-se que a DM1 se manifestaria aos cinco anos, em 100% dos que tinham três anticorpos positivos. Concluiu-se que a presença de dois ou mais anticorpos foi altamente preditiva do desenvolvimento de DM1[7].

Um outro estudo sueco em diabéticos com diagnóstico recente da doença[8], no diagnóstico diferencial entre DM1 e DM2 – que nem sempre é fácil, em especial em adultos jovens –, verificou-se que dos que necessitaram de insulina a curto prazo (menos de 2 anos), a maioria era ICA positivo e esse marcador tinha sensibilidade, especificidade e valor preditivo positivo para o diagnóstico de DM com requerimento de insulina de 72%, 96% e 84%, respectivamente.

Assim, os marcadores sorológicos serão indicadores de evolução da destruição imunológica, das células betapancreáticas produtoras de insulina e sua monitorização facilita a identificação de doentes de risco para DM1, mesmo antes da expressão clínica da doença[7,9].

Em alguns diabéticos tipo 1, ICA, anti-GAD, IAA e IA-2 podem desaparecer ao longo dos anos após a perda do tecido endócrino-pancreático e em outros permanecem positivos em títulos variáveis.

O QUE PODE INFLUENCIAR O REAPARECIMENTO DA AUTOIMUNIDADE?

Que fatores poderão associar-se ao risco da recidiva da doença autoimune no pâncreas transplantado, ou de ilhotas, é mais uma questão que persiste por esclarecer.

POSITIVIDADE DOS AUTOANTICORPOS

Após o transplante, os autoanticorpos pancreáticos podem comportar-se de modo variado, tal como se verifica na doença inicial do pâncreas nativo: permanecer negativos, positivos, descer, ou subir de título[10]. Se, por um lado, o doente volta a ter tecido endócrino funcionante e a expressar os antigênios pancreáticos que havia muito não existiam, por outro lado fica sujeito à imunossupressão.

Do equilíbrio desses dois fatores e provavelmente de muitos outros que se desconhecem, resultará a expressão humoral. A positividade, subida de título ou a manutenção de títulos altos sustentados dos diferentes anticorpos mostraram, em vários trabalhos, correlação com a perda do pâncreas[4,6,10,11]. Outros autores, apesar de reportarem uma positividade dos diversos anticorpos numa porcentagem elevada de doentes com transplante duplo de rim e pâncreas[12], não verificaram associação entre estes e falência do pâncreas[12-14].

IMUNOSSUPRESSÃO

A utilização de drogas imunossupressoras, como a ciclosporina, numa fase precoce em doentes identificados como tendo DM1, na tentativa de controlar a doença autoimune e evitar a destruição das células produtoras de insulina, resultou apenas no atraso da dependência de insulina exógena[15].

Alguns casos reportados após o transplante indicaram correlação entre redução da imunossupressão e recidiva da autoimunidade e perda do enxerto, documentada histologicamente e acompanhada de reaparecimento ou subida dos títulos dos autoanticorpos pancreáticos[3,16].

Segundo alguns autores, o tipo de imunossupressão (ciclosporina *versus* tacrolimo) e a suspensão de corticoides pós-transplante es-

tiveram relacionados com a positividade dos autoanticorpos[12], mas noutros trabalhos a imunossupressão de manutenção não diferiu entre os que tinham os anticorpos positivos ou negativos[13].

Em alguns casos, no TP com expressão da autoimunidade e disfunção do enxerto[3], o reforço da terapêutica imunossupressora resultou na resolução da insulite e preservação da secreção de insulina. Noutras séries, a imunossupressão não impediu a subida ou manutenção da positividade dos autoanticorpos[10,13,14,17], ainda que também em algumas delas não se tenha verificado disfunção do enxerto associada[13,14,17].

PARTICULARIDADES DA AUTOIMUNIDADE NO TRANSPLANTE DE ILHOTAS

O transplante de ilhotas parece estar mais sujeito à imunoativação quer humoral, quer celular[6], ainda que a observação histológica seja mais difícil no transplante de ilhotas. Isso ocorre provavelmente porque a exposição antigênica das células beta é maior, uma vez que as células não estão resguardadas pelo tecido pancreático circundante, como no transplante de pâncreas vascularizado, mas também eventualmente por outros fatores, isquêmicos, infecciosos, maior apoptose, ou maior exposição à toxicidade dos calcineurínicos. Também no transplante de ilhotas é mais frequente a correlação entre reaparecimento ou subida dos autoanticorpos e a falência endócrina[17,18] e a imunossupressão terá menor sucesso no controle do processo autoimune[18].

RECOMENDAÇÕES

A monitorização do título de autoanticorpos pancreáticos, seriada, ao longo do seguimento dos doentes, pode ser útil. Desconhecendo que evolução poderão ter a longo prazo os doentes que têm esses anticorpos positivos, mesmo que sem disfunção atual, será certamente aconselhável a vigilância atenta do perfil dos anticorpos e da função pancreática. A praticabilidade do seu doseamento bioquímico, sem requerer métodos invasivos, vem ao encontro dessa recomendação, reservando-se a biópsia, procedimento invasivo, a situações em que clínica ou analiticamente haja fundamento para a sua realização.

Contudo, sem indicadores de disfunção do enxerto (doseamento da insulina, peptídeo C em jejum ou estimulado, glicemia) e/ou sem comprovação por biópsia do ataque inflamatório às células betapancreáticas, na ausência de rejeição, não existem elementos que fundamentem uma alteração da imunossupressão. A decisão da intensificação da imunossupressão deve estar embasada numa evidência histológica de doença aloimune (rejeição) ou autoimune.

CONCLUSÕES

A eficácia crescente dos protocolos de imunossupressão levou à redução da rejeição dos vários tipos de enxertos e também do pâncreas. Em alguns casos, observou-se perda do enxerto pancreático sem que histologicamente existissem sinais de rejeição aguda ou crônica, com insulite isolada, como na expressão inicial de DM1. Em alguns desses doentes, documentou-se

recidiva da autoimunidade humoral anti-ilhota pancreática, traduzida por aumento do título dos anticorpos anteriormente enumerados (ICA, anti-GAD, IAA ou IA-2) ou por se terem tornado positivos quando antes eram negativos.

Apesar de escassos, existem estudos de análise de sobrevivência que mostram maior prevalência de doentes com esses autoanticorpos positivos e, em título mais elevado, no grupo de doentes que perderam o enxerto pancreático. Se os referidos anticorpos são de fato um fator de risco para perda do enxerto pancreático a curto, médio ou longo prazo, está por determinar e existem resultados contraditórios nas diferentes séries publicadas.

A intensificação da imunossupressão pela recidiva da autoimunidade humoral, sem evidência histológica que documente a ausência de rejeição e a presença do processo autoimune, não tem bases de apoio, pelo risco de imunossupressão excessiva e suas complicações. A biópsia é crucial para fundamentar essa decisão.

A monitorização dos autoanticorpos e a vigilância estreita dos doentes com títulos positivos parece ser recomendável, até porque não requer técnicas invasivas. Interessará certamente estudar o contexto epidemiológico em que surge a recidiva ou agravamento da autoimunidade, identificar os fatores de risco para o seu desenvolvimento, bem como as suas implicações na função do enxerto pancreático ao longo do seguimento pós-transplante. Esses conhecimentos futuros poderão permitir ter uma atitude preventiva, ou terapêutica, no controle da resposta autoimune anti-ilhota pancreática.

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Resumo VI.3

Este segundo artigo publicado aborda de modo mais amplo a nossa experiência com os marcadores serológicos de autoimunidade pancreática após TRP. Se por um lado permanece incerto o papel real destes anticorpos no mecanismo de lesão, por outro é cada vez menos questionado que a sua subida acompanha normalmente a exacerbação da autoimunidade.

De entre 135 doentes, monitorizamos prospetivamente os autoanticorpos pancreáticos em 105 casos que permaneceram com enxerto pancreático funcionante. As amostras séricas foram colhidas à data do transplante, e pelo menos aos 6 meses e anualmente.

Com um seguimento máximo de até 138 meses, o nº médio de amostras analisadas por doente foi de 8. No final do estudo 43,8% dos doentes tinham ICA, anti-GAD ou IAA positivos, por persistência ou por recidiva. Anti-GAD foi o anticorpo mais prevalente (31,4%). Comparando os doentes positivos com os doentes negativos para qualquer dos anticorpos, eles não diferiam em termos de compatibilidades HLA em A e DR, imunossupressão, rejeição aguda, função renal e amilasemia ou lipasemia. Porém, os doentes positivos tinham maior compatibilidade em HLA-B; glicemia em jejum e HbA1c mais altas; e Pept-C mais baixo. Dividimos os doentes em 4 grupos de acordo com a positividade ou não dos anticorpos no início e no fim do estudo: negativo/negativo; positivo/negativo; positivo/positivo; e negativo/positivo. Este último tendia a ter HbA1c mais alta.

A análise multivariada confirmou a associação significativa entre a positividade para estes anticorpos e os níveis de HbA1c e Pept-C. Observámos que a probabilidade de terem estes marcadores positivos era 5,2 vezes superior nos doentes com HbA1c>5,6%; e 35% mais baixa nos doentes com Pept-C mais elevado.

Apesar destes achados, não encontrámos pior sobrevivência do enxerto pancreático no tempo de seguimento deste estudo. Ainda assim, e comparando com os resultados do estudo anterior em menos doentes e com menor seguimento, parece haver um agravamento do perfil glicémico nos doentes que apresentam positividade para os autoanticorpos pancreáticos. Baseados nestes factos, entendemos que este é um assunto que merece maior atenção e investigação no futuro.

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Clinical Transplantation

Pancreatic autoantibodies after pancreas–kidney transplantation – do they matter?

Martins LS, Henriques AC, Fonseca IM, Rodrigues AS, Oliveira JC, Dores JM, Dias LS, Cabrita AM, Silva JD, Noronha IL. Pancreatic autoantibodies after pancreas–kidney transplantation – do they matter?

Abstract: Type 1 diabetes recurrence has been documented in simultaneous pancreas–kidney transplants (SPKT), but this diagnosis may be underestimated. Antibody monitoring is the most simple, noninvasive, screening test for pancreas autoimmune activity. However, the impact of the positive autoimmune markers on pancreas graft function remains controversial. In our cohort of 105 SPKT, we studied the cases with positive pancreatic autoantibodies. They were immunosuppressed with antithymocyte globulin, tacrolimus, mycophenolate, and steroids. The persistence or reappearance of these autoantibodies after SPKT and factors associated with their evolution and with graft outcome were analyzed. Pancreatic autoantibodies were prospectively monitored. Serum samples were collected before transplantation and at least once per year thereafter. At the end of the follow-up (maximum 138 months), 43.8% of patients were positive (from pre-transplant or after recurrence) for at least one autoantibody – the positive group. Antiglutamic acid decarboxylase was the most prevalent (31.4%), followed by anti-insulin (8.6%) and anti-islet cell autoantibodies (3.8%). Bivariate analysis showed that the positive group had higher fasting glucose, higher glycated hemoglobin (HbA1c), lower C-peptide levels, and a higher number of HLA-matches. Analyzing the sample divided into four groups according to pre-/post-transplant autoantibodies profile, the negative/positive group tended to present the higher HbA1c values. Multivariate analysis confirmed the significant association between pancreas autoimmunity and HbA1c and C-peptide levels. Positivity for these autoantibodies pre-transplantation did not influence pancreas survival. The unfavorable glycemic profile observed in the autoantibody-positive SPKT is a matter of concern, which deserves further attention.

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Key words: autoantibodies – autoimmunity – pancreas transplantation – recurrence

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Type 1 diabetes results from an autoimmune disorder leading to pancreatic beta-cell destruction (1, 2). With the advance of pancreas transplantation, questions have been raised about the possibility of disease recurrence in the graft. Cases of pancreas loss without rejection criteria increased this suspicion. Biopsy findings of pancreas grafts have identified similarities with the pathology of the native pancreas at the onset of diabetes, confirming the autoimmune relapse in some pancreas transplant patients (3, 4). It is still a matter of discussion whether this has a significant impact on pancreas graft survival. Some authors estimate that the recurrence of autoimmune diabetes may account for half of the global 10% immunological pancreas failures and that it has been underappreciated (5). Such a cause (autoimmunity) contributing to graft loss at the same rate as alloimmunity (approximately 5%) undoubtedly requires further investigation.

The factors that may ignite this phenomenon are not known. Several risk factors may be involved and are being investigated. The decision of whether to treat the autoimmune recurrence in pancreas

grafts remains controversial. Similar to other authors (5), we also believe that this is an underestimated problem, and given the limited knowledge and therapeutic choices, further research is needed to develop new treatment regimens that can control autoimmunity.

The aim of this work was to study cases with positive pancreatic autoimmune markers from a cohort of 135 simultaneous pancreas–kidney transplants (SPKT) and identify risk factors possibly associated with the recurrence and evolution of pancreatic autoantibodies and with patient and graft outcomes.

Research design and methods

Patients

We studied a cohort of 135 SPKT performed in 135 patients with more than six months of follow-up. Table 1 describes the demographic and clinical characteristics of baseline study population.

All SPKT were performed at our Transplantation Unit. Cytomegalovirus (CMV) prophylaxis was always performed, for at least six months, with oral valgancyclovir. Steroids were completely withdrawn in 53.3% of the patients after completion six months post-transplantation. In terms of surgical technique, systemic-enteric drainage was performed in all cases.

Baseline analysis was performed for the 135 SPKT patients. In 30 cases, the pancreas allograft failed. A longitudinal analysis of the pancreatic autoantibodies and graft outcome was conducted on the 105 SPKT patients with a functioning pancreas.

Table 1. Demographic and clinical characteristics of baseline study population

n = 135 SPKT	
Male gender (number%)	60 (44.4%)
Age at transplantation date (mean ± SD)	35 ± 6 yr
Duration of diabetes before SPKT (mean ± SD)	24 ± 6 yr
Duration of dialysis before SPKT*(mean ± SD) (* except 5 pre-emptive)	31 ± 23 months
Patients transplanted with 6 HLA-mismatches	25.9%
Initial immunosuppressive protocol	Antithymocyte globulin, tacrolimus, mycophenolate, steroids
Follow-up after SPKT (range)	6–138 months

SPKT, simultaneous pancreas–kidney transplants.

Autoantibody monitoring

Blood samples were systematically collected to prospectively measure pancreatic autoantibodies. The initial samples were obtained during the pre-transplant evaluation, on the admission date for transplantation or in the early days thereafter. Samples were then collected at six and 12 months and once per year after the SPKT. The mean number of samples analyzed per patient was eight (3–15 samples). Glutamic acid decarboxylase antibodies (anti-GAD) were measured using the radioligand assay CentAK® anti-GAD65 (MEDIPAN GMBH, Berlin, Germany). Islet cell autoantibodies (ICA) were determined by ELISA test, using the Isletest™-ICA (BIOMERICA, Irvine, CA, USA). Anti-insulin antibodies (IAA) were measured using the radioimmunoassay RiaRSR™ IAA (RSR Limited, Cardiff, UK).

Patients were considered positive for pancreatic autoantibodies when anti-GAD antibodies were >1.45 U/mL, ICA > 1.05, and IAA > 0.4 U/mL. The normal range for HbA1c was 3.8–5.6% (18–38 mmol/mol) and for C-peptide 1.1–4.4 ng/mL.

Statistical analysis

Results are presented as mean ± standard deviation for continuous, normally distributed variables and as percentages for categorical data. Normality was tested using the Kolmogorov–Smirnov test. Continuous variables were analyzed using two-tailed *t* test for independent observations. One-way ANOVA with Bonferroni's post hoc test was used for comparisons between more than two groups. Categorical data and proportions were analyzed using chi-square or Fisher's exact test when required. Patient survival was determined from the time of SPKT until death or end of follow-up. Death-censored kidney graft survival was determined from the time of kidney transplantation until kidney retransplantation, return to dialysis, or end of follow-up. Death-censored pancreas graft survival was determined from the time of SPKT until pancreas failure or end of follow-up. Survival analysis was performed using the Kaplan–Meier (product-limit) estimator of survival and the log-rank test to compare survival rates. A p value ≤ 0.05 was considered statistically significant. Multivariable logistic regression analysis was used to determine factors independently associated with autoimmune positivity. The independent variables that were significantly ($p \leq 0.05$) or marginally significantly ($p < 0.1$) associated with glycemic indicators in the bivariate analyses were then entered into the multivariate logistic regression

model. These models were run using backward stepwise elimination procedures, and the Hosmer–Lemeshow test was used for assessing the goodness of fit of the models. Results were expressed as odds ratio (OR) and their respective p value. Statistical analysis was performed using SPSS software version 20.0 (SPSS, Chicago, IL, USA).

Results

Study population

The incidence of rejection in the initial cohort of 135 SPKT patients for either of the two grafts was 16.3%; in 8.9%, pancreas rejection was diagnosed. Pancreatic biopsy was only recently initiated; pancreas rejection was previously inferred based on clinical, analytical, and imaging data. Among the pancreatic rejections (12 episodes), there were two cases of humoral rejection, treated with plasmapheresis, immunoglobulin, and rituximab. The other cases were cellular rejections, responsive to steroids in seven and needing antithymocyte globulin in three.

Six patients died: two from cardio-cerebrovascular disease; two from sepsis in the first months after transplantation; one from invasive aspergillosis; and one from an unknown cause.

Fourteen kidneys were lost: seven due to acute or chronic rejection; three due to patient death with a functioning kidney; three due to graft thrombosis; and one due to sepsis.

Pancreas graft loss occurred in 30 patients for the following reasons: thrombosis or bleeding in 12 patients; local infection in seven; rejection in six (4.4%); patient death with a functioning pancreas in two; unclear cause in three (2.2%). In the three cases with an unclear cause of death, all available data were consistent with diabetes recurrence after excluding other possible causes; kidney or pancreas rejection was not proven, and infection was excluded. However, progressive endocrine failure occurred, and pancreatic autoantibodies increased, but amylase and lipase remained normal. In two of these cases, pancreas biopsies showed a scarce lymphocytic infiltrate, but Langerhans islets almost disappeared, probably corresponding to a late phase of autoimmune destruction.

At the last follow-up, the pancreas graft was functioning in 105 SPKT patients, our study population for the purpose of prospective analysis of pancreatic autoantibodies and outcome.

Autoimmunity – baseline data and longitudinal profile

Of the 135 SPKT patients, 21.5% were positive for anti-GAD antibodies before transplantation; 10.4% were ICA-positive; and 3% were IAA-positive. Fig. 1 shows the evolution of pancreatic autoantibodies from pre-SPKT to the end of follow-up (of 4.9 ± 3.1 yr).

The patients were separated into two groups based on the presence or absence of autoantibodies. The one defined as the "positive" group contained the 33 anti-GAD-positive, the four ICA-positive, and the nine IAA-positive patients, totalizing 46 patients. The other defined as the "negative" group contained the remaining 59 patients of the 105 prospectively analyzed patients with a functioning pancreas.

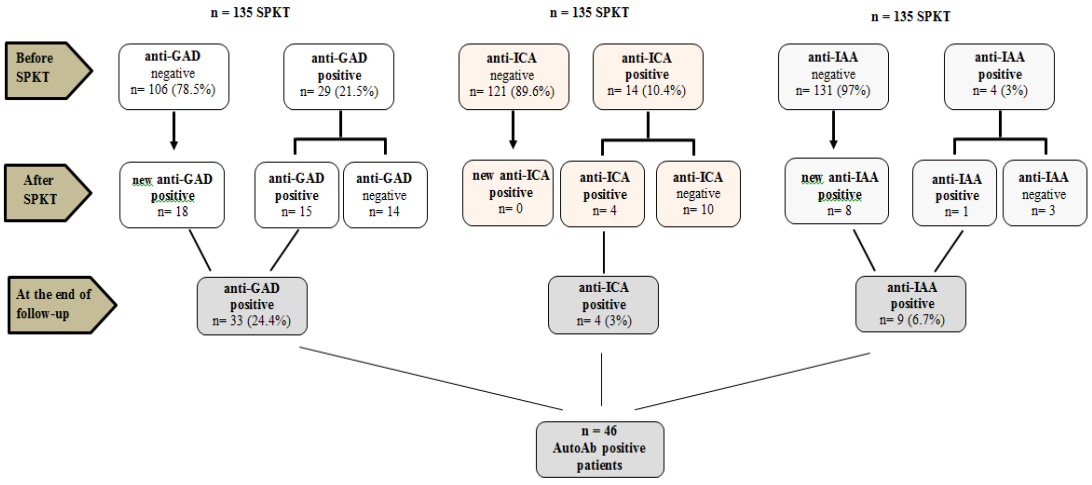


Fig. 1. Pancreatic autoantibodies in type 1 diabetic patients before and after pancreas transplantation.

Autoimmunity – prospective analysis of clinical associations and risk factors

Age, gender, and length of diabetes evolution at the date of transplantation were not significantly associated with autoantibody positivity. In positive patients, we found a higher number of HLA-matches on loci A and B, from the possible four matches. HLA-DR matching, and specifically for the alleles DR3 and/or DR4, was not associated with autoantibody positivity in the positive group.

Compared with the negative group, the positive group had higher HbA1c, higher fasting glucose, and lower C-peptide levels. Fourteen patients had an HbA1c level higher than 5.6% (38 mmol/mol). HbA1c above 5.6% (38 mmol/mol) was significantly more frequent among positive patients. A similar result was obtained for those who were only anti-GAD-positive: HbA1c was higher and HbA1c above 5.6% (38 mmol/mol) was significantly more prevalent in the positive than in the anti-GAD-negative patients.

We also classified our sample into four groups according to pre-/post-transplant autoantibodies profile (positive/positive; positive/negative; negative/positive; and negative/negative), and we analyzed the differences in HbA1c, C-peptide, and fasting glucose between them. Using ANOVA to compare the four groups, no significant differences were found in mean values of fasting glucose and C-peptide, but a nearly significant difference was found regarding HbA1c ($p = 0.053$), specifically higher between the negative/positive vs. negative/negative group, respectively, 5.44% (36 mmol/mol) and 5.18% (33 mmol/mol). Additionally, HbA1c > 5.6% (38 mmol/mol) was more frequently found in the negative/positive group: Among the fourteen patients with HbA1c above this value, 42.9% belonged to this group. Using the negative/negative group as the reference, the likelihood of presenting HbA1c > 5.6% (38 mmol/mol) was higher in the negative/positive group (OR = 12.6, $p = 0.023$) and in the positive/positive (OR = 9.9, $p = 0.047$).

Concerning the body mass index (BMI), within the positive group, eight patients (17.4%) had excessive weight (BMI > 25 kg/m^2), but none was obese (maximum = 29.6 kg/m^2). Within the negative group, the prevalence of excessive weight was not different (18.6%). Steroid withdrawal was assessed as another possible risk factor. The results showed that the rate of patient withdrawal from steroids was not significantly different between the positive and negative groups.

Seven cases of minor/moderate CMV disease and one case of BK virus nephropathy were registered. All CMV episodes were successfully treated with intravenous gancyclovir. The case of BK virus nephropathy, confirmed by biopsy 10 yr ago, remained with stable graft function (serum creatinine = 1.3 mg/dL) and with negative viral load after mycophenolate discontinuation and treatment with cidofovir and immunoglobulin. We could not find any association between these viral infections and pancreatic autoimmunity.

Graft rejection of the pancreas, the kidney, or both grafts was not significantly associated with pancreas autoimmunity, for any of the antibodies studied. We also analyzed the possible correlation between autoantibodies positivity and pancreatic or peri-pancreatic inflammation and infection that needed surgical reintervention, but no significant association was found. Serum amylase and lipase and kidney graft function, assessed by serum creatinine, did not differ between the positive and the negative patients. Table 2 summarizes the relevant clinical associations with autoimmunity, by bivariate analysis.

By multivariable logistic regression, after including variables significantly associated with autoimmune positivity by bivariate analysis (fasting glucose; HbA1c; C-peptide; A and B HLA-matches), C-peptide and HbA1c > 5.6% (>38 mmol/mol) remained significantly associated. The odds of having positive autoimmunity were 5.2 times higher among those with HbA1c above this value (OR = 5.2, $p = 0.03$) and less likely among the participants with higher levels of C-peptide (OR = 0.65, $p = 0.039$) – Table 3.

We did not observe that the presence of the pancreatic autoantibodies was associated with poor pancreas graft survival ($p = 0.971$). Kidney graft survival also was not affected by the presence of these autoantibodies ($p = 0.962$).

Finally, we evaluated patients who were positive for autoantibodies before the SPKT to determine whether they had poorer pancreas survival than the negative patients. Because only one patient was consistently IAA-positive and the majority of ICA-positive patients became negative (10 in 14) within months after transplantation, we focused the analysis on patients who were anti-GAD-positive pre-transplantation. Pre-transplant anti-GAD autoantibodies were not determinant for an increased rate of pancreas graft loss ($p = 0.731$) – Fig. 2.

Survival and function

In our study sample of SPKT patients, the patient survival rate was 97% at one yr and 95% at five and 10 yr. The death-censored kidney graft survival was 97% at one yr, 93% at five yr, and 81%

Table 2. Relevant clinical associations with pancreatic autoimmunity by bivariate analysis

	"Positive" (n = 46)	"Negative" (n = 59)	p Value
HLA-match			
HLA-A/B/DR (0–6 matches)	1.30 ± 1.07	1.05 ± 0.76	0.163
HLA–A/B (0–4 matches)	0.84 ± 0.77	0.55 ± 0.60	0.032
HLA-DR (0–2 matches)	0.49 ± 0.55	0.50 ± 0.57	0.921
Matching HLA-DR3/DR4 with the donor (% of patients)	6.5	5.1	0.769
Glucose metabolism			
HbA1c (%; [and mmol/mol])	5.41 ± 0.46; (36 ± 5)	5.22 ± 0.31; (34 ± 3)	0.011
HbA1c > 5.6% (>38 mmol/mol) (% of patients)	21.7	6.8	0.028
Fasting glycemia (mg/dL)	84 ± 12	79 ± 9	0.032
C-peptide (ng/mL)	2.54 ± 0.96	3.30 ± 2.32	0.038
Immunosuppression			
Withdrawn from steroids (% of patients)	56.5	52.5	0.754
Acute rejection			
Graft rejection [any graft] (% of patients)	15.2	11.9	0.640
Pancreas graft rejection (% of patients)	6.5	5.1	0.769
Kidney graft rejection (% of patients)	10.9	8.5	0.699
Other			
Pancreas reinterventions (% of patients)	6.5	10.2	0.491
Serum amylase (U/L)	70 ± 37	77 ± 29	0.834
Serum lipase (U/L)	30 ± 15	38 ± 16	0.761
Serum creatinine (mg/dL)	1.1 ± 0.3	1.2 ± 0.4	0.122

Table 3. Multivariate analysis of factors associated with pancreatic autoimmunity

				95% CI for Exp (B)	
Variables	B	Sig.	Exp (B)	Lower	Upper
HbA1c > 5.6% (>38 mmol/mol)	1.656	0.030	5.240	1.176	23.351
HLA-match DR	-0.166	0.675	0.847	0.389	1.843
HLA-match A/B	0.610	0.061	1.841	0.972	3.487
C-peptide	-0.426	0.039	0.653	0.436	0.979
Glycemia	0.034	0.110	1.035	0.992	1.079
Pancreas acute rejection	0.930	0.341	2.535	0.373	17.230
Constant	-2.455	0.191	0.086		

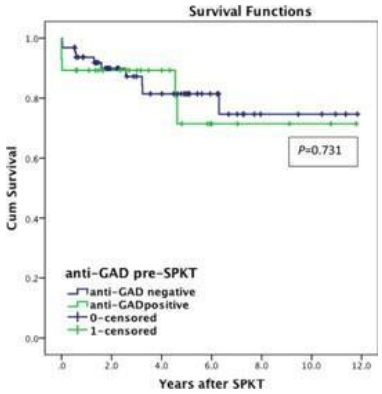


Fig. 2. Log-rank test comparing pancreas graft survival curves (Kaplan–Meier method) for the presence of absence of anti-GAD before transplantation).

at 10 yr. The death-censored pancreas graft survival was 85% at one yr, 79% at five yr, and 74% at 10 yr post-transplant.

At the end of follow-up, patients with functioning kidney grafts (n = 121, 89.6%) had mean serum creatinine level = 1.16 ± 0.38 mg/dL and creatinine clearance = 75.7 ± 24.2 mL/min. The 105 SPKT patients with functioning pancreas grafts (77.8%) had mean fasting blood glucose = 81 ± 11 mg/dL, HbA1c = 5.30 ± 0.39% (34 ± 4.5 mmol/mol), and C-peptide = 3.0 ± 1.9 ng/mL.

Discussion

The autoimmune nature of type 1 diabetes (DM1) has been largely documented (1). At the onset of the disease, the majority of patients present detectable pancreatic autoantibodies in their blood and a lymphocytic infiltrate selectively targeting pancreatic beta cells.

Recent studies have identified an important role of autoimmune cellular activation in the graft recurrence of type 1 diabetes (6, 7), in addition to the previously observed autoimmune humoral response (4, 8–10). Pancreatic autoantibody relapse is accompanied by a selective mononuclear lymphocytic infiltrate attacking Langerhans islets. It was demonstrated that these lymphocytes have the same phenotype at the time of initial diagnosis of diabetes and at the time of diabetes recurrence (6, 7, 11); that they may disappear after antilymphocyte therapy and can relapse months or years later, again with the same phenotype (6, 7). Both

the importance and the difficulty to control the memory cell response are evident from the above-mentioned studies.

There is substantial knowledge about the genetic susceptibility for developing DM1, and several polymorphisms of multiple genes have been reported. The greatest susceptibility is associated with the inheritance of the HLA–DR3 and/or HLA-DR4 haplotypes (12, 13). The same phenomenon may occur after pancreas transplantation. Previous reports have described autoimmune recurrence and pancreas loss in HLA-identical patients with minimal immunosuppression (3). After examining 100 pancreas grafts, Sibley et al. (14) did not find evidence of autoimmune diabetes recurrence in grafts from non-HLA-identical donors maintained with immunosuppression. It has been suggested that HLA-matching could be a risk factor for pancreatic autoimmunity relapse.

By bivariate analysis, we have found that patients positive for any of the antibodies studied had a better HLA-match with the donor on the A or B loci, but not on the DR locus. However, multivariate analysis did not confirm such a significant association. Additionally, positive and negative patients equally shared the DR3 or DR4 alleles with the donor (three SPKT in each group).

More recent studies have provided evidence of selective beta-cell loss in pancreas transplants under immunosuppression (4). Exocrine pancreas tissue was spared and rejection was excluded. Eisenbarth (15) pointed out some questions that may arise from these data: Why does autoimmunity not occur more frequently? Can autoimmunity be a great contributing factor to a poor outcome of pancreas or islet transplantation? Currently, it is believed that diabetes recurrence is more frequent than initially thought. It has most likely been underdiagnosed, because it is not routinely assessed (5), perhaps due to scarce knowledge and low awareness of clinicians for this problem.

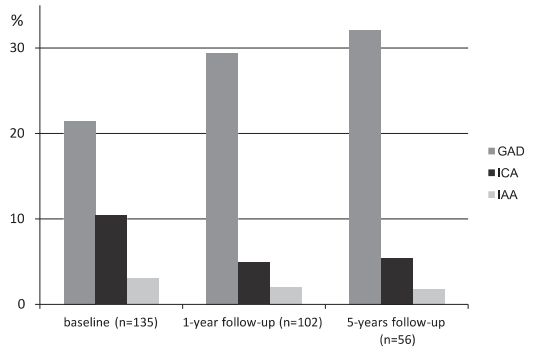
In one previous report, we have (16), like others (5, 9, 17), already reported persistence, disappearance, or reappearance of pancreatic autoantibodies after pancreas transplantation. We have found the same in the present study. The observation of autoantibodies reenhancement in immunosuppressed pancreas transplants and its correlation with an increased rate of graft failure was initially described for ICA (8). Although ICA may persist or even reappear, they mostly tend to become negative after transplantation (18). The same was observed in this study: ICA tended to disappear in the majority of the pre-transplant ICA-positive patients.

Subsequent reports (9, 10) have shown a more consistent association between anti-GAD and/or IA-2 (antityrosine phosphatase) antibodies and recurrent beta-cell destruction. Autoantibody reappearance, more than their persistence, is associated with a high risk of diabetes recurrence (5). IAA are additional immunological markers for type 1 diabetes that can be found in pancreas transplant patients, but not all authors have identified an association between immunoreactivity and impaired graft function (19). IAA may be positive in patients under exogenous insulin; however, only 3% were positive before transplantation in our cohort of patients. In a recently published paper (20), the authors confirmed the diagnosis of diabetes recurrence and beta-cell destruction without an increase in pancreatic autoantibody levels. This means that these serological markers may not be necessarily present in all patients who have autoimmune loss, although normally they are positive in the majority of the cases.

Other autoantibodies, such as IGRP (islet-specific glucose-6-phosphatase catalytic subunit-related protein), and the cation efflux transporter ZnT8 have been described and are considered pancreas autoimmune markers (7). When we started to analyze the samples of this study, more than 10 yr ago, tests for these new antibodies were not available and we used the three tests available at that time (ICA, anti-GAD, IAA). It is believed that autoantibody testing will become the mainstay of type 1 diabetes recurrence diagnosis and prediction (5). Given the wide availability of these assays, they should be included in the routine assessment in most pancreas transplant centers (5).

Anti-GAD antibodies were the most frequently positive autoimmune markers in our population. Despite apparently adequate immunosuppression, anti-GAD antibodies persisted in a considerable number of patients. Graphic 1 highlights the rate of patients with positive pancreatic autoantibodies, for each one of those searched in this study, at each follow-up time. As in other reports (9), in this study, we could not prove that the positivity of the pancreatic autoantibodies before transplantation was a risk factor for pancreas graft loss.

We routinely perform enteric diversion of pancreatic exocrine secretion, the surgical technique most commonly used worldwide (21), more physiological and associated with fewer long-term complications. However, it increases the difficulty to assess the pancreas graft. Thus, we cannot monitor urinary amylase, as others do, using the urinary diversion. Nonetheless, we can monitor serum amylase and lipase profiles, which usually change during situations of whole pancreas inflammation, like rejection. Compared with the negative patients, no significant changes on pancreas



Graphic 1. Rate of patients with positive pancreatic autoantibodies during the follow-up (from patients at risk, at each follow-up time).

enzymatic profile were observed in our positive patients. Moreover, they had stable kidney graft function, favoring the assumed absence of rejection and controlled alloimmunity. However, only pancreas biopsy can definitely establish the diagnosis of pancreas graft rejection. In more recent years, we have proceeded to pancreas graft biopsy whenever indicated. In some cases of pancreas dysfunction, the biopsy was performed on both grafts, even without kidney dysfunction, when the pancreas histology results were not clear.

We observed a lower C-peptide level in the global positive group, and this was significant. HbA1c was also higher in the positive patients, and more positive patients had an HbA1c > 5.6% (38 mmol/mol), compared with the negative group. A higher rate of patients with HbA1c > 5.6% and a tendency to higher HbA1c values was found in the negative/positive group, after splitting the sample in four groups according to the profile of pre-/post-transplant autoantibodies. Despite a small number of patients in each group, the difference found was nearly significant. These differences are uncomfortable findings, and we must be alert and keep continuously monitoring their evolution. Pancreas autoimmune humoral activity may precede endocrine failure by months or years (8, 9).

As the rate of patients with excessive weight was similar in both groups, it is unlikely that the insulin resistance could explain the worst glycemic profile among the positive patients.

Minimization of immunosuppression was also initially described as a possible determinant for diabetes recurrence (3). In our cohort, the immunosuppression regimen was similar in the positive and negative patients, with tacrolimus plus mycophenolic acid being the most common protocol. We found no association between the presence of pancreatic autoantibodies and steroid withdrawal.

Vendrame et al. (7) described in one patient the coexistence of later rejection and autoimmune reactivation. One can hypothesize that rejection and tissue lesion may lead to a more extensive exposure of pancreas autoantigens, facilitating the autoimmune attack. However, there are no consistent reports confirming that rejection can facilitate autoimmunity recurrence. In this study, we also could not associate rejection and pancreatic autoantibodies positivity. Islet transplant patients are even more prone to exhibit autoimmune activity and destruction than the whole pancreas (10, 15). One explanation may be the lower exposure of the islets in the whole pancreas, conferring more efficient resistance to the autoimmune process (10).

Based on the above-mentioned theories, we can speculate that pancreas transplants with infectious complications and pancreatitis – possibly losing their pancreatic tissue integrity, increasing antigen exposure – may more frequently present signs of autoimmune activity. In our study group, we could not find such association. Patients with pancreatic or peri-pancreatic infection, abscess, and surgical reinterventions showed rates of pancreatic autoantibody positivity not different than the others without these complications.

Very interesting studies emerged in the last years on the role of the cellular autoimmune response in type 1 diabetes recurrence in pancreas transplant patients (5–7). Memory autoreactive CD4+ and CD8+ T cells were detected in patients around the time of diabetes recurrence, not only in the circulation, but also in peri-islet pancreatic tissue and in pancreatic lymph nodes. The authors were able to clone these cells and to prove, *in vivo*, their capacity to cause beta-cell destruction (5–7). They demonstrated a correlation between the identified autoreactive T cells, specific for the islet autoantigen GAD65, and disease recurrence and progression. In patients who received additional immunosuppression to treat recurrence, with antilymphocyte therapy, an initial T-cell disappearance was followed by a later reappearance and then total endocrine failure. Pancreatic autoantibodies accompanied the T cell fluctuations.

The current immunosuppressive protocols cannot sufficiently suppress and maintain islet autoimmunity under control. Further research is needed, to develop treatment strategies that can specifically target the different pathways of the autoimmune process and stop their progression. It is not easy to decide which patients are candidates for treatment or how to treat them. Before that, it is essential to be aware of this pathology and able to make the diagnosis. We share the same opinion of Pugliese et al. (5–7), who stated that type 1 diabetes

recurrence in pancreatic transplant patients has most likely been underdiagnosed.

In sum, we confirmed that pancreatic autoantibodies may vary during follow-up and can disappear, persist, or reappear after SPKT. We also demonstrated that their presence pre-transplantation did not influence pancreas graft survival.

A less favorable glycemic profile was observed in the positive patients: Compared with the negative group, lower levels of C-peptide and higher HbA1c were recorded in the positive group. Although their mean values were still within the normal range, this is a matter of concern and a major focus of our attention.

Antibody monitoring is the most simple and non-invasive test used to identify individuals with autoimmune activity. Although these autoantibodies may not be present in all cases, they are an important initial tool. After this screening test, further workup is required to establish the definitive diagnosis. The triggers for autoimmunity recurrence are not known. Factors facilitating or associated with the process deserve more attention because they may be controllable and the ideal therapeutic targets for disease prevention. We still believe this problem has not been sufficiently considered and deserves further investigation.

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Authors' contributions

L.S.M.: Designed the study, performed research, analyzed data, contributed to discussion, and wrote the manuscript; A.C.H.: Performed research and contributed to discussion; I.M.F.: Performed research, analyzed data, and contributed to discussion; J.C.O., J. M. D., L.S.D., A.M.C., and J.D.S.: Contributed to discussion and interpretation of data; A.S.R.: Contributed to discussion and edited the manuscript; I.L.N.: Contributed to discussion, wrote, and edited the manuscript. All the authors reviewed and approved the final version of the manuscript.

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Resumo VI.4

Dados mais atuais parecem confirmar que a recidiva da autoimunidade anti-ilhota pancreática tem sido subestimada. Pensa-se que poderá representar metade das perdas imunológicas do pâncreas transplantado, ou seja 5% do total das perdas, o que multiplicado pelo número total dos transplantes deste órgão realizados por ano é de grande relevo clínico e económico.

O artigo de revisão que se segue revisita os aspetos da autoimunidade na DM1, relembrando a necessidade da deteção precoce da recorrência da doença. Para que tal aconteça é necessário o reconhecimento dessa entidade por parte dos clínicos e o seu rastreio.

Haverá certamente um papel quer da autoimunidade humoral quer da celular na recidiva, tal como documentado na diabetes inaugural. Salientam-se os achados mais recentes encontrados nesta área, assim como as estratégias terapêuticas ensaiadas para controlar ou fazer remitir a doença. Múltiplas abordagens têm sido utilizadas, desde a intensificação dos imunossupressores já em curso, até variados anticorpos mono e policlonais (já em uso, ou ainda em fase experimental), anti-linfócito B e/ou T. Os resultados têm sido pouco promissores. Porém, estes têm sido usados em fase terciária de intervenção, potencialmente com dano já avançado das células beta pancreáticas. A intervenção secundária, baseada em marcadores da autoimunidade presentes, mas sem diabetes clínica, poderá aumentar a taxa de sucesso do tratamento. Ainda assim, continua por definir qual o tratamento mais eficaz.

Os resultados da experiência da nossa Unidade de TRP, na avaliação prospetiva da autoimunidade pancreática, são relatados nesta revisão. Os 2 trabalhos publicados anteriormente são consistentes na demonstração de que haverá uma evolução progressiva da doença correlacionada com a presença dos anticorpos: no 2º trabalho (VI.3) com maior nº de doentes e seguimento mais longo, tornou-se mais evidente que o perfil glicémico é mais desfavorável nos doentes “positivos”.

Concluímos que a monitorização periódica destes anticorpos, que pode anteceder muito a doença clínica, bem como o conhecimento de todo o processo que conduz à exacerbação da autoimunidade, poderão no futuro contribuir para encontrar um esquema eficaz na prevenção ou no tratamento da recidiva da diabetes autoimune no transplante pancreático.

Esta publicação faz uma revisão dos artigos mais relevantes publicados sobre este tema.

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MINIREVIEWS

Autoimmune diabetes recurrence should be routinely monitored after pancreas transplantation

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Abstract

Autoimmune type 1 diabetes recurrence in pancreas grafts was first described 30 years ago, but it is not yet completely understood. In fact, the number of transplants affected and possibly lost due to this disease may be falsely low. There may be insufficient awareness to this entity by clinicians, leading to underdiagnosis. Some authors estimate that half of the immunological losses in pancreas transplantation are due to autoimmunity. Pancreas biopsy is the gold standard for the definitive diagnosis. However, as an invasive procedure, it is not the ideal approach to screen the disease. Pancreatic autoantibodies which may be detected early before graft dysfunction, when searched for, are probably the best initial tool to establish the diagnosis. The purpose of this review is to revisit the autoimmune aspects of type 1 diabetes and to analyse data about the identified autoantibodies, as serological markers of the disease. Therapeutic strategies used to control the disease, though with unsatisfactory results, are also addressed. In addition, the author's own experience with the prospective monitoring of pancreatic autoantibodies after transplantation and its correlation with graft outcome will be discussed.

Key words: Autoantibodies; Autoimmune type 1 diabetes; Pancreas transplantation; Type 1 diabetes recurrence

Core tip: Recurrence of pancreatic autoantibodies after kidney-pancreas transplantation is a disturbing finding. It was estimated that half of the immunological losses of pancreas grafts may be due to autoimmunity. There is a rising investigational effort concerning this issue. At our unit, we have designed a protocol of prospective monitoring of pancreatic autoantibodies after transplantation. In our experience, patients with positive pancreatic autoantibodies, compared to negative patients, were more likely to present higher HbA1c and lower C-peptide levels. A review of the most important publications in this field, and about the interest of pancreatic autoantibodies monitoring after transplantation, was made.

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TYPE 1 DIABETES MELLITUS AND AUTOIMMUNITY

Type 1 diabetes mellitus (DM1), a disease with an evident underlying autoimmune process[1], may recur after pancreas transplantation. The first cases described by Sutherland *et al*[2] were documented only in patients who have received grafts from highly HLA-matched donors (siblings) and with minimized immunosuppression[3]. Few years later, diabetes recurrence was also documented in recipients of

pancreas grafts with HLA-mismatches with the donor and maintaining standard immunosuppression[4].

There is a recognized genetic susceptibility for DM1. The disease is strongly associated with *HLA* genes, specifically with alleles DR3 and DR4[5,6]; with polymorphisms of the proinsulin/insulin gene[7]; and with the PTP gene (*PTPN22*), a gene coding for a lymphocyte-specific tyrosine phosphatase[8]. However, only about 50% of HLA identical twins inheriting alleles DR3 and/or DR4 develop the disease[1,9]. It means that inheritance of the *HLA* gene is not a sufficient condition and susceptibility is most certainly polygenic.

It is not known which individuals are at higher risk for DM1 recurrence on pancreas graft and what are the important risk factors for the disease. Additionally, there is also no consensus about the best screening tests to identify patients at risk.

DIAGNOSIS OF AUTOIMMUNE DIABETES RECURRENCE ON PANCREAS GRAFTS

A pancreas graft biopsy showing an inflammatory T-cell infiltrate, specifically targeting the beta-cells (aspect designated as "insulitis") and sparing the exocrine tissue, remains the gold-standard for the diagnosis of DM1 recurrence[10]. However, it is not easy to justify such an invasive procedure, carrying a non neglectable risk of complications, in patients without a misfunctioning pancreas graftor, at least, without reliable data favouring the hypothesis of reactivated autoimmunity.

Serological markers of the autoimmune process, the islet cell autoantibodies (ICA)[4] have been proposed as a basic tool, the first screening test to identify the activity of autoimmune disease. The anti-glutamic acid decarboxylase (anti-GAD antibodies)[11]; the anti-insulin antibodies (IAA)[12]; the anti-IA2 (anti-tyrosine phosphatase) antibodies[13]; and the most recently described anti-ZnT8 (cation efflux zinc transporter) antibodies[14], have also been identified as autoimmune markers of DM1. The positivity for these immune humoral markers is considered a good predictor of the enhancement of autoimmune diabetes. The association of several markers (two or more) increases its predictive value[15]. As yet, pancreas biopsy is the confirmatory procedure when suspecting for recurrence on pancreas graft.

There is some controversy about the real role of these autoantibodies: do they have a direct participation in the process? Or are they surrogate markers, merely testifying the lesion? Although the pancreatic autoantibodies were not detected in a recent case documented with insulitis in the biopsy[16], they are usually present in the vast majority of the cases confirmed by biopsy.

The new onset or rising levels of these autoantibodies in pancreas transplant patients has been pointed out as a serious indicator of recurrence and progression of the disease. In fact, several studies reported worse pancreas outcome in patients with these humoral markers[10,17-20]. It has been suggested that half of the immune losses of pancreas grafts may be due to autoimmunity[21]. Based on these data, monitorization of pancreatic autoantibodies has been recommended in all pancreas transplants[21] as a primary test to identify patients at risk for autoimmune graft loss. My personal opinion is concordant with these authors, stating that the disease may currently remain underdiagnosed. This may be the cause of pancreas graft failure in some cases with unclear etiology, probably because this is not sufficiently investigated.

IMMUNOSUPPRESSION AND AUTOIMMUNITY

Immunosuppressive protocols designed to prevent rejection in the pancreas transplant are not capable of containing autoimmunity[21]. This is a disturbing finding in organ transplantation. Remembering autoimmune disorders affecting the kidney, such as ANCA-associated vasculitis or lupus, they may relapse after kidney transplantation despite apparently adequate immunosuppression to control alloimmunity. One condition in kidney transplantation, which is quite similar to the pancreatic autoimmunity recurrence, is that observed in some patients with Alport syndrome: they may develop anti-glomerular basement membrane disease post-transplantation, after a new exposition to glomerular basement membrane antigens (type 4 collagen antigens), for which they were natively defective. Immune attack against the newly presented beta-cells may occur after a pancreas transplant.

The Miami group has tried to treat autoimmune relapse in pancreas transplants with anti-lymphocyte (anti-B and/or anti-T cell) therapies[21-23]. After a transient response in a few cases, autoimmune activity has recurred within a short period of time. At the time of the second recurrence they were able to identify the same clone of autoreactive GAD-specific T cells which has been found in the first recurrence. Pancreatic autoantibodies followed the reappearance of the T cells, with a new rise[23]. Therefore, it seems that immunosuppressive agents available at the moment cannot prevent this immune memory response. To date, there are no studies reporting effective and sustained treatment of pancreatic autoimmunity in DM1 patients with diabetes recurrence.

Efforts are needed to find therapeutic strategies to control this process. Can protocols used in kidney transplant hypersensitized patients be advantageous? Combined therapies, like plasmapheresis, immunoglobulin and rituximab have been successfully used in kidney transplants with HLA donor-specific antibodies; and also in systemic autoimmune diseases, such as lupus, with severe expression. The results from trials using new drugs (abatacept, etanercept, teplizumab, rituximab) have failed to prove long lived efficacy in native pancreas after DM1 onset[24]. However, intervention after clinical disease (tertiary intervention) may be too late, since overt disease corresponds to extensive beta-cell destruction. The most promising long-term results were achieved with hematopoietic stem-cell transplantation, in patients pre-

senting autoimmune markers, but before clinical diabetes (secondary intervention)[24]. Another serious worry are the important side effects of each drug. Toxicities and efficacy, if used in combination, remain to be assessed. We have learned the benefits of the association of complimentary therapies from the oncology research, allowing the use of smaller doses, with fewer side effects and with gains in terms of efficacy. Could this be an efficient strategy in pancreas transplant patients with recurred autoimmune diabetes?

OUR OWN EXPERIENCE

We have designed a prospective monitoring protocol of pancreatic autoantibodies after pancreas-kidney transplantation at our unit. Anti-GAD, IAA and ICA were analysed before or on admission for transplantation, at 6 and 12 mo, and at least once a year thereafter. In a cohort of 135 patients[25], 34.5% were positive for any of these antibodies before surgery, anti-GAD being the most prevalent (> 20%). Nearly half of these became negative within months or years, but in some others (previously negative) we verified a new appearance of anti-GAD antibodies. After a mean time of 6 years (ranging from 1 to 12 years), among the 78% of the patients with functioning pancreas, 44% are positive for at least one autoantibody. Anti-GAD remain the most common (31%). The frequency of patients with IAA before transplantation was surprisingly low, 3%, considering that patients under exogenous insulin may present anti-insulin antibodies. At our last follow-up the prevalence of IAA-positive is three-fold higher and none of the patients was under exogenous insulin. ICA, present in 10% of the patients before transplantation, tended to disappear and there was no new onset of these antibodies. Less than 3% maintain ICA positive now. In 10% of the patients more than one pancreatic autoantibody was present.

Pancreas graft survival was not significantly different in the group of patients with some positive pancreatic autoantibody, compared to the patients who were negative for these autoantibodies. The immunosuppression used in the positive and in the negative patients did not differ (tacrolimus and mycophenolic acid mostly used) and the frequency of patients withdrawn from steroids was also similar in both groups.

Positive patients for any pancreatic autoantibody tended to have a higher HLA-match with the donor, though not reaching statistical significance.

Concerning the glycemic control, our data are not so tranquilizing. The group of patients with at least one positive pancreatic autoantibody, compared to negative patients, was more likely to have higher HbA1c and lower C-peptide levels and this difference was statistically significant. And, more important, our results showed a more than 5 times higher probability to find positive autoimmunity among the pancreas transplants with normal-high HbA1c. Kidney graft function was similar in both groups, with or without pancreatic antibodies, which strengths the argument that decline in glycemic control was not due to alloimmunity. In a former report from our centre[26], analysing the glycemic profile in both groups (positive and negative for pancreatic autoantibodies), the difference did not (yet) reach statistical significance. In fact, in this former study, a few number of patients with positive antibodies showed normal-high fasting glucose levels and the lowest C-peptide. Comparing results from our former study to the more recent one, it probably means that we are facing an evolutive process. The number of patients has almost doubled and the follow-up period is now much longer, and we could now associate pancreatic autoimmunity with less favourable glycemic profile. It will be interesting to analyse data with a more extended follow-up.

CONCLUSION

In conclusion, we think it is advisable to routinely monitor pancreatic autoantibodies after transplantation. There is substantial evidence that DM1 can reappear after pancreas transplantation, but may have been underdiagnosed for the last decades[21,27], mainly because it is a forgotten question. The awareness of the entity will certainly lead the majority of pancreas transplant units to search for the disease and to prospectively assess these antibodies. Pancreas graft biopsy remains the gold standard for the diagnosis of DM1 recurrence but, as it is an invasive procedure, it is not the ideal as a screening methodology, without other clinical or analytical (metabolic or immunological) data. On the other hand, pancreas biopsy only after impaired endocrine function is most of the times too late. Antibody monitoring is a non-invasive basic screening test, available in most units, and may bring the necessary information to proceed to pancreas biopsy before dysfunction. Islet autoantibodies are currently the most robust biomarker of DM1[28].

Additionally, pancreatic autoantibodies assessment may be of interest in other areas. The authors of a very recent study[29] have also proposed the use of the GAD-autoantibody status before pancreas-kidney transplantation as a guide to choose the kind of prophylactic antibody induction.

Positivity for pancreatic autoantibodies after transplantation may never occur, or it may be intermittent or persistent, not always correlated with graft function, at least in a short period of time. However, beta-cell destruction was documented in other cases and autoantibody rising may have preceded in several years the graft dysfunction, has it been searched for. An early diagnosis gives the clinician more time for some kind of intervention.

Another critical point is the lack of effectiveness of the treatments tried so far in DM1 patients. The recognition of the role of islet autoreactive CD4+ T cells[22,23] and CD8+ T cells[27,30] on beta-cell destruction, as well as all the targets of humoral activity[28], may lead to other treatment opportunities. Novel therapies, namely targeting proinsulin-reactive CD8+ T cells, were recently pro-

posed as a potential therapeutic approach[31].

In the meantime, we must improve our ability to make an early diagnosis and to increase our knowledge about all the processes of the disease. These may be some of the missing steps to find the most advantageous strategy to treat, or even to prevent, the autoimmune diabetes recurrence in pancreas grafts.

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Resumo VI.5.

A rejeição aguda no TRP tem vindo a decrescer ao longo das décadas, tal como noutros transplantes de órgãos sólidos, consequências dos avanços na imunossupressão e no seu diagnóstico precoce. Dos estudos no TR isolado, sabe-se que o aparecimento de anticorpos HLA dador-específicos (DSA) tem impacto negativo na sobrevivência do enxerto. No TRP, estudos a correlacionar os DSA de novo com rejeição e perda de rim e/ou pancreas são escassos.

Neste trabalho, em 136 TRP com um seguimento alargado, fomos estudar a relação entre a positividade para os DSA e a evolução dos enxertos. Estes doentes cumprem um protocolo pré-estabelecido de doseamento destes anticorpos (por Luminex) aos 3, 6 e 12 meses pós-TRP e depois anualmente - ou quando clinicamente justificado.

Foram encontrados anticorpos HLA em 28 doentes (20,6%), mas em apenas 18 (13,2%) eles eram DSA, tendo positivado em média ao fim de 3,3 anos após o TRP. Verificámos que a idade do recetor (mais jovem), a sensibilização anti-HLA classe I ou classe II pré-transplante, 2 incompatibilidades em HLA-DR e rejeição de qualquer dos enxertos foram fatores de risco para o aparecimento de DSA. Os DSA observaram-se mais frequentemente nos doentes com rejeições agudas mais graves, grau II e III de Banff.

A presença de DSA associou-se a menor sobrevivência do rim (P=0.007), do pâncreas (P=0.016) e de ambos os enxertos (P=0.024). Esta associação foi independente da ocorrência de rejeição aguda, uma vez que ambos (DSA e rejeição) foram fatores preditores independentes de falência de enxerto. Estratificando a sobrevivência de cada enxerto segundo os DSA e a rejeição aguda, confirmámos esses resultados. Dentro do grupo com DSA positivos, quer o número médio dos DSA somados (P=0.011), quer a intensidade desses anticorpos (P=0.030) - medida em MFI (*mean flourescence intensity*) – foram significativamente crescentes consoante se tinham perdido 0, 1 ou os 2 enxertos. Apurámos ainda que o tempo médio entre a deteção dos DSA e a perda do enxerto foi 10 meses.

Concluímos, assim, que os TRP em que os DSA surgiram de novo tinham maior risco de perder os enxertos (um ou ambos); que este efeito se mantinha independentemente de ter ocorrido rejeição ou não; e que o número e a intensidade desses DSA podem permitir identificar os indivídios com maior risco de perda de enxerto.

Title

DETRIMENTAL EFFECT OF *DE NOVO* DONOR-SPECIFIC HLA ANTIBODIES ON GRAFTS SURVIVAL IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION: DOES IT GO BEYOND ACUTE REJECTION?

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Footnotes

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Abbreviations

dDSA: *de novo* donor-specific antibodies; SPK: simultaneous pancreas-kidney; AR: acute rejection; HLA: human leukocyte antigen; IDDM: insulin-dependent diabetes mellitus; DSA: donor-specific antibodies; SAB: single-antigen bead; MFI: mean fluorescence intensity; ATG: anti-thymocyte globulin; MMF: mycophenolate mofetil; BPAR: biopsy-proven acute rejection; ACR: acute cellular rejection; AMR: antibody-mediated rejection; SD: standard deviation; IQR: interquartile range; h: hours; CI: confidence interval; min: minimum; max: maximum

Abstract

Background. *De novo* donor specific antibodies (dDSA) relevance in simultaneous pancreas-kidney (SPK) transplantation has been scarcely investigated. Particularly, data on dynamics of dDSA appearance and kidney and pancreas grafts acute rejection (AR) and survival are lacking. We analyzed dDSA relationship with grafts outcomes in a long-term follow-up SPK transplanted cohort.

Methods. In 136 patients that received SPK transplant between 2000 and 2012, posttransplant anti-human leukocyte antigen (HLA) antibodies were screened and identified using Luminex-based assays in sera collected at 3, 6, 12 months, then yearly.

Results. Anti-HLA antibodies were detected in 28 patients (20.6%), but only 18 (13.2%) had dDSA, which appeared at a median 3.3 years after transplant. Recipient age, pretransplant anti-HLA sensitization, DR HLA mismatches and previous AR were significant risk factors for dDSA. dDSA was significantly associated with double, kidney-only and pancreas-only graft lower survival. Both dDSA (HR=3.30) and AR (HR=4.11) were independent predictors of graft failure. Stratification of each graft survival analysis by dDSA and AR corroborated this finding. In DSA+ patients, those with any graft failed presented more frequently DSA against HLA class I+II (P=0.002), and had a higher median dDSA number (P=0.011) and mean fluorescence intensity (P=0.030) than patients with both grafts functioning. Median time between dDSA emergence and graft failure was 10 months.

Conclusions. SPK transplanted patients who develop dDSA have an increased risk of losing their grafts. This effect remained even in the absence of a previous AR episode. DSA characteristics might help identify patients at a higher risk of graft failure.

Introduction

Simultaneous pancreas-kidney (SPK) transplantation has become the mainstay treatment in selected patients with insulin-dependent diabetes mellitus (IDDM) and end-stage renal failure, liberating them both from insulin and dialysis (1, 2). Improvements in immunosuppression protocols have allowed SPK grafts recipients to experience an incidence of acute rejection (AR) similar to kidney-only transplantation (3). However, medium-term graft attrition rates in SPK transplantation remain significant (4, 5), calling for an improvement in graft monitoring. Pancreas graft immunological surveillance has been pursued using several biomarkers and pathological evaluations (5). In kidney transplantation, there is wider experience and knowledge about graft pathology evaluation and easily available biomarkers (eg, creatinine, proteinuria) that allow a closer monitoring of graft function and lesion (6). Nevertheless, undiagnosed immune-mediated injury has been shown to be responsible for many cases of late kidney graft failure (7, 8).

In the past decade, screening of antibodies against human leukocyte antigens (HLA) by solid-phase assays after transplantation, particularly for *de novo* donor-specific antibodies (DSA), has been used to track recipient alloimmune reactivity in organ transplantation (9). Improvements in these solid-phase assays have allowed detection and specification of anti-HLA antibodies to be done with greater precision and reproducibility (10). Several published studies have shown that posttransplant formation of DSA is associated with lower graft survival in kidney (11-13), heart (14) and liver transplantation (15). In pancreas transplantation, only two long-term series have addressed this issue (16, 17). However, its importance is clear given that alloimmune sensitization after SPK transplantation is fairly stronger than in kidney-only transplants, as a larger amount of immunogenic tissue is transplanted (kidney, exocrine and endocrine pancreatic tissues, and a segment of donor's duodenum) and because it is performed frequently with a poor HLA matching for logistic reasons, resulting in a higher incidence of AR (18).

Thus, we decided to analyze, in our cohort of SPK transplanted patients, which factors were related with *de novo* formation of DSA and their potential role as predictors of kidney and/or pancreas graft failure. Furthermore, DSA characteristics were detailed in the search of a potential association with grafts outcomes.

MATERIALS AND METHODS

Patients

All 150 consecutive adult patients who received a SPK transplant in our unit between May 2000 and December 2012 were investigated. Fourteen patients were excluded because of insufficient or unavailable anti-HLA antibodies data, leaving 136 patients (90.7%) for the final analysis. Ten patients experienced pancreas graft failure from surgical reasons within the first 15 days after surgery. As such, for the analysis of double graft and pancreas graft survival only the remaining 126

patients were considered. Pancreas transplants were performed using a systemic-enteric drainage. All patients were transplanted with a negative pretransplant T- and B-lymphocyte complement-dependent cytotoxicity crossmatch in current and peak sera and without presence of preformed DSA. The Institutional Review Board at Centro Hospitalar do Porto approved this study.

Anti-HLA Antibodies Screening and Specification

Anti-HLA antibodies screening was performed before transplantation in the last pretransplant sera and after transplantation at 3, 6, 12 months and then yearly posttransplant, as well as at the time of significant clinical events.

Screening of HLA-antibodies was performed by ELISA until 2005 and by Luminex since 2006. For ELISA screening (LAT-M One Lambda Inc.) or identification (LAT ID-1288 One Lambda Inc.), wells were coated with purified HLA antigens derived from human B-cell lines. Specific antibodies were detected by optical density signal. Cutoffs were calculated as the percentage of the reactivity of the provided serum used as a positive control minus the nonspecific background of the test serum analyzed.

For Luminex assays, anti-HLA antibodies were tested by multiplex microsphere based on Luminex Xmap® Technology (LABScreen® Mixed kit, OneLambda Inc., Canoga Park, CA, USA). The cut-off for positive samples was the Normalized Background (NBG) ratio recommended by the manufacturer and performed by the HLA fusion® software (One Lambda Inc.). To determinate the specificity of the HLA antibodies, single-antigen bead (SAB) assays (LabScreen Single Antigen Beads®, OneLambda Inc.) were performed in patients with a positive screening. The sera used for the SAB assay were the same pretransplant sera used for the screening. Mean fluorescence intensity (MFI) of each bead was analyzed using HLA fusion® software (One Lambda Inc.) and a cut-off for a positive reaction were set in MFI raw value of ≥1000.

Donor HLA Typing and DSA Assignment

Samples of all deceased donors had been routinely typed before recipient selection in loci HLA-A*, B* and DR* using polymerase chain reaction amplification with specific sequence primers (SSP; Olerup SSP® low resolution HLA typing kits, Stockholm, Sweden). HLA-C* and -DQ* antigens were also typed by SSP DNA-typing, when a potential donor-specific antibody was present. High resolution was performed in those cases in which it was necessary in order to establish whether the anti-HLA antibodies were DSA. Donor HLA-DP typing was not performed.

With the information of the donor HLA typing, we performed a virtual crossmatch allowing for the assignment of anti-HLA antibodies as donor-specific. In each individual DSA, the strength was based on the MFI of one SAB. In the case of several DSA against different HLA-antigens, we usually considered the DSA with the highest MFI; occasionally, we also used the cumulative strength of all DSA by adding the individual MFI values, as stated in the results.

Induction Protocol and Maintenance Immunosuppression

Per protocol, all patients received induction therapy using a polyclonal anti-thymocyte globulin (ATG Fresenius®, 3 mg/Kg for 5-7 days). All enrolled recipients had similar triple maintenance immunosuppression, consisting of oral tacrolimus, mycophenolate mofetil (MMF) and methylprednisolone/prednisolone. Tacrolimus was started at the dose of 0.1 -0.15 mg/kg/day, and the dose was adjusted to maintain a trough level of tacrolimus in whole blood between 8 and 12 ng/ml during the first month postoperatively, between 7 and 10 ng/ml during 2–3 months after transplant and between 5 and 8 ng/ml thereafter. MMF was started at the dose of 2000 mg/day, with the dose decreasing to 1000–1500 mg/day during the first month postoperatively, depending on white blood cells count. Methylprednisolone was administered intravenously at doses of 500, 250 and 125 mg/day on the day of transplantation, day 1-2 and day 3-4 after the operation, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg, being then tapered to 5-10 mg/day within 2–3 months after transplant. Steroids were completely withdrawn in 75 (55.1%) patients at 6 months posttransplant.

Data Collection and Outcomes

Data regarding recipient and donor characteristics, and pre- and post transplantation variables were collected retrospectively. Delayed kidney graft function was defined as dialysis requirement in the first week posttransplant. All patients were followed-up from time of transplant until death, single or both graft failure or June 30, 2014. Graft survival was analyzed using two different approaches: double graft survival analysis considered graft failure when one of the grafts failed (the first to occur) and time until that first graft failed; kidney or pancreas graft survival analysis considered that specific graft failure and survival time. All grafts survival analysis considered graft failure censored for death with a functioning graft.

Rejection Diagnosis and Treatment

Kidney graft rejection was defined as biopsy proven acute rejection (BPAR), with specimens being evaluated by light microscopy and immunofluorescence staining for C4d and classified according to Banff classification as updated in 2013 (19). Pancreas graft rejection was defined both as suspected (if kidney graft BPAR was present concomitant with an increase in serum amylase and lipase levels) and BPAR (specimens were evaluated by light microscopy and immunofluorescence staining for C4d) and classified according to Banff classification as updated in 2011 (20).

Banff grade I acute cellular rejection (ACR) was treated with pulse steroids (500 mg MP for 3 days) and increased maintenance immunosuppression. All other ACR were treated with ATG. Antibody-mediated rejection (AMR) was also treated with pulse steroids, intravenous immunoglobulin 2g/Kg (maximum 140 g) divided in 2-4 doses associated with plasmapheresis (at least 3-5 sessions) and rituximab (single-dose of 375 mg/m^2).

Statistical Analysis

Continuous data were described using mean (±standard deviation) or median (and interquartile range) and categorical data were expressed as number (and percentages). Categorical data including demographic, clinical, immunological features and DSA detection were compared using Pearson χ^2 test or Fisher´s exact test, as appropriate. Continuous variables were compared with Student t-test or Mann–Whitney U test (Kruskal–Wallis test for variables with 3 categories), as appropriate. Logistic regression analysis was used to determine significant associations between studied variables and *de novo* DSA appearance, using a multivariable model that included clinically significant variables and those presenting $P \leq 0.2$ in univariable analysis (recipient gender and age, presensitizing events, time on dialysis, pretransplant anti-HLA sensitization, acute rejection in any graft, AB and DR HLA mismatches). Graft survival curves were done using Kaplan-Meier method and compared by log-rank test. Multivariable Cox proportional hazards analysis was applied to assess independent predictors of double graft failure censored for patient death, including variables presenting $P \leq 0.2$ in univariable analysis to adjust for potential confounders: recipient gender and age, donor age, time on dialysis, years of IDDM, pretransplant anti-HLA sensitization, acute rejection in any graft, ABDR HLA mismatches, *de novo* DSA.

A two-sided *P*-value <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

In our sample of 136 SKP grafts recipients, anti-HLA antibodies were detected posttransplant in 28 (20.6%) patients, with 18 (13.2%) of them having antibodies directed against donor HLA molecules (DSA+). Timing of DSA detection ranged from 0.5 and 8.7 years posttransplant (median 3.3 years).

Patient, donor and transplant baseline characteristics are given in table 1. DSA+ patients were younger at transplant (P=0.052) and more frequently sensitized against HLA class I (P<0.001) or II (P=0.003) before transplantation. No significant differences between groups were found regarding recipient gender, donor age or gender, presensitizing events, steroids withdrawal, HLA mismatches and cold ischemia time.

Posttransplant events

Clinical events after transplantation are detailed in table 2. Studied population mean follow-up was 6.7 years, with no significant differences between groups. The occurrence of AR in any graft was more common in DSA+ (38.9%) in comparison with DSA- (10.2%) patients (P=0.001). ACR-only episodes preceded in all cases DSA emergence. In a multivariable logistic regression analysis, recipient younger age [OR=0.90 per year, P=0.040), pretransplant anti-HLA sensitization

(OR=11.48, *P*=0.003), 2 (vs 0-1) DR HLA mismatches (OR=3.91, *P*=0.049) and AR in any graft (OR=5.41, *P*=0.018) were shown to be significantly associated with *de novo* DSA (see Table S1, SDC).

The presence of DSA was associated with poorer death-censored double graft survival as depicted in Fig. 1. At 6-years follow-up, pancreas-kidney graft survival was 87.7% in patients without detectable anti-HLA antibodies, 100% in those with non-donor-specific anti-HLA antibodies and 67.3% in DSA+ patients (*P*=0.024). In a multivariable Cox regression analysis, DSA presence (HR=3.30 per year, *P*=0.039) and AR in any graft (HR=4.02, *P*=0.003) were shown to be independent predictors of graft failure (see Table S2, SDC).

Patient death occurred more frequently in DSA+ (n=3, 16.7%) than in DSA- (n=4, 3.4%) group (*P*=0.018). Infectious (n=3) and vascular (n=3) events were the more common causes of death, with one patient dying from a digestive bleeding.

Kidney graft outcomes

Biopsy-proven acute rejection in the kidney graft (Table 2) occurred more frequently in DSA+ (33.3%) than in DSA- (8.5%) patients (*P*=0.002). Noteworthy, ACR was more common in DSA+ patients, if classified as Banff grade 2/3 (P=0.003); no significant difference between groups was detected for grade 1 ACR. As expected, AMR occurred only in DSA+ patients (n=3).

Death-censored kidney graft survival according to DSA presence is shown in Fig. 2. At 6-years follow-up, kidney graft survival was 99.1% in DSA- and 79.9% in DSA+ patients (*P*=0.007). Furthermore, we analyzed death-censored kidney graft survival according to both DSA presence and AR occurrence in the kidney graft (Fig. 3). At 6-years follow-up, kidney graft survival was 100% in DSA- AR-, 87.5% in DSA- AR+, 90% in DSA+ AR- and 62.5% in DSA+ AR+ patients (*P*<0.001).

Causes of kidney graft failure are presented in Table 2. All graft failures (n=4) in DSA+ patients were deemed as rejection-driven (acute or chronic) in comparison with only one (out of 6) in DSA- patients (*P*=0.048).

Pancreas graft outcomes

The occurrence of AR in the pancreas graft (Table 2) occurred more frequently in DSA+ (33.3%) than in DSA- (4.6%) patients (*P*<0.001). Biopsy-proven acute rejection was also more frequent in DSA+ (27.8%) in comparison with DSA- (1.9%) patients. Only Banff grade 2/3 BPAR was significantly more common in DSA+ patients (*P*=0.001). Noteworthy, one DSA+ patient had C4d positive staining in the pancreas graft biopsy.

Death-censored pancreas graft survival according to DSA presence is shown in Fig. 4. At 6-years follow-up, pancreas graft survival was 88.7% in DSA- and 65.6% in DSA+ patients (*P*=0.016). Furthermore, we analyzed death-censored pancreas graft survival according to both DSA presence

and AR occurrence in the pancreas graft (Fig. 5). At 6-years follow-up, pancreas graft survival was 90.2% in DSA- AR-, 60.0% in DSA- AR+, 66.8% in DSA+ AR- and 62.5% in DSA+ AR+ patients (*P*=0.019).

Causes of pancreas graft failure are presented in Table 2. Five out of 6 graft failures in DSA+ patients were deemed as rejection-driven (acute or chronic) in comparison with only 2 (out of 13) in DSA- patients (*P*=0.010).

Graft failure and DSA characteristics

Comparison of DSA characteristics in patients with both grafts (n=3), one graft (n=4) and no graft failing (n=11) is shown in table 3. Presence of DSA against both HLA classes was more common in patients in whom both grafts failed (*P*=0.002). DSA directed to HLA *loci* A, C and DR were significantly more common in patients with graft loss, with DSA against HLA *locus* B showing a similar trend (*P*=0.059); notably, the presence of DSA against HLA *locus* DQ, though being the most common, was similar between groups (*P*=0.755). An increasing median MFI of the highest DSA bead (*P*=0.030), of the sum of all DSA beads (*P*=0.021), and of the number of DSA present (*P*=0.011) was observed in patients with one or both grafts failing, in comparison with patients without any graft failure. All graft failures in DSA+ patients occurred within 2 to 15 months (median 10 months) after DSA detection.

Discussion

We report the longest follow-up time (mean 6.7, maximum 14 years) cohort published to date, addressing the issue of *de novo* DSA impact in SPK transplantation, presenting critical data about risk factors for DSA emergence and its effect on SKP grafts. We detected 18 patients (13.2%) with *de novo* DSA at a median time posttransplant of 3.3 years. In one series with 167 pancreas graft recipients (152 patients also received a kidney graft), 15.6% developed *de novo* DSA at a median of 1 year posttransplant (16). Another study reported that 12.8% of SPK grafts recipients developed DSA between months 1-35 after transplant (17). Incidence of DSA in our cohort was similar to these studies, although our DSA onset time was comparably later. This difference may be related with our immunosuppression protocol, which was distinct from these series; the former study used a maintenance regimen with cyclosporine or sirolimus in 31% of patients and no patient was on steroids beyond the third month posttransplant, and the latter one used alemtuzumab for induction and no steroids were used for maintenance. The use of ATG induction and a tacrolimus-based maintenance therapy has been shown to be associated with a low incidence of DSA in comparison with cyclosporine- or everolimus-based maintenance therapy (21-24). Additionally, we are able to discern risk factors for DSA appearance like younger recipients, HLA DR mismatches, pretransplant anti-HLA sensitization and previous episodes of acute rejection, as several other studies have shown previously (12, 13, 17, 25).

De novo DSA formation was clearly associated with a significant detrimental effect on grafts outcomes in SPK transplantation. First, we demonstrated that previous BPAR episodes both in kidney as, for the first time, in pancreas graft were associated with DSA formation, specifically if there was presence of vascular injury (ACR Banff grade 2/3). An association of ACR and later development of *de novo* DSA may be related with the degree of microcirculatory inflammation present at the time of the ACR, in particular the sensitizing effect of upregulated HLA proteins expression in the peritubular capillaries (26). Moreover, histopathological analysis of vascular rejection biopsies showed that concomitant presence of peritubular capillaritis was very common (around 90%) (27). Second, we showed, as others (16, 17), that kidney and pancreas graft survival was significantly reduced in DSA+ patients, by analyzing each graft or a double graft survival curves. No adverse graft outcomes were associated with the presence of non-DSA anti-HLA antibodies (n=10). Besides, significantly more kidney and pancreas graft failures were deemed as rejection-driven in DSA+ patients, in some with the occurrence of an acute episode while in others as an indolent chronic process. Lastly, as several studies have shown that DSA association with graft failure is mainly related to AR occurrence (11, 22), we decided to stratify our survival analysis considering these two events. Kidney graft survival was significantly lower in patients with DSA and/or BPAR in comparison with those without any of the two. Pancreas graft survival was only significantly reduced in patients with both DSA and AR, although a trend towards a detrimental effect of one without the other event was foreseeable. In both grafts survival analysis, it was noticeable that presence of DSA and AR had a particularly harmful and seemingly synergic adverse effect. Furthermore, multivariable Cox survival analysis showed that both DSA and AR were the sole independent predictors of double graft failure. To the best of our knowledge, we are the first to point up these results in SPK transplantation, leading us to speculate that chronic antibody-mediated injury evolved in the presence of DSA even without clinically evident AR, in a similar model to the one already described in kidney-only transplantation (28).

The characteristics of DSA, namely number, MFI value and HLA class, have been associated with early events after kidney transplantation (e.g., AMR) in the context of preformed DSA (29-31). Clinical correlations of these characteristics in *de novo* DSA with graft outcomes have been less analyzed. In kidney transplantation, some have shown that the presence of DSA against HLA class I and II was associated with poorer graft survival (11, 13), while others demonstrated that same deleterious effect for DSA against HLA-DQ (25). In SPK transplantation, no significant association between graft outcomes and DSA MFI (16) or HLA class (17) has been shown. Seven out of 18 DSA+ patients from our cohort lost at least one of their grafts. It was noticeable that, as shown by others, the presence of DSA against both HLA classes was more common in patients with graft lost, as were the prevalence DSA against most HLA *loci*, with the notable exception of *locus* DQ. Furthermore, median DSA number and MFI values increased markedly between patients with a surviving double graft and those with one or two grafts failed. These results should be considered with caution given the small sample of DSA+ patients involved. Nonetheless, we consider that they suggest a

correlation between the amount of donor-specific alloreactivity, as read by DSA number and strength, and the degree of graft injury and, ultimately, with graft failure. These novel observations in posttransplant DSA formation seem to mimic the better understood relationship between preformed DSA characteristics and graft outcomes (29-31). Graft failure occurred within 2 to 15 months after DSA detection indicating that, at least in some patients, a clinical intervention directed against DSA would have been feasible. Unfortunately, the management of *de novo* DSA outside an episode of acute AMR is still undetermined. Some have reported the use of high-dose intravenous immunoglobulin with or without rituximab in patients with chronic kidney graft dysfunction and detectable DSA with limited (32) or even null effect (33).

We recognize that this study has important limitations. First, given its long-term retrospective design, changes in patients' clinical management and in anti-HLA antibodies detection techniques occurred resulting in some data biases. Second, anti-HLA antibodies surveillance schedule was not thoroughly carried out. So, temporal relationship between DSA formation and graft failure cannot be accurately determined. Third, no information about compliance with immunosuppression was available for this study, a known risk factor for *de novo* DSA formation (13). Fourth, we considered a MFI≥1000 as positive, although no definitive MFI cut-off exists, with published levels ranging from 500 to 2000. This issue should be considered while interpreting our results. Lastly, DSA complement-fixing ability was not studied in this cohort. It has been shown that detection of complement-binding DSA after transplantation by C1q Luminex assay pertains a significant adverse effect on kidney graft survival (34). However, recently, Schaub et al demonstrated a close relationship between DSA MFI and C1q assay positivity, with a MFI>14,154 being able to predict a positive C1q with a high (>90%) sensitivity and specificity (35). We would like to restate that we found a strong association between very high MFI levels and graft failure in DSA+ patients.

In conclusion, we consider that our results demonstrate a strong association between *de novo* DSA and kidney and pancreas graft survival in SPK transplantation. We also emphasize, as a novel observation, the non-dependence of *de novo* DSA adverse effect on graft survival from AR occurrence. The definition of all kidney graft AR and many pancreas graft AR episodes as BPAR strengthen this finding. Analysis of DSA characteristics might have a role in selecting patients particularly at risk for graft failure, although further studies are necessary. We would recommend anti-HLA antibody screening every 3 to 6 months in SPK transplantation, given the results here presented. Nevertheless, only new and efficacious therapeutic strategies would clearly change the ominous prognosis associated with *de novo* DSA emergence.

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Table 1. Baseline characteristics

	Total N=136	DSA - N=118	DSA + N=18	*P*-value
Recipient				
Age (years), mean±SD	34.9±6.1	35.3±6.0	31.9±6.5	0.052
Female gender, n (%)	72 (52.9)	61 (51.7)	11 (61.1)	0.456
Years of IDDM, mean±SD	23.7±5.9	23.8±6.0	22.7±5.6	0.428
Previous blood transfusions, n (%)	46 (33.8)	41 (34.7)	5 (27.8)	0.561
Previous pregnancies, n (%)	27 (19.9)	24 (20.3)	3 (16.7)	1.0
Months on dialysis, median (IQR)	24.0 (17.0–37.8)	26.5 (17.8–38.0)	17.0 (9.8-36.0)	0.073
Peak panel reactive antibody >5%, n (%)	9 (6.6)	7 (5.9)	2 (11.1)	0.339
Pretransplant anti-HLA sensitization, n (%)				
Class I				<0.001
Undetected	123 (90.4)	111 (94.1)	12 (66.7)	
Third party	13 (9.6)	7 (5.9)	6 (33.3)	
Class II				0.003
Undetected	130 (95.6)	116 (98.3)	14 (77.8)	
Third party	6 (4.4)	2 (1.7)	4 (22.2)	
Donor				
Age (years), mean±SD	28.1±10.8	27.9±10.8	29.3±11.2	0.640
Female gender, n (%)	56 (41.2)	49 (41.5)	7 (38.9)	0.832
Transplant				
AB HLA mismatches, n (%)				0.695
0-2	40 (29.4)	34 (28.8)	6 (33.3)	
3-4	96 (70.6)	84 (71.2)	12 (66.7)	
DR HLA mismatches, n (%)				0.210
0-1	64 (47.1)	58 (49.2)	6 (33.3)	
2	72 (52.9)	60 (50.8)	12 (66.7)	
Cold ischemia time (h), mean±SD	14.5±5.9	14.4±5.8	14.8±6.3	0.801

DSA, donor specific antibody; SD, standard deviation; IDDM, insulin-dependent diabetes mellitus; IQR, interquartile range; HLA, human leukocyte antigen; h, hours.

Table 2. Posttransplant clinical events

	Total N=136[§]	DSA - N=118[§]	DSA + N=18[§]	*P*-value
Follow-up time (years), mean±SD	6.7±3.4	6.6±3.5	6.7±3.1	0.915
Steroids withdrawal at 6 months, n (%)	75 (55.1)	65 (55.1)	10 (55.6)	0.970
Anti-HLA antibodies posttransplant, n (%)	28 (20.6)	10 (8.5)	18 (100)	<0.001
Class I	22 (16.2)	10 (8.5)	12 (66.7)	<0.001
Class II	14 (10.3)	3 (2.5)	11 (61.1)	<0.001
Acute rejection in any graft, n (%)	19 (14.0)	12 (10.2)	7 (38.9)	0.001
Delayed kidney graft function, n (%)	19 (14.0)	14 (11.9)	5 (27.8)	0.070
BPAR in kidney graft, n (%)	16 (11.8)	10 (8.5)	6 (33.3)	0.002
ACR, n (%)	15 (11.0)	10 (8.5)	5 (27.8)	0.015
Banff grade 1, n (%)	8 (5.9)	8 (6.8)	1 (5.6)	1.0
Banff grade 2/3, n (%)	7 (5.1)	2 (1.7)	4 (22.2)	0.003
AMR, n (%)	3 (2.2)	0	3 (16.7)[*]	0.002
Kidney graft failures, n (%)	10 (7.4)	6 (5.1)	4 (22.2)	0.028
Causes:				
Infection, n	2	2	0	0.467[^]
BKV nephropathy, n	1	1	0	1.0[^]
Rejection (acute or chronic), n	5	1	4	0.048[^]
Unknown, n	2	2	0	0.467[^]
AR (suspected/BPAR) in pancreas graft, n (%)	11 (8.7)	5 (4.6)	6 (33.3)	<0.001
BPAR in pancreas graft, n (%)	7 (5.6)	2 (1.9)	5 (27.8)	<0.001
Banff grade 1, n (%)	2 (1.6)	1 (0.9)	1 (5.6)[#]	0.266
Banff grade 2/3, n (%)	5 (4.0)	1 (0.9)	4 (22.2)	0.001
Pancreas graft failures, n (%)	19 (15.1)	13 (12.0)	6 (33.3)	0.019
Causes:				
Infection, n	3	3	0	0.517[¶]
Rejection (acute or chronic), n	7	2	5	0.010[¶]
Vascular, n	2	2	0	0.544[¶]
Unknown, n	4	3	1	1.0[¶]
Pancreatic fistula, n	3	3	0	0.517[¶]

DSA, donor specific antibody; SD, standard deviation; HLA, human leukocyte antigen; BPAR, biopsy-proven acute rejection; ACR, acute cellular rejection; AMR, antibody-mediated rejection.

[§]For analysis of pancreas graft events, 10 patients with early (≤15 days) pancreas graft failure were excluded: Total n=126; DSA- n=108; DSA+ n=18.

[*]One patient presented AMR without ACR.

[^]*P*-value calculated considering only patients with kidney graft failure.

[#]This patient had a C4d positive staining in the pancreas graft biopsy.

[¶]*P*-value calculated considering only patients with pancreas graft failure.

SDC, Table S1. Multivariable logistic regression analysis for predictors of *de novo* DSA

	OR (95% CI)	*P*-value
Recipent gender (female vs male)	1.87 (0.53-6.63)	0.332
Recipient age, per year	0.90 (0.81-0.98)	0.040
Any presensitizing event	0.38 (0.10-1.49)	0.166
Time on dialysis, per month	0.96 (0.93-1.01)	0.087
Pretransplant anti-HLA sensitization (vs no)	11.48 (2.35-56.12)	0.003
AB HLA mismatches (3-4 vs 0-2)	0.72 (0.20-2.56)	0.609
DR HLA mismatches (2 vs 0-1)	3.91 (1.01-15.20)	0.049
Acute rejection in any graft (yes vs no)	5.41 (1.39-21.84)	0.018

CI, confidence interval; HLA, human leukocyte antigen.

SDC, Table S2. Multivariable Cox regression survival analysis for predictors of censored double graft failure

	HR (95% CI)	*P*-value
Recipent gender (female vs male)	0.50 (0.19-1.35)	0.172
Recipient age, per year	0.97 (0.88-1.06)	0.459
Donor age, per year	1.03 (0.99-1.08)	0.181
Time on dialysis, per month	1.01 (0.99-1.03)	0.256
Years of IDDM, per year	0.96 (0.86 -1.06)	0.378
Pretransplant anti-HLA sensitization (vs no)	0.26 (0.05-1.43)	0.257
ABDR HLA mismatches (5-6 vs 0-4)	3.82 (1.03-14.13)	0.312
Acute rejection in any graft (yes vs no)	4.02 (1.63-9.93)	0.003
De novo DSA (yes vs no)	3.30 (1.06-10.25)	0.039

CI, confidence interval; HLA, human leukocyte antigen; IDDM, insulin-dependent diabetes mellitus; DSA, donor specific antibody.

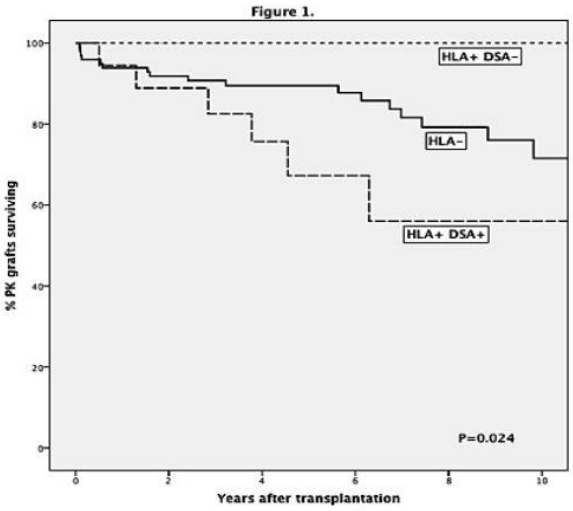
Table 3. Comparison of *de novo* DSA characteristics according to the occurrence graft failure.

	No graft failed N=11	One graft failed[§] N=4	Both grafts failed N=3	*P*-value
By HLA class				
DSA class I, n	4	1	0	0.455
DSA class II, n	6	3	0	0.129
DSA class I+II, n	1	0	3	0.002
By HLA *locus*				
Anti-HLA-A, n	2	1	3	0.026
Anti-HLA-B, n	3	1	3	0.059
Anti-HLA-C, n	1	0	2	0.036
Anti-HLA-DR, n	1	1	3	0.008
Anti-HLA-DQ, n	6	3	2	0.755
Number of DSA, median (min-max)	1 (1-4)	2 (1-5)	6 (4-6)	0.011
Highest MFI DSA bead, median (IQR)	2251 (1164-4241)	5931 (1664-10922)	20712 (16943-22210)	0.030
MFI sum of all DSA beads, median (IQR)	3082 (1164-4439)	7274 (2283-15731)	50823 (50398-82204)	0.021
Months from DSA detection until graft failure, median (IQR)	-	11.4 (6.7-13.5)	6.5 (4.4-8.1)	0.157

HLA, human leukocyte antigen; DSA, donor specific antibody; min, minimum; max, maximum; MFI, mean fluorescence intensity; IQR, interquartile range.

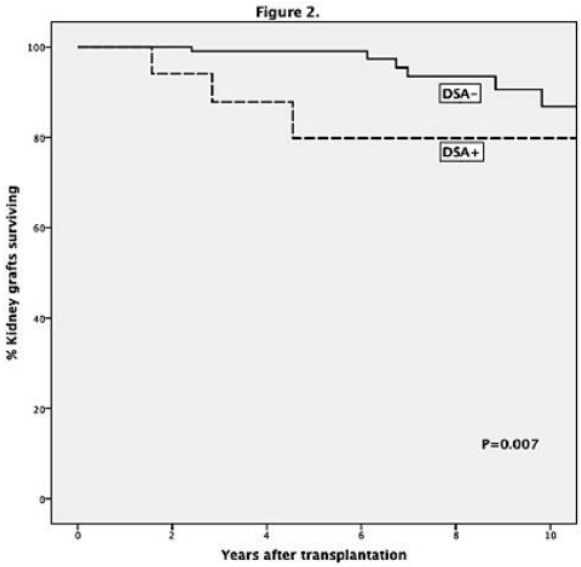
[§] Three patients presented pancreas graft loss and 1 kidney graft loss.

Figure 1. Double graft (kidney and pancreas, KP) death-censored survival in patients without anti-HLA antibodies (HLA-), with non-donor-specific anti-HLA antibodies (HLA+ DSA-) and those with donor-specific anti-HLA antibodies (HLA+ DSA+). HLA+ DSA+ patients presented significantly lower double graft survival in comparison with HLA+ DSA- (*P*=0.039) and HLA- patients (*P*=0.024).



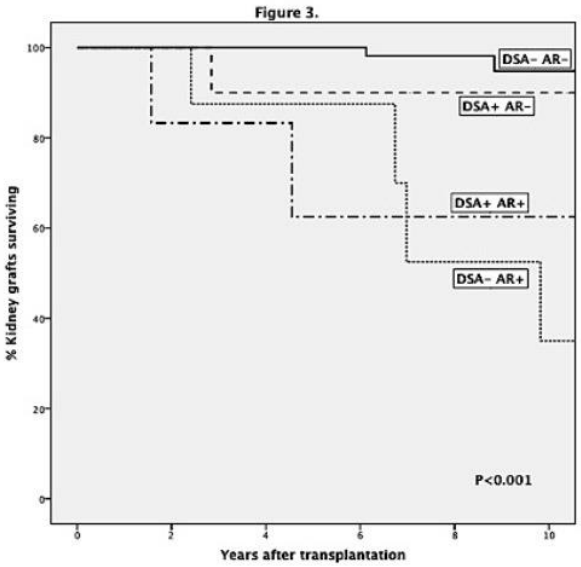
HLA-	Number at risk	98	85	65	46	30	15
	% PK grafts surviving		91.8	89.5	87.7	79.2	71.6
HLA+ DSA-	Number at risk	10	7	4	3	2	2
	% PK grafts surviving		100	100	100	100	100
HLA+ DSA+	Number at risk	18	15	11	7	3	2
	% PK grafts surviving		88.9	75.7	67.3	56.0	56.0

Figure 2. Death-censored kidney (K) graft survival in patients without donor-specific (DSA-) and those with donor-specific anti-HLA antibodies (DSA+).



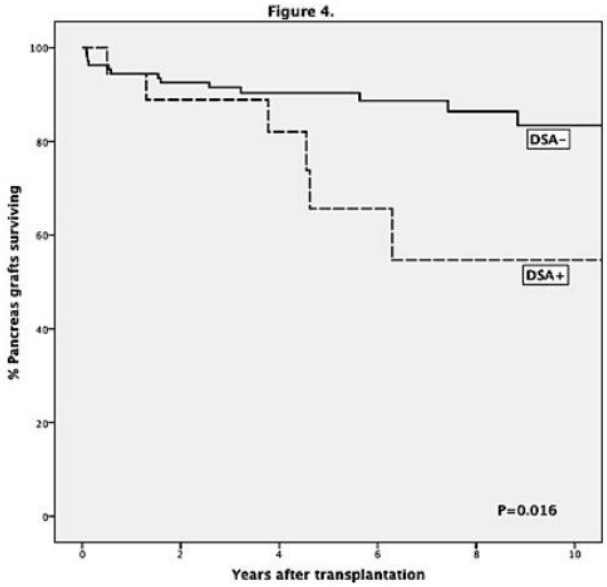
DSA-	Number at risk	118	109	84	61	39	21
	% K grafts surviving		100	99.1	99.1	93.5	86.8
DSA+	Number at risk	18	16	13	8	5	4
	% K grafts surviving		94.1	87.8	79.9	79.9	79.9

Figure 3. Death-censored kidney (K) graft survival according to DSA detection and the acute rejection (AR) occurrence in the kidney graft. Comparing with DSA- AR- patients, all groups showed a significant decrease in graft survival (DSA- AR+, *P*<0.001; DSA+ AR-, *P*=0.007; DSA+ AR+, *P*<0.001).



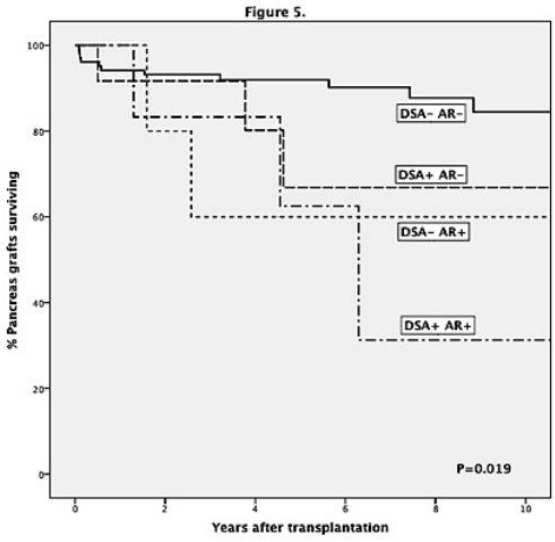
DSA- AR-	Number at risk	108	101	78	56	36	19
	% K grafts surviving		100	100	100	98.2	94.8
DSA- AR+	Number at risk	10	8	6	5	3	2
	% K grafts surviving		100	87.5	87.5	52.5	35.0
DSA+ AR-	Number at risk	12	11	8	6	4	3
	% K grafts surviving		100	90	90	90	90
DSA+ AR+	Number at risk	6	5	5	2	1	1
	% K grafts surviving		83.3	83.3	62.5	62.5	62.5

Figure 4. Death-censored pancreas (P) graft survival in patients without donor-specific (DSA-) and those with donor-specific anti-HLA antibodies (DSA+).



DSA-	Number at risk	108	92	69	49	35	19
	% P grafts surviving		92.6	90.4	88.7	86.4	83.4
DSA+	Number at risk	18	15	12	7	3	2
	% P grafts surviving		88.9	82.1	65.6	54.7	54.7

Figure 5. Death-censored pancreas (P) graft survival according to DSA detection and the acute rejection (AR) occurrence in the pancreas graft. Comparing with DSA- AR- patients, all groups showed a decrease in graft survival, although only significant for DSA+ AR+ patients (*P*=0.005); a similar trend was observed in DSA- AR+ (*P*=0.074) and DSA+ AR- patients (*P*=0.118).



DSA- AR-	Number at risk	103	88	66	47	33	17
	% P grafts surviving		93.2	91.9	90.2	87.7	84.5
DSA- AR+	Number at risk	5	4	3	2	2	2
	% P grafts surviving		80.0	60.0	60.0	60.0	60.0
DSA+ AR-	Number at risk	12	10	7	5	2	1
	% P grafts surviving		91.7	80.2	66.8	66.8	66.8
DSA+ AR+	Number at risk	6	5	4	2	1	1
	% P grafts surviving		83.3	83.3	62.5	31.3	31.3

VII. Evolução dos AGE após TRP

- **La Salete Martins**, José C Oliveira, José Ramón Vizcayno, Isabel Fonseca, Carlos Gouveia, Donzília Silva, António C Henriques, Irene L Noronha, Anabela Rodrigues. **AGE levels evolution after pancreas-kidney transplantation: plasmatic and cutaneous assessments.**

  (*submetido*)

Resumo VII

A acumulação dos AGE está comprovadamente associada à DM, embora não seja uma condição exclusiva desta doença. A uremia e o próprio envelhecimento, entre outros processos, levam à sua acumulação. Os AGE, por sua vez, foram associados às complicações secundárias da DM, quer à doença microvascular que à macrovascular. Nos DM1 submetidos a TRP, recuperando a euglicemia e normalizando a função renal, poderá em teoria esperar-se uma descida dos AGE, mas tal facto não é conhecido.

Fomos estudar prospetivamente a evolução dos AGE durante o 1º ano pós-TR. Medimos seriadamente em 20 doentes, no dia do transplante (dia 0), aos 3 (T3), 6 (T6), e 12 meses (T12) os AGE globais plasmáticos e a CML, e ainda os AOPP. Em 15 desses doentes, obtivemos uma biópsia de pele no dia 0 e repetimos nova biópsia aos 12 meses. Nas biópsias de pele, por imuno-histoquímica utilizando um anticorpo policlonal anti-AGE, fomos observar a variação da marcação dos AGE nesse período.

Os doentes estudados apresentaram normal e estável função dos enxertos renal e pancreático. O valor médio dos AGE foi de 16.83±6.39µg/mL no T0; 17.14±3.76µg/mL no T3; 17.46±5.64µg/mL no T6; e 15.99±5.17µg/mL no T12. A CML variou de 0.94±0.36ng/mL no T0; 1.11±0.48ng/mL no T3; 0.99±0.42ng/mL no T6; para 0.78±0.38ng/mL no T12. Os AOPP apresentaram uma média de 130.09±76.83µMol/L no T0; 137.25±110.60µMol/L no T3; 116.39±51.20µMol/L no T6; e 106.40±57.93µMol/L no T12. A variação registada para a CML foi estatisticamente significativa (*P*=0.022); a variação dos AOPP aproximou-se da significância (*P*=0.076). Nas biópsias de pele, verificou-se - em 11 dos 15 doentes - uma mudança do padrão inicial de marcação dos AGE de citoplasmático difuso para um padrão periférico interqueratinocítico, aos 12 meses,. Em 7 casos, observou-se uma redução da intensidade dessa marcação (de 3+ ou 2+, para 2+ ou 1+, respetivamente). De todos os fatores estudados (idade, tempo de diabetes, nível da HbA1c, hipertensão, dislipidemia) potencialmente influenciadores do processo de glico-oxidação, apenas a correlação entre tempo de diálise e o nível da CML se aproximou do significado estatístico (*P*=0.071).

Com base nestes dados, concluímos que a descida dos marcadores de de glico-oxidação pode observar-se ainda durante o 1º ano, em doentes com DM1 submetidos a TRP que mantenham ambos os enxertos funcionantes. Estudos a mais longo prazo serão necessários para confirmar estes resultados.

AGE levels evolution after pancreas-kidney transplantation: plasmatic and cutaneous assessments

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Informed consent was obtained from all the participants. This work was approved by the appropriate ethics committee. Therefore, it has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments; and in accordance with the Declaration of Istanbul.

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Abstract:

Diabetes mellitus (DM) leads to increased Advanced-Glycation Endproducts (AGE) production, which has been associated with secondary diabetic complications. Type-1 DM patients undergoing pancreas-kidney transplantation (SPKT) can restore normoglycemia and renal function, eventually decreasing AGE accumulation.

Aims: To prospectively study AGE evolution after SPKT.

Methods: Circulating AGE were assessed in 20 patients, at time 0 (T0), 3 (T3), 6 (T6) and 12 (T12) months after successful SPKT. Global AGE and carboxymethyllisine (CML) were analyzed, as well as advanced oxidation protein products (AOPP). Skin biopsies were obtained in 15 patients at T0 and T12. Immunohistochemistry with anti-AGE antibody evaluated skin AGE deposition.

Results: AGE mean values were 16.83±6.39µg/mL on T0; 17.14±3.76µg/mL on T3; 17.46±5.64µg/mL on T6; and 15.99±5.17µg/mL on T12. CML mean values were 0.94±0.36ng/mL on T0; 1.11±0.48ng/mL on T3; 0.99±0.42ng/mL on T6; and 0.78±0.38ng/mL on T12. AOPP mean values were 130.09±76.83µMol/L on T0; 137.25±110.60µMol/L on T3; 116.39±51.20µMol/L on T6; and 106.40±57.93µMol/L on T12. CML variation was statistically significant (*P*=0.022); AOPP variation nearly significant (*P*=0.076). Skin biopsies evolved mostly from a cytoplasmic diffuse immunoreaction pattern to a peripheral interkeratinocytic pattern; in 7 cases, a reduction on AGE immunoreaction intensity was observed one T12. With the exception of euglycemia and renal function recovery, we couldn't find factors (diabetes or dialysis time, glycated hemoglobin, age) significantly associated with the variation of these markers.

Conclusion: Glycoxidation markers decrease, not only at plasmatic but also at tissue level, may start early after SPKT. Studies in larger samples with prolonged follow-up are needed to confirm these data.

Introduction:

Patients with diabetes mellitus (DM) have increased production of AGE, the Advanced Glycation Endproducts([1,2]) . AGE accumulation is only one of the proposed mechanisms for cell and tissue injury in diabetes([3]). There are other possible described mechanisms, such as increased sorbitol formation through the polyol pathway([2,4]), increased protein-kinase C activation([1,2,5]) and the hexosamine pathway([2]). However, AGE have been the most investigated and may play a central role([1]).

Studies did correlate plasmatic and tissue AGE levels to the principle micro and macrovascular complications of DM([1,3]). AGE formation and deposition have been deeply searched in type 2 (DM2) and type 1 diabetes (DM1), with more recent focus directed to therapeutic possibilities. AGE receptors (RAGE) antagonists([6,7,8,9]) and other potential targets, possibly preventing AGE formation([1,2,7,10]) or promoting AGE degradation and removal([3,7,8]) are still under investigation.

DM1 patients submitted to simultaneous pancreas-kidney transplantation (SPKT) can restore normoglycemia and renal function, two concurrent ways to decrease AGE deposition: reducing AGE formation and increasing their renal elimination. Data on AGE levels after successful SPKT are very scarce. One might aspire that AGE stabilization, or even removal, can be achieved once uremia and hyperglycemia are reverted. However, presuming that it is possible, still the dynamic back-process is not known.

With this study we aimed to collect data on AGE evolution after SPKT. For this purpose, we prospectively measured AGE in the plasma and in skin biopsies, in a group of SPKT patients during the first year after the procedure. The overall protein oxidation has also been assessed, through a test measuring advanced oxidation protein products (AOPP) plasmatic levels.

Research design and methods:

Patients

Consecutive patients undergoing SPKT at our center between January 2012 and July 2013, who gave their informed consent, were enrolled in this study. Only patients with well-succeeded pancreas and kidney transplants were considered. Twenty SPKT patients were included for measurement of plasmatic AGE levels; in 15 of these, skin biopsies were obtained to perform the histological and immunohistochemistry analysis of epidermal and dermal AGE deposition.

SPKT was performed with systemic-enteric diversion. Immunosuppression comprised anti-thymocyte globulin, tacrolimus, mycophenolate and steroids. Steroid withdrawal after the sixth month is a general practice in our Unit, if immunological events are not observed.

Sample collection

AGE were prospectively analyzed in skin deposits and in plasma in these SPKT, from time 0 (T0) to 12 months (T12) after the procedure. T0 values (date of transplantation) obtained for each studied marker were considered their basal (reference) levels.

Blood samples were collected in evacuated tubes without additive at T0 and thereafter at 3 months (T3), 6 months (T6) and T12 after the transplant. The first skin biopsy was obtained at T0, during the kidney transplantation surgery; the second one was obtained through a 5mm skin punch at T12, from the left abdominal wall, 2-3 cm below the scar of the surgical incision used to perform the kidney transplantation. The lower abdominal wall is a part of the body with low chronic UV-exposure and the local of the two biopsies was very close to each other.

Samples (blood and tissue) collection was differed at least one week for T3 samples; two weeks for T6 samples; or 2-4 weeks for T 12 samples, whenever there was an infection and/or transient mild graft dysfunction.

Besides AGE evaluation, in the 4 blood samples collected from each patient, we also analyzed fasting blood glucose, glycated hemoglobin (HbA1c), total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c) and C-reactive protein (CRP). Additionally, 24-hour urinary protein excretion was measured on T12; and estimated-glomerular filtration rate (e-GFR) was calculated based on MDRD equation. Blood pressure was recorded in each visit. Hypertension (>130/85mmHg), hypertriglyceridemia (>150mg/dl), hypercholesterolemia (>200mg/dl), high LDL-c (>130mg/dl) and low HDL-c (<40 in men, <50mg/dl in women) were defined according to the National Cholesterol Educational Program (NCEP/ATPIII) criteria for metabolic syndrome.

Skin samples from healthy subjects have been provided by the Pathology Department of Santo Antonio Hospital, Porto, from its archive. These were obtained from margins of biopsies made to analyze skin lesions, which were of benign origin (nevus). Healthy skin from 6 non-diabetic subjects aging between 30 and 45 years-old were then used as control samples, to assess AGE deposition in the absence of diabetes and within this age range.

Biochemical studies

Blood samples were centrifuged without delay and the serum aliquoted and stored frozen at -80ºC, until analysis.

Global plasmatic Advanced Glycation End Products (AGE) were evaluated using a competitive ELISA-Kit, OxiSelect™ AGE (STA-817, Cell Biolabs, Inc, San Diego, CA). N^{ϵ} –(Carboxymethyl) lysine (CML), a specific AGE, was evaluated using a competitive ELISA-Kit, OxiSelect™ CML (STA-816, Cell Biolabs, Inc, San Diego, CA). Oxidative state was evaluated with a colorimetric kit for the detection of Advanced Oxidation Protein Products, OxiSelect™ AOPP (STA-318, Cell Biolabs, Inc, San Diego, CA). Biochemical analyses were performed according manufacturer indications for each assay.

Skin Histological / Immunohistochemistry studies

After excision, skin samples were immediately fixed in 10% neutral buffered formalin for 24h and embedded in paraffin wax. Serial 3µm cuts were obtained from each block. These sections

were stained with hematoxylin-eosin and others used to analyze AGE deposition, through immunohistochemistry assay. After deparaffinization and rehydration, these sections were incubated with the primary polyclonal IgG antibody anti-AGE (ab23722, Abcam, Cambridge, UK) diluted 1:5000, for 20 minutes at room temperature – according to the manufacturer's instructions. Immunohistochemistry protocol from Ventana Benchmark Ultra (Ventana Medical Systems, Inc – Roche) has been followed, using detection system Optiview DAB, IHC Detection Kit. Sections were counterstained with Haematoxylin, then dehydrated and coverslipped under DPX mountant.

A semiquantitative AGE assessment was made based on its immunoreaction intensity, and graded on a scale from 0 (absent), 1+ (weakly positive), 2+ (moderately positive) to 3+ (strongly positive).

Statistical analysis

Variables distribution was studied by Kolmogorov-Smirnov test. Results are presented as mean ± standard deviation for continuous, normally distributed variables, or as medians and 95% confidence interval for non-normal distribution variables (e.g. AOPP). Percentages were used for categorical data. A repeated-measures ANOVA was used to compare AOPP, AGE and CML between time-points. Multiple comparisons were adjusted using Bonferroni's test. The effect of potential confounding variables, such as age, gender, previous time on dialysis, diabetes time, dyslipidemia, HbA1c and creatinine clearance was then analyzed on longitudinal changes of the three markers also using repeated measures ANOVA.

Statistical analysis was performed using SPSS software version 22.0 (SPSS, Chicago, IL, USA) and $P<0.05$ was considered statistical significant.

Results:

Demographic and clinical patients' characteristics

Baseline and post-SPKT patients' characteristics are presented in table 1. Their age at transplantation date ranged from 28 to 47 (mean 36.7) years; their time on dialysis from 2 to 40 (mean 18) months; and their diabetes evolution time from 17 to 33 (mean 26) years. Excessive weight was not observed in this sample of DM1 patients; 20% were active smokers before SPKT; very poor glycemic control was evident in 35%, who presented HbA1c ≥9%. All of these patients have kept both grafts functioning during the study follow-up.

After SPKT, graft function remained stable. The rate of actively smoking patients has decreased. Hyperlipidemia and hypertension prevalence was low: the percentage of patients taking anti-hypertensive medication or statins was 15% and 5%, respectively. Mean BMI pre and post-SPKT was similar, although we have noted weight gain in 9 and weight loss in other 8 patients. Two patients had a BMI >25kg/m^2 at T12. Table 2 shows graft function and lipid profile evolution after SPKT.

AGE, CML and AOPP plasmatic levels after SPKT

AGE, CML and AOPP results during the first year, at different 4-time points, are represented in figure 1-A, 1-B and 1-C, respectively.

An increase in the mean values of AGE, CML and AOPP was registered from T0 to T3. AGE levels have also increased from T3 to T6, a fact not observed for CML and AOPP, for which the decrease started after the 3rd month. From T6 to T12, all the 3 markers have decreased, reaching levels below those registered pretransplantation. AGE mean values were 16.83±6.39µg/mL at T0; 17.14±3.76µg/mL at T3; 17.46±5.64µg/mL at T6; and 15.99±5.17µg/mL at T12 measurements. These variations did not reach statistical significance. CML mean values were 0.94±0.36ng/mL at T0; 1.11±0.48ng/mL at T3; 0.99±0.42ng/mL at T6; and 0.78±0.38ng/mL at T12 measurements. The observed variation from T0 to T12 was statistically significant (*P*=0.022). AOPP mean values were 130.09±76.83µMol/L at T0; 137.25±110.60µMol/L at T3; 116.39±51.20µMol/L at T6; and 106.40±57.93µMol/L at T12 measurements. AOPP variation was almost statistically significant (*P*=0.076).

Diabetes duration and age at transplantation date did not significantly correlate with T0 and T12 AGE, CML or AOPP levels. Time on dialysis was the single factor with nearly significant positive correlation with CML values (*P*=0.071). In this group, poor glycemic control (fasting glucose and HbA1c) before transplantation also did not influence the values of these 3 markers, pre or post-SPKT. The same was found for pretransplant hypertension. Additionally, we couldn´t find any association between T12 values of HbA1c and T12 values of the 3 markers. The number of patients with active smoking (n=1), not taking aspirin (n=1), taking ACEI (n=3) or with any marker of dyslipidemia (n=4) on T12 was too small to study their correlation with AGE, CML or AOPP at last evaluation (T12).

AGE skin deposits from time 0 to 12 months post-SPKT

On skin histological examination we verified that the AGE immunostaining was invariably negative in some specific cells/areas: the outer epidermal layer (stratum corneum), the erector pili muscle and the eccrine sweat glands. In other cells/areas, immunoreaction for AGE was invariably positively, such as: fat cells, vascular endothelial cells, dermal collagen fibers (on superficial dermis 2+/3+, on deeper dermis 3+), and perivascular collagen. The other layers of the epidermis (granular, spinous and basal) and the hair follicle presented several distinct AGE immunostain patterns and intensity. Hair follicle layers – whenever hair follicle was present in the section - normally follows the same pattern and the intensity of the epidermal layers immunoreaction. On hematoxylin-eosin staining, no relevant changes were found. In young healthy controls, AGE immunostaining was negative.

Table 3 explains the specific sites with positive immunoreaction for AGE and the respective intensity. The most common finding, observed in 11 among the 15 cases, was a change from a cytoplasmic diffuse immunoreaction pattern on T0, to an interkeratinocytic pattern on T12,

saving the central cytoplasmic area and only peripherally staining the cells, with an aspect usually described as “chicken wire” pattern. At least in 7 cases, we have also observed a decrease on the intensity of AGE immunoreaction one year after SPKT (from 3+ to 1+, or from 2+ to 1+). To illustrate these changes we present 4 cases in Figure 2, which exemplify the modifications observed from pretransplant to one year later.

Discussion:

AGE are a group of heterogeneous compounds that represent the ultimate product of multiple reactions occurring in several conditions, namely in the hyperglycemic state of DM. Non-enzymatic glycation begins with interaction and link between the carbonyl group of a reducing sugar and an aminoterminal group of a protein([1,3]). Complex rearrangements result in early AGE forms, called Amadori products (HbA1c is one of such); progressively they result in more stable AGE precursors (like methylglyoxal)([11]); and lately in irreversible long-lasting glycoxidation of the proteins, such as carboxymethyllysine (CML), carboxyethyllysine (CEL) and pentosidine (an AGE with fluorescent properties), among others([1,3,11]). This process may affect not only proteins (plasma and tissue proteins, such as collagen), but also lipids and nucleic acids([1,11]), then being a measure of overall metabolic and oxidative stress([1,3]). AGE formation, lipoxidation and reactive oxygen species (ROS) generation can activate inflammation with consequent tissue damage([3]).

Several studies did confirm the association between AGE accumulation and diabetic microvascular complications([1,2,3,7,11]), namely retinopathy([12,13]), neuropathy([10,14]), nephropathy([15,16]); and also macrovascular disease, such as cardiovascular (CV)([7,17,18]) and peripheral artery disease([7,19]). Additionally, it seems that AGE can be directly toxic to pancreatic beta-cells([9,11]). Exogenous sources of AGE, from diet or smoking, are other contributors to their imbalance and accumulation([1,3,11]). AGE formation is not an exclusive mechanism of diabetes. Many other diseases may induce AGE overexpression, such as renal diseases evolving to renal failure([1,6]), neoplasms([6]), Alzheimer’s disease([1,6,10]), arthritis([1,6]) and CV disease itself([1,9]). Furthermore, even unspecific inflammation and aging promote AGE production([1,10]). Since AGE depend on renal function for their excretion, chronic renal insufficiency also leads to AGE accumulation([1]).

RAGE are activated by increased AGE exposure, they respond with overexpression and contribute to ROS formation and inflammation([1,6,11]). There are several AGE receptors, some of them with protective antioxidant effects, working to control excessive oxidative stress, whereas others – like RAGE – have prooxidant properties([11]). The search for efficient RAGE blockers is still ongoing.

SPKT treats two diseases, DM1 and renal failure, and is performed in young patients (most under 50 years of age). Therefore, this is certainly an interesting group of patients to study AGE evolution. Results from AGE levels are very difficult to interpret and there are not

standardized methods of detection. Moreover, it remains unclear which AGE should be measured, and where to obtain more reliable results – whether in plasma or in tissues([7,20]). Plasma levels reflect AGE linked to proteins with higher turnover rate (circulating proteins); tissue levels probably reflect better those AGE linked to low turnover proteins such as collagen and, consequently, the tissue damage([20]). For this reason, stabilization or improvement of diabetic secondary complications, thought to be associated to AGE formation and deposition, may eventually occur lately after SPKT([20]). This is the reversal face of the "metabolic memory" phenomenon observed in diabetic patients, a concept that came from the Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) research: several studies demonstrated a slower progression of diabetic complications in the group of patients who have received intensive insulin treatment, a persistent benefit more than 10 years after the end of the treatment([21,22,23]). Established tissue lesions certainly are not easily and rapidly reverted, even under maintained normal glycemia and renal function, after successful SPKT([20]).

The few studies in transplanted patients([24,25]), one of them comparing a small number of SPKT to kidney alone transplants, have not been able to demonstrate additional benefits with the pancreas graft and euglycemia, besides the correction of renal failure with a kidney transplant. The authors could find a decrease in pentosidine plasma levels either in kidney and pancreas-kidney transplants, but not in tissue pentosidine levels([25]).

AGE measurement can in fact be made in plasma([26,27,28,29,30]), in urine([26]) or in tissues, skin being the most often used tissue([31,32]). Among several compounds already studied, CML is the best characterized AGE([29]), and the most consistently assessed one in plasma analysis([26,27,28,29,30]). Higher plasmatic CML levels correlated with higher thickening rate of the glomerular basement membrane([26]), increased arterial stiffness([27]), increased coronary artery calcification([28]) and with higher incidence of fatal and non-fatal CV events([30]) in diabetic patients; they even correlate with CV events in elderly non-diabetics subjects([33]). In studies performed in chronic kidney disease patients, AOPP plasmatic levels have also been associated with atherosclerotic events in the predialysis stage([34]). Additionally, it was demonstrated that these levels increase after dialysis([35]) and AOPP have been proposed as a reliable marker of oxidant-mediated protein damage. AGE accumulation may be directly assessed in tissues, by immunohistochemistry methods([31,32,36]); or extracted through acid hydrolysis and enzymatic digestion, and then measured by biochemical assays([37]). High cutaneous AGE expression has been correlated with skin damage due to sun exposure([31]) in non-diabetic patients. In diabetic patients, it has been correlated with dermal inflammation and denervation([32]) and with faster progression of microvascular([37]) and macrovascular([36]) disease.

These were the main reasons why, in our study, we decided to use the assays explained above. One assay was chosen to assess global plasma AGE levels; another one to specifically assess

CML levels; and AOPP assay to evaluate protein oxidation. With these 3 markers we can evaluate the overall oxidative status in these patients. Skin deposits were determined by immunohistochemistry, a manner to evaluate profound tissue lesion and its progression after SPKT.

SPKT patients in our center are strongly encouraged to abolish smoking and to avoid non-healthy food, external sources of AGE. Smoking habits in these transplanted patients were very rare: only 1 out of 20 remained as an active smoker. Given that, the possible interference of smoking in AGE results in our study is very unlikely. The same assumption can be made regarding inflammation: CRP was almost always steadily low after the first months – data shown only for time 12.

Our study group presented stable pancreas and kidney graft function. CV risk factors, such as hypertension and hyperlipidemia were generally well-controlled. Hypertension frequency decreased after SPKT, from 65% to 30%. Only 10% presented hypertriglyceridemia and 5% hypercholesterolemia after SPKT. The rate of patients needing statins and ACEI was low. Statins([20]) and ACEI have been proposed as potential preventers of AGE formation and accumulation([1,7,8]), as well as aspirin([1,10]). Per protocol, all of our SPKT are under aspirin after discharge. In this study group, all the patients but one were under aspirin. This homogeneity doesn't allow us to confirm or to exclude the contribution of these drugs (ACEI, statins, aspirin) for the results. Still, based on all these facts, we have assumed that the changes observed in our study, regarding AGE, CML and AOPP levels, may be attributed mainly to normoglycemia restoration and to renal function normalization.

What we observed was a transient increase in AGE, CML and AOPP after SPKT, instead of an immediate decrease. However, during this initial period after SPKT there are several well-known inflammatory/infectious insults, or even high-doses of new drugs, such as immunosuppressors, that may explain the initial increment of these markers. Major surgery, indwelled catheters, episodes wound, urinary, abdominal or systemic infections, among other possible complications, they all may contribute for an initial inflammatory state in SPKT patients. Inflammation usually leads to an increase in the oxidative processes. The decrease of the oxidative markers after the 3rd or after the 6th month, although statistically significant only for CML, has been an interesting finding. Once both the rapidity or the reversibility of glycoxidation and protein oxidation processes are not known in the short term post-SPKT, we cannot say these were expected results; yet these were not totally surprising results. The limited sample size may also explain the lack of significance of markers' variation.

The same interpretation can be made for skin results. Changes observed from T0 to T12 are in accordance with a reduction in cutaneous AGE deposits. In the majority of patients we have observed a modification from an initial diffuse cytoplasmic immunoreaction to an immunoreaction only at the periphery of the cells one year later. Besides this change in

pattern, intensity has also decreased, clearly in 7 out of 15. There are inherent limitations of this semiquantitative assessment from 0 to 3+, however, this is currently the most often used method to subjectively quantify the immunoreaction intensity in immunohistochemistry.

Certainly, it will be of interest to extend the follow-up of these patients and AGE measurements, in order to analyze their progressive evolution in the mid and long-term after SPKT, in those maintaining normoglycemia and good renal function. Another future point of interest will be to find if obtained biochemical and immunohistochemistry data will lately correlate with the intensity of microvascular disease manifestations.

Skin autofluorescence (SAF) measure is a promising non-invasive method to evaluate AGE deposition which correlated with AGE levels determined by biochemical analysis of skin biopsies([38]). In uremic patients under dialysis, data obtained on AGE levels through the AGE-Reader were associated with CV mortality([39]). Even in early stages of chronic renal disease, several studies could find a correlation between SAF and CV disease([20]) . Additionally, in diabetic patients, data from SAF could be associated with vascular damage([40]). SAF reading needs, however, some adjustments that can affect the accuracy of the method. There are no standardized units; it has to be corrected to ethnic, gender, age and skin phototype characteristics; and it should be measured in the same part of the body in consecutive measurements, to avoid biases from different UV-exposure zones. Even so, when widely available and taking into account the necessary adjustments, this may be a practical method to measure AGE accumulation, also in SPKT patients.

We have not been able to find any factors clearly associated with the variation of AGE, CML and AOPP levels in our group of patients.

Based on our results, we can conclude that skin and plasmatic glycoxidation markers, in DM1 patients, may in fact start to decrease during the first year after SPKT. Further studies in a larger sample and with extended follow-up are needed to confirm these results.

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Table 1 – Patients' demographic and clinical characteristics

	Total group (n=20)
Before SPKT:	
Age	36.7±5.4
Female gender	11 (55%)
Time of Diabetes (years)	26.0±5.3
Time on dialysis (months)	18±11
HbA1c (%)	8.29±1.61
HbA1c ≥9%	7 (35%)
Fasting glucose (mg/dl)	304±129
Active smoking (n/%)	4 (20%)
Body mass index (BMI) (kg/m^2)	22.4±2.6
BMI >25kg/m2 (n/%)	0 (0%)
Hypertension (>130/85mmHg) (n/%)	13 (65%)
After SPKT (12 months)	
HbA1c (%)	5.34±0.31
HbA1c ≥6%	0 (0%)
Fasting glucose (mg/dl)	83±9
e-GFR* ($ml/min/1.73m^2$)	77.5±15.5
Urinary protein excretion (g/24h)	0.071±0.093
Active smoking (n/%)	1 (5%)
BMI (kg/m^2)	22.4±2.5
BMI >25kg/m^2 (n/%)	2 (10%)
Taking aspirin (n/%)	19 (95%)
Hypercholesterolemia (>200mg/dl) (n/%)	1 (5%)
Hypertriglyceridemia (>150mg/dl) (n/%)	2 (10%)
Low HDL-c**(n/%)	2 (10%
High LDL-c (n/%)	0 (0%)
Taking statins (n/%)	1 (5%)
Hypertension (n/%)	6 (30%)
Taking ACEI (n/%)	3 (15%)
C-Reactive protein (CRP) (mg/l)	1.58±1.05
CRP >5 (mg/l)	0 (0%)

*e-GFR: estimated glomerular filtration rate (MDRD calculation)

** low HDL-cholesterol defined as <40mg/dl in men and <50mg/dl in women

ACEI: angiotensin converting enzyme-inhibitors

Table 2 – Patients' graft function and lipid profile evolution during the first year

Pts	Creat 3m	Creat 6m	Creat 12m	GFR 3m	GFR 6m	GFR 12m	Gluc T0	Gluc T3m	Gluc T6m	Gluc T12m	HbA1c T0	HbA1c 3m	HbA1c 6m	HbA1c 12m	Chol T_0	Chol T_3m	Chol T_6m	Chol T_12m	TG T0	TG 3m	TG 6m	TG 12m	LDL-c 12m	HDL-c 12m	CRP 12m
n=1	0,95	1	1,17	71	67	64	469	78	72	75	10	5,5	5,3	5,1	136	212	158	170	229	84	130	91	95	55	1.4
n=2	1,33	1,24	1,18	65	69	72	268	78	89	81	6,8	5,6	5,4	5,4	161	141	156	176	100	91	81	67	86	75	1.6
n=3	0,92	0,85	0,96	103	107	104	246	76	82	79	7,4	5,4	5,2	4,9	186	110	112	147	203	78	82	59	84	61	1.2
n=4	1,4	1,35	1,45	59	61	58	169	87	85	85	6,5	5,3	5,2	5,1	97	126	151	122	77	108	83	98	58	57	2.6
n=5	1,05	1,02	0,93	88	91	95	315	91	90	88	8,5	5,4	5,3	5,1	80	101	113	97	96	39	27	20	16	67	3.3
n=6	1,09	1,04	1,02	64	67	68	345	72	84	79	11	5,3	5,6	5,3	230	226	212	207	155	99	76	84	115	58	2.1
n=7	0,75	0,81	0,85	88	85	83	217	69	71	64	9,8	6,1	5,5	5,5	168	147	139	155	151	127	88	59	70	70	1
n=8	1,27	1,05	1,3	58	66	57	482	88	77	90	7,3	5,8	6	5,9	190	188	177	158	140	78	98	76	79	64	2.5
n=9	1,13	0,95	0,93	59	71	74	110	86	82	81	6,7	5,4	5,2	5,2	192	213	271	192	263	255	245	158	120	40	1.2
n=10	0,84	1,01	0,9	89	76	87	162	79	81	73	10	4,9	5,4	5,3	139	183	202	172	191	229	146	96	86	76	1.3
n=11	0,91	1,01	0,95	81	72	77	189	77	71	88	8,2	5,6	5,1	5,2	171	176	183	181	141	106	89	65	111	65	0.7
n=12	0,75	0,8	0,65	91	88	110	277	90	86	84	6,1	6	5,7	5,4	146	215	193	188	104	118	97	72	99	67	1.5
n=13	1,9	1,8	1,36	36	39	51	523	100	104	96	7,2	6,3	6,2	5,9	190	188	185	189	322	265	131	136	94	57	0.9
n=14	1,37	1,39	1,29	66	65	70	116	83	80	69	8	5,4	5,2	5	195	186	179	160	106	107	99	69	71	79	0.7
n=15	1,27	1,24	1,2	70	73	76	487	91	93	92	9,3	6,2	5,9	5,7	171	117	122	148	100	96	92	87	76	55	0.7
n=16	1,38	1,3	1,3	64	69	69	405	98	86	87	6,7	6,4	6,3	5,8	152	149	144	137	113	106	95	91	59	60	1.1
n=17	0,85	0,88	1,09	87	85	82	279	84	75	101	9,7	6,1	5,5	5,6	181	129	137	139	183	56	62	66	77	52	0.7
n=18	0,86	1,09	0,99	110	95	97	405	79	100	91	11	6,4	6,2	5,1	138	188	221	161	79	136	145	149	100	24	1.5
n=19	0,9	0,89	0,95	79	79	76	212	63	72	75	6,6	5,2	5,3	5	151	225	175	193	107	111	115	74	123	55	0.7
n=20	1,13	1,25	1,16	84	77	79	398	100	86	88	8,6	5,7	5,4	5,3	134	202	196	188	134	172	168	156	113	52	4.8
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	1,1	1,1	1,08	76	75	77,5	304	83	83,3	83	8,3	5,7	5,55	5,3	160	171	171	164	150	123	107	89	87	59	1.6
	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD
	0,29	0,24	0,2	18	14	15,5	129	10	9,15	9,2	1,6	0,44	0,38	0,3	35	40	39,5	27	65	62	46	36	25	13	1.0

Creat: Serum creatinine(mg/dl); GFR: e-GFR (ml/min - MDRD calculation); Gluc: Fasting Glucose (mg/dl); HbA1c: glycated hemoglobin (%); Chol T: total cholesterol (mg/dl); TG: triglycerides (mg/dl); LDL-c: Low-density lipoprotein-cholesterol (mg/dl); HDL-c: High-density lipoprotein-cholesterol (mg/dl); CRP: C-Reactive Protein (mg/l); Pts: patients, SD: standard deviation; T0: time 0; 3m: time 3 months; 6m: time 6 months; 12m: time 12 months.

Table 3 – Skin biopsies: AGE immunoreaction pattern and intensity before and after SPKT

Case number	**SPKT**	**Epidermis**	**Epidermis**	**Epidermis**
		AGE immunoreaction	**Immunoreaction pattern**	**Intensity**
(patients)		(layers with immunostain)	(Peripheral; Diffuse; Mixt- both coexist)	(From 0 to 3+)
1	before	basal, spinous, granular	Diffuse cytoplasmic	2+ (basal layer 1+)
	after	basal, spinous, granular	Peripheral/interkeratinocytic	2+
2	before	basal, spinous	Peripheral/interkeratinocytic	1+
	after	Basal	Peripheral/interkeratinocytic	1+
3	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Diffuse cytoplasmic	1+
4	before	basal, spinous, granular	Diffuse cytoplasmic	3+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	2+
5	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	1+/2+ (only spinous layer 2+)
6	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Mixt	1+/2+
7	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Mixt	1+/2+
8	before	basal, spinous, granular	Diffuse cytoplasmic	3+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	1+
9	before	basal, spinous, granular	Mixt	1+
	after	Basal	Peripheral/interkeratinocytic	0/1+ (only basal layer 1+)
10	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	Basal	Peripheral/interkeratinocytic	1+
11	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	2+
12	before	None		0
	after	None		0
13	before	basal, spinous, granular	Diffuse cytoplasmic	2+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	1+
14	before	basal, spinous, granular	Diffuse cytoplasmic	1+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	1+
15	before	basal, spinous, granular	Diffuse cytoplasmic	1+
	after	basal, spinous, granular	Peripheral/interkeratinocytic	1+

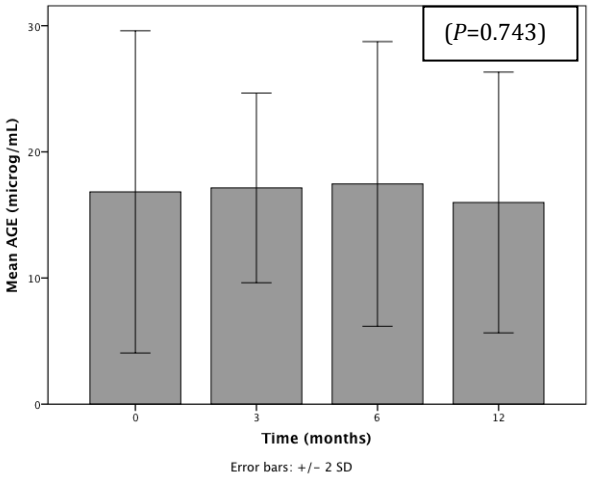

Figure 1-A – AGE variation from time 0 to time 12

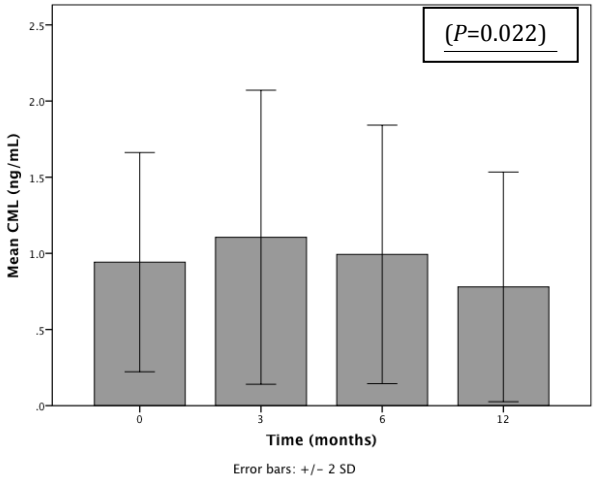

Figure 1-B – CML variation from time 0 to time 12

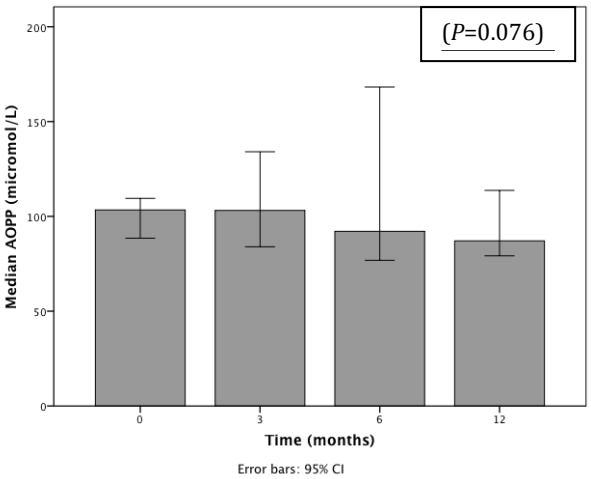

Figure 1-C –AOPP variation from time 0 to time 12

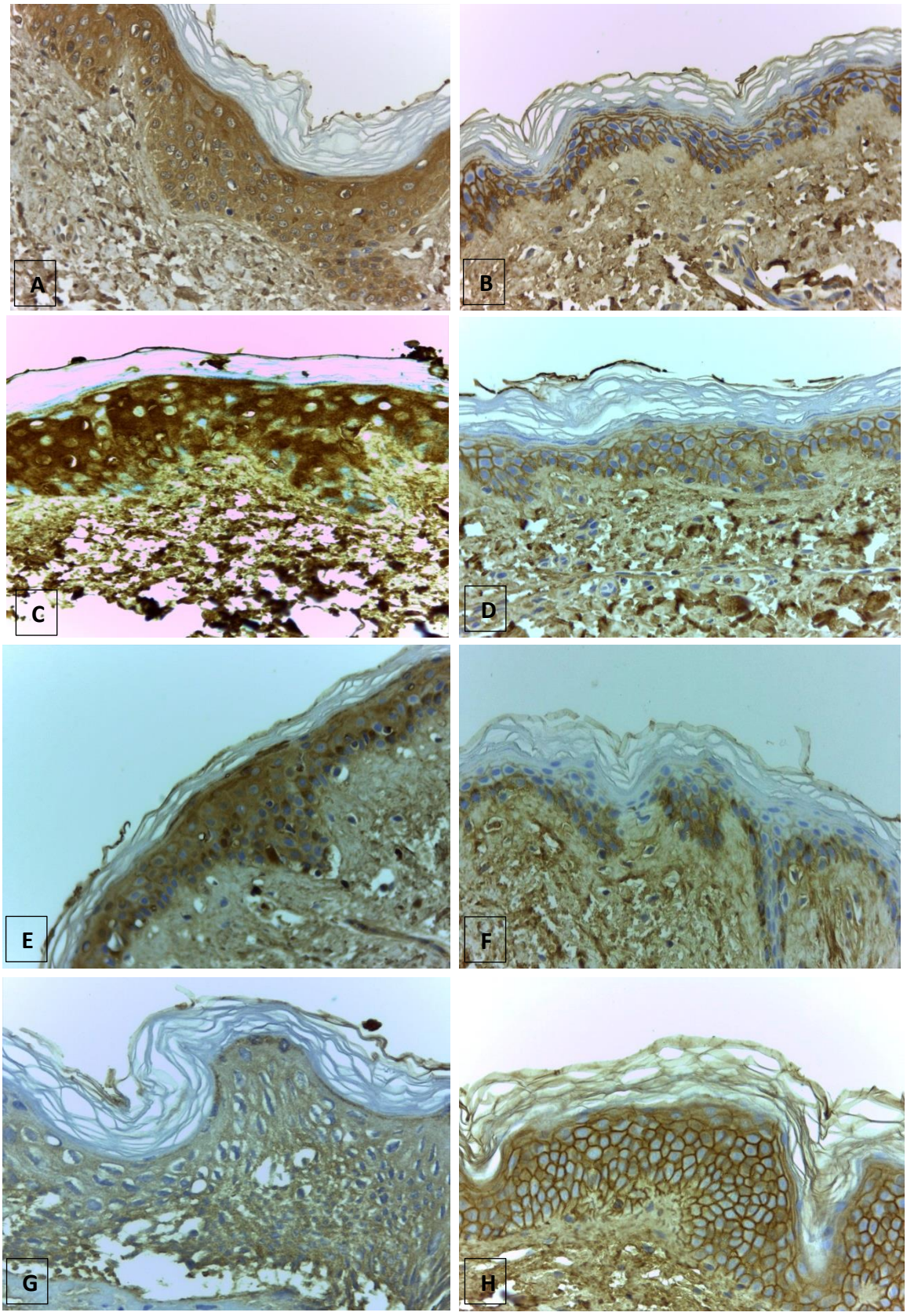

Figure 2 – **Epidermal Immunostaining for AGE**: patient 4 before (**A**) and after SKPT (**B**); patient 8 before (**C**) and after SKPT (**D**); patient 10 before (**E**) and after SKPT (**F**); patient 11 before (**G**) and after SKPT (**H**). (400x amplified, hematoxylin counterstained). Images showing the main immunostaining changes, from a diffuse cytoplasmic to an interkeratinocytic or peripheral pattern, often less intense, at time 12.

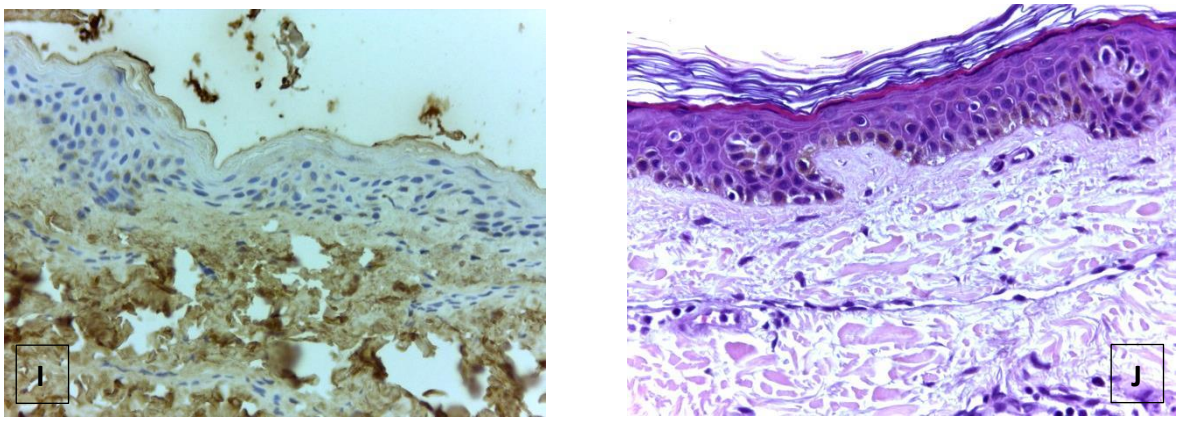

Figure 2S - (supplementary data) - Image **I** represents a negative control, from a young healthy individual. Irrelevant changes were found on hematoxylin-eosin staining in our patient population (exemplified on image **J**).

VIII. Impacto do TRP na qualidade de vida

- **Martins LS**, Outerelo C, Malheiro J, Fonseca I, Henriques AC, Dias L, Rodrigues A, Cabrita A, Davide J, Noronha I L. **Health-related quality of life may improve after transplantation in pancreas-kidney recipients.**

  (*submetido*)

Resumo VIII

A qualidade de vida (QoL) relacionada com a saúde é muitas vezes negligenciada e não avaliada. Alguns autores defendem que deveria fazer parte do processo de acreditação de todas as Unidades de Transplantação. O TRP, resolvendo 2 doenças crónicas e graves como a diabetes e a doença renal crónica, poderá melhorar a QoL dos doentes com DM1, ultrapassada a fase inicial pós-operatória de maior morbilidade e mortalidade.

Em 126 doentes que deram o seu consentimento, avaliámos a sua QoL através de 2 questionários: o *Euro-QoL 5-Dimensions* (EQ-5D) e o *Gastrointestinal Quality of Life Index* (GIQLI). Foi solicitado a cada doente que pontuasse a perceção do seu estado de saúde e de QoL à data do TRP e no final do estudo. Estes questionários foram aplicados de forma prospetiva num subgrupo e retrospetivamente nos restantes. Todos tinham recebido o TRP há mais de 6 meses e a média do tempo de seguimento destes doentes foi de 5 anos. Em 84,1% dos doentes ambos os enxertos estavam funcionantes; apenas 1 dos enxertos em 15,9%.

Em todos os 5 domínios do EQ-5D (mobilidade; autocuidado; atividades normais; dor/desconforto; ansiedade/depressão) se verificou uma melhoria significativa após o TRP. A escala (*VAS-scale*) de avaliação do seu estado global de saúde, de 0% (pior estado de saúde possível) a 100% (melhor possível) melhorou de 40 para 79%. Relativamente ao GIQLI, das 36 questões que abrangiam 5 domínios major (geral; social; físico; psicológico; e gastrointestinal), em todas se verificou melhoria após o TRP, à exceção de 1 única questão sobre os ruídos intestinais. A questão específica sobre o incómodo com o tratamento foi a que mais melhorou com o transplante.

O subgrupo prospetivo foi avaliado também de modo retrospetivo, tendo-lhes sido pedido no segundo inquérito que reclassificassem o seu estado pré-TRP e o comparassem com o atual. Verificámos concordância entre os dados obtidos prospetivamente e retrospetivamente.

O TRP permitiu que a taxa de doentes incapazes de trabalhar ou estudar descesse de 50,8% para 36,5%.

Das diversas variáveis estudadas no modelo de análise multivariada, somente a permanência de apenas 1 enxerto funcionante (em vez dos 2) foi preditor de piores resultados nos inquéritos de QoL.

Assim, o nosso estudo comprovou que o TRP pode aportar uma melhoria significativa na QoL e até na retoma de uma vida laboral ativa, nestes doentes.

Original article

HEALTH-RELATED QUALITY OF LIFE MAY IMPROVE AFTER TRANSPLANTATION IN PANCREAS-KIDNEY RECIPIENTS

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Key words: EQ-5D; GIQLI; pancreas transplantation; outcomes; quality of life. Abbreviations: PKT (pancreas-kidney transplantation); DM1 (type 1 diabetes); QoL (Quality of Life); HRQOL (health-related quality of life); GIQLI (Gastrointestinal Quality of Life Index questionnaire); EQ-5D-5L (EuroQol-5Dimensions-5 Levels questionnaire); VAS-scale (Visual Analogue Scale); GI (gastrointestinal); CV (cardiovascular); KTA (Kidney transplantation alone); PAK (Pancreas after kidney)

Running Title: Quality of Life after a Pancreas-Kidney Transplant

Author: Martins LS.

Title: **HEALTH-RELATED QUALITY OF LIFE MAY IMPROVE AFTER TRANSPLANTATION IN PANCREAS-KIDNEY RECIPIENTS**

Abstract:

Pancreas-kidney transplantation (PKT) may significantly improve quality of life (HRQOL) in type1 diabetic patients. We have assessed the changes felt by PKT patients, using the GIQLI and EuroQol-5D questionnaires. Patients were asked to compare how their HRQOL had changed from pretransplantation to the last visit. The 60 men and 66 women enrolled had a mean follow-up of 5 years; 84.1% with both grafts, 15.9% with one graft functioning. In all domains of EuroQol-5D scores improved after PKT, as well as the VAS-scale health state (from 38% to 84%, P<0.001; effect size 3.34). In GIQLI, physical function was felt better after PKT than before (14.83±3.86 vs 7.86±4.43, P<0,001; effect size 1.68); the same was observed for psychological status; social function; and GI complaints. Concerning the burden of medical treatment, the score significantly improved (from 1.31 to 3.63, P <0.001, effect size 2.02). The rate of unemployed patients decreased after PKT (from 50.8% to 36.5%, P<0.001). Multivariate analysis showed that having only 1 functioning graft was associated with worse HRQOL scores (B=-5.157, p=0.015). In conclusion, for all assessed domains, patients reported a significant improvement in HRQOL after PKT. Maintenance of the two grafts functioning predicted higher improvement of HRQOL scores.

Introduction:

Pancreas-kidney transplantation (PKT) is considered the best treatment for selected type 1 diabetic (DM1) patients with end-stage renal disease. PKT offers the potential benefit of independence from multi-daily glucose monitoring and insulin injections; avoidance of hypoglycemic episodes; freedom from dialysis; and also reduced risk of long-term diabetic complications([1,2,3]). The drawbacks are the morbidity and mortality associated with the procedure; the lifelong immunosuppression and its side-effects; the regular blood tests and medical appointments; and the need to be constantly vigilant for signs and symptoms of rejection and infection([4]). Despite these handicaps, with the evolution of surgical technique of pancreas transplantation and with the improved immunosuppressive protocols, PKT nowadays offers considerable survival benefits in DM1 patients([5,6,7]). It has been assumed that PKT is associated with higher costs, when compared to kidney transplantation alone (KTA) in these patients([3,8]). To become advantageous, PKT has to be capable of outweighing these increased costs and risks([2,3,8]), as it does, even though much later than in KTA([8]); and ideally it should improve patients quality of life (QoL)([1,6,7]).The bigger the improvement in QoL, the stronger the arguments favoring the option for PKT, instead of KTA, in DM1 patients.

Rejection and survival rates as well as posttransplant complications are frequently used as references of outcome after transplantation. In the last years it has become increasingly frequent to assess patients' perception of the impact of transplantation on their daily life. QoL assessment through patient-reported outcome (PRO) instruments is used to measure the global success of transplantation([4]). It has already been suggested that some kind of health-related QoL (HRQOL) measurement should be made in all transplant centers, as part of their accreditation process([9]).

QoL questionnaires may be generic or disease-specific. Short-form survey SF-36 is one of the most used in transplantation([4,10,11,12,13]), a 36-item questionnaire assessing two major component scores, physical and mental. Some of the items may be irrelevant to measure QoL after transplantation and may neglect other issues likely to be important([4]). Several other generic instruments have been used to evaluate QoL in transplanted patients, namely the Karnofsky Index([1]) and the EuroQol-5 Dimensions (EQ-5D)([14,15,16]). Disease-specific surveys have been developed to measure changes in particular diseases, mostly after a treatment. Diabetes Quality of Life Questionnaire (DQOL) is often used in DM1 patients([4]). However, some of its items were also judged as irrelevant or outdated when applied to transplanted

patients([4]). The 36-item Gastrointestinal Quality of Life Index (GIQLI) questionnaire was conceived to assess gastrointestinal (GI) complaints and QoL in patients with GI problems([17]). The GIQLI focuses not only on the GI symptoms that may affect QoL, but also assesses other domains: emotional, physical and social function, and stress of medical treatment([17,18]).

Our day-to-day experience is that GI complaints are very frequent and may be very disabling in DM1 patients referred to us for transplantation. GI complaints are mainly due to diabetic neuropathy, which is thought to significantly improve after PKT([19]). We conducted a study to analyze the QoL changes perceived by the PKT patients of our program. Because GIQLI assesses GI-specific symptoms and HRQOL in its several dimensions (social, emotional and physical) as well as the burden of treatment, we decided to use this questionnaire. We also used the Euroqol EQ-5D-5L questionnaire. We asked each patient who consented to participate in this study, the changes felt from pretransplantation to the present, after transplantation. The EQ-5D-5L has already been validated for Portuguese population([20]) and in renal transplants without([14]) or with diabetes([15]). The GIQLI has also been used and validated in renal transplants([14,18]) for the Spanish population([21]). To our knowledge, this is the first study using GIQLI survey in PKT to assess changes in QoL and in GI symptoms.

Patients and methods:

This study comprises two sub-studies: one retrospective and one prospective.

From a cohort of 152 PKT patients, with systemic-enteric drainage as surgical technique, we enrolled those who gave informed consent and who met the following criteria: more than 3 months post-discharge (considered as the minimal time for the patient to appreciate the benefits of transplantation); at least one functioning graft (defined as not needing dialysis or not needing insulin administration); and not having experienced a rejection, infection or admission episode for the last 3 months (to avoid biases from recent “crisis episodes”). One hundred twenty six patients fulfilled these criteria. They were requested to score the two questionnaires, GIQLI and EQ-5D-5L, posttransplant (at date of completion, or time 1) and for comparison, to score them for their pretransplant period (pretransplant, or time 0). The questionnaires were applied at a mean follow up of 4.8±3.5 years (all >6 months) from PKT.

A sub-study was done in the last patients 20 enrolled - the prospective cohort. In this subsample the surveys were administered prospectively, on admission for transplantation (called time 0) and 6-12 months after the procedure (time 1). When scoring the items at time

1, they were also requested to score again, for each item, their pretransplant situation ("Please compare your present status with that before transplantation. Keeping in mind your last week, how do you score your present status? Remembering the last week before transplantation, how do you score your pretransplant status?"). Thus, in these 20 patients we could measure the changes from time 0 to time 1 prospectively. Moreover, we could analyze if the answers given at time 1 concerning their pretransplant status (called time 0 retrospective), were significantly different from those given previously at time 0.

The patients were also inquired about their employment status before PKT and at the time of survey completion (time 1).

Outcome measurements:

The GIQLI questionnaire, with 36-items, includes 5 subscales: core symptoms, psychological status, physical function, social function and GI symptoms, as well as a single question addressing burden of the treatment. Item scores range from 0 to 4, resulting in a total score from 0-144 points. Higher scores represent better QoL.

The EQ-5D-5L is a generic instrument to measure health-related QoL with two sections. The first one consists of 5 items to assess level of functioning: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Patients score each item from 1 (no problem) to 5 points (extreme problems). Higher scores represent worse QoL. The second section is a 20-cm vertical visual analogue scale (VAS-scale) running from 0 (the worst imaginable health state) to 100% (the best imaginable health state). Patients have two identical scales, to draw a line on the scale point which they feel most accurately reflects their health, before PKT and at present. Permission to use this questionnaire was obtained from the Euroqol group.

The surveys were self-administered on a pen-and-paper basis, in a single session coinciding with one medical appointment. Blind patients (n=7) or those with severely impaired vision (n=5) were assisted in completing questionnaires by their relatives or friends who were accompanying them to the medical visit – a possible, but inevitable, bias. The surveys were collected from February 2012 to September 2013.

Statistical analysis:

Patient's characteristics were summarized by computing QoL and subscale scores of EQ-5D-5L and GIQLI 36-item questionnaires. Descriptive statistics included arithmetic mean and standard deviation for continuous and frequencies for categorical variables.

Student's **t** test for paired samples was undertaken to calculate changes in quality of life and scores between pre-transplant and post-transplant evaluation, and also to compare the differences between mean scale scores in the sub-study of the prospective cohort. McNemar test was used to compare the unemployment rate before and after transplantation. Cohen's d effect sizes and 95% confidence intervals were calculated to assess the magnitude of changes between mean scale scores [Cohen's d = (mean of change)/(standard deviation of change)]. Differences were regarded as meaningful but small at Cohen's *d* of 0.20, medium at 0.50, and large at 0.80([22]).

Multivariable linear regression analysis was done to identify the main predictors of QoL, with the posttransplant GIQLI-36 score as the dependent variable. The independent variables tested were recipient gender, age at transplantation, duration of diabetes prior to PKT, number of functioning grafts, acute rejection, cardiovascular (CV) disease, time on dialysis before PKT, duration of PKT hospitalization and time since PKT to the survey.

Analysis was performed using statistical software SPSS 21.0 and P-values <0.05 were considered statistically significant.

Results:

Demographic and clinical characteristics of the sample

Population characteristics of the global group of 126 PKT are shown in table 1. This table also describes, separately, the characteristics of the prospective group. Besides the differences in time since PKT to the survey and time on dialysis before PKT, no other significant differences between groups were noted.

QoL outcomes

Prospective study – performed in 20 patients, during the first year after PKT. We observed improvements from time 0 to time 1, in all 5 main domains of GIQLI assessment; in the 5 questions of EQ-5D-5L assessment; and in the VAS-scale score (results shown in table 2).

We compared the answers obtained from patients at time 0 to those obtained retrospectively at time 1, re-evaluating their QoL status prior transplantation (called “time 0 retrospective”). We observed that 94.4% of the answers coincided, meaning that only 5.6% of patients scored differently their QoL pretransplant status in the second questionnaire (table 2). However, the mean values for each domain did not significantly differ, between “time 0” and “time 0 retrospective” (with the exception of physical items that showed a small but significant higher scoring in the time 0 retrospective evaluation).

Retrospective study – The results presented here are relative to the global sample, retrospectively assessed (including the last 20 patients with their retrospective evaluation). The two assessments, GIQLI and EQ-5D-5L, obtained from the 126 patients were analyzed. For all the major assessed domains of both questionnaires, we observed a significant improvement in the patient reported QoL after PKT. The results are presented in table 3. Scores obtained for each particular question of the GIQLI questionnaire are shown in table 4. In all the items, with the exception of those two concerning flatulence and abdominal noises, there was an improvement. The biggest improvements (largest effect sizes) were observed in the physical, psychological and social functions, rather than in GI symptoms. Only in one question (abdominal noises) the patients scored it lower after PKT, meaning they felt it worsened comparatively to their situation before PKT but not significantly (2.71 vs 2.79, P=0.559; effect size -0.07). Concerning the stress with the treatment, there was a very significant improvement, from 1.50 points before PKT to 3.56 after PKT (P<0.001; effect size 2.02).

We also analyzed the employment status before and after transplantation (graphic 1). Before transplantation, 64 of PKT patients (50.8%) didn’t have a job. The majority of them were unable to work due to disease. Severely impaired vision or blindness was an important cause (in 18.8% of those not working). Only 3 of those 64 (4.7%) answered that they couldn’t get a job, although feeling able to work. After transplantation, the rate of unemployed patients significantly decreased to 36.5% (46 patients, P<0.001). In fact, 5 of these have considered themselves able to work; 4 lost their jobs. Other 22 could find a job after PKT.

We analyzed separately the possible impact of the two symptoms which did not improve after PKT, on the ability to return to work. Patients with a score <3 for flatulence or abdominal noises, or <6 for both, were categorized as having “relevant” symptoms; patients scoring ≥3 in each one (3 means mild, 4 means absence of symptoms), or ≥6 in both were categorized as having “irrelevant” symptoms. Relevant flatulence was registered in 44 (34.9%); relevant

abdominal noises in 42 (33.3%) and both symptoms were relevant in 25 patients (19.8%). Return to work rate was similar in patients with relevant or irrelevant flatulence (54.5% vs 68.3%, p=0,127); relevant or irrelevant abdominal noises (57.1% vs 66.7%, p=0.295); and relevant or irrelevant abdominal noises plus flatulence (52% vs 66.3%, p=0.183).

In a multivariable linear analysis (table 5), only the functioning status of both grafts was an independent predictor of GIQLI-36 score. Patients with only 1 functioning graft presented a significantly lower score (B=-5.157, p=0.015).

Discussion

PKT may enhance more life years from transplant (LYFT) than other therapeutic modalities, in DM1 patients([23]). Costs associated with PKT are not easy to compare to renal replacement therapy by dialysis and insulin treatment. These patients have a high rate of admissions and it may be difficult to separate which are due directly to transplantation or due to the evolution of diabetic complications. The break-even point, for KTA, has been to be found around 2.5 years: from there on KTA is less expensive than dialysis([24]). According to the 5-year cost-utility model presented by Douzdjian V et al([8]), PKT is the most cost-effective treatment for DM1 patients with renal failure. It means, PKT certainly has a break-even point much later than KTA, but at 5 years PKT is clearly superior. Despite having later cost-efficacy compensation, the fact that PKT brings clear benefits in QoL strongly validates the option for PKT in DM1.

Mortality risk for DM1 patients in the waiting list is several times higher than that associated with PKT([5]), even if the wait-listed patients have shorter duration of diabetes and similar CV risk factors([5]). Among the distinct modalities of pancreas transplantation, better survival results are achieved with simultaneous PKT, compared to pancreas after kidney (PAK) and to pancreas transplantation alone([25]).

HRQOL has emerged as another important measure of the global success of transplantation. The level of QoL achieved with a successful PKT in DM1 patients, was sometimes described as comparable to the QoL levels of the healthy population([26,27]). Nevertheless, most of the papers, reported results below the normal levels for the standard population([11,13,28]). Some studies were able to demonstrate that PKT confers significant improvement overtime in general health([1,13]), or at least in the physical domains([12,13]). Others([11,29,30]) have found that this improvement was not different from the achieved with KTA in DM1 patients. However, concerning the diabetes-specific QoL, there is consensus that better results are obtained in the

PKT than in the KTA patients([13,29,30,31]). PKT patients, compared to KTA, tend to perceive more benefits related with diabetes secondary complications([13]), less anxiety, less dietary restrictions and better living conditions([32]); although experiencing similar limitations associated with transplant, such as rejection or fear of graft loss([13]). Studies to assess the additional benefit of a functioning pancreas graft (in PKT who lost their pancreas or maintained both grafts), showed inferior HRQOL results in those who lost the pancreas graft([1,32,33]). DM1 patients who receive a PAK may achieve comparable health status to the PKT patients([10]). Loss of the kidney in PKT may result in a significant decrease in QoL([26]). Compared to the wait-listed DM1 patients, there is no doubt that PKT have not only better diabetes-specific QoL([30]), but also better general HRQOL([33,34]).

In our study, we observed significant improvement in all major domains of the GIQLI and EQ-5D-5L questionnaires used, from PKT to the present. As shown in table 3, only in the EQ-5D-5L items about mobility and self-care there was a small to medium effect size. In all the other EQ-5D-5L items, as well as in the GIQLI items, a large effect size was found. It has been suggested that maximum scores can be obtained in the most recent transplants([34]) or during the first year after transplantation([12,13,35]), which stabilize([12]) or worsen([13]) in the mid to long-term. On the contrary, other studies in DM1 after KTA demonstrate comparable perceived health in short, mid and long-term, despite more comorbidities and symptoms in the long-term([36]), probably reflecting less influence of these symptoms in the perceived health in the long-term. In our global study group we have found that HRQOL markedly improved since PKT: improvement occurred within the first year in the prospective subgroup; and also in the global group with a mean follow-up of almost 5 years. The mean time from PKT to the survey was significantly higher in the retrospective group and, logically, it was one of the variables included in the linear regression multivariable analysis. However, it was not found to be a predictor of QoL results. The same was observed for time on dialysis prior to PKT: although higher in the retrospective group, multivariate analysis showed it was not a factor influencing QoL scores.

Adang et al([37]) have presented a study in which the patients retrospectively scored their pretransplant QoL in each assessment after PKT. In these retrospective re-evaluations pretransplant QoL the scores were significantly lower overtime. In our study, in the prospective subgroup of 20 patients we did not find significant differences in the pretransplant QoL scores, assessed on admission for transplantation or assessed up to 12 months later, with the exception of the GIQLI physical items, which scored higher in the time 0 retrospective

evaluation. However, it is not possible to exclude that the results may be different with longer follow-up time intervals between re-evaluations.

The VAS-scale of the EQ-5D-5L is an easy to use instrument, to quantify QoL from the worst to the best possible health status. Our results from the pretransplant to the present situation, after PKT, are very elucidative of the global improvement in the patients' health condition.

Differently from other studies([12,34]), we did not observe lower scores in the female patients. The positive predictor of better QoL scores in our study was the presence of the two grafts functioning.

Despite the possible negative effects of the immunosuppressive drugs on the GI symptoms, we also found an improvement in GI complaints after PKT. The effect sizes were not so large in GI items, but still improved. These findings go along with the improvement of autonomic digestive dysfunction after PKT observed by others([19]). The single items that did not score better after PKT were those concerning abdominal noises and flatulence. Albeit this, return to work significantly augmented after PKT and these two symptoms did not prove to significantly influence it. More disabling GI symptoms such as bowel urgency, uncontrolled stools, diarrhea, and nausea did in fact significantly improve, which may explain why GI symptoms did not affect the ability to work. Some of the immunosuppressive drugs can cause GI complaints. Mycophenolate is the one most commonly associated with diarrhea, nausea, abdominal pain, bloating, among others. In fact, in our study population, digestive intolerance was the main cause for switching some patients (7.9%) from mycophenolate to sirolimus. GI complaints improved, and 6 out of 10 were able to work after PKT, not differently from the global population. However, we cannot prove whether the ability to return to work was related to the medication change. Additionally, it is not possible to exclude the contribution of the medication to the lack of improvement of those two GI symptoms (flatulence and abdominal noises).

Unsurprisingly, a positive result was obtained in the question about the burden by treatment: the difference between scores, prior to PKT and after PKT, was highly significant. The employment status was another pleasant result. For each patient, maintenance or achievement of a new job after PKT reflects his good general health condition. In this group, the number of patients working or able to work after PKT is significantly higher. Our results are in accordance with others([12,34]), who reported an improvement in the work status in these patients.

We acknowledge the limitations and the strengths of this study. For the global sample (126 patients), this was a retrospective study assessing pretransplant QoL status for comparison to the posttransplant QoL status. Only in the last 20 patients a prospective study was also conducted. We are aware that a recall bias may be present in all retrospective surveys. In order to check for such bias, we also looked for it in the prospective group, assessing them prospectively and retrospectively. With the exception of physical items in GIQLI, for all the other items from both questionnaires, there were no significant differences. However, with larger intervals since PKT, the accuracy of the pretransplant evaluation may not be the same and we cannot assume that results would be similar if prospectively collected. The strengths of the study are also highlighted: the number of patients included, 126; the focus on quality of life as a relevant clinical outcome often neglected; the use of two validated QoL instruments; the consistent results in both surveys; the statistical analysis, with the effect-size calculation, a valuable tool to interpret the meaningfulness of changes observed between scale scores at two different time points; and the inclusion of particular questions assessing changes in the employment status and satisfaction with PKT.

This study provides evidence that PKT may significantly improve QoL in DM1 patients: markedly in physical, psychological and social functions; and at a least moderately the GI symptoms. Several confounding factors, such as the immunosuppressive agents, may act against GI symptoms improvement. In spite of that, most GI complaints did in fact improve. The improvement in satisfaction with treatment and in the employment status was very significant. Maintenance of both grafts functioning was a positive predictor for better HRQOL scores.

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All the patients gave their informed consent prior to their inclusion in the study. This work was approved by the appropriate ethics committee. Therefore it has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments; and in accordance with the Declaration of Istanbul.

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Title of **Table 1** – demographic characteristics of PKT patients
Title of **Table 2** – QoL results in the prospective subgroup
Title of **Table 3** – QoL results in the total sample.
Title of **Table 4** - GIQLI scores for each item.
Title of **Table 5** – Linear regression multivariable analysis of predictors of posttransplant GIQLI-36 score.
Title of **Graphic 1** – Employment status of PKT patients.

Table 1 – demographic characteristics of PKT patients

	n=126 (retrospective)	**n=20 (prospective)**
Male sex	60 (47.6%)	8 (40%)
Mean age at PKT	35.7 ±6.0 years	37.7 ±5.3 years
Median duration of PKT hospitalization	19 (IQR 13-28) days	18 (IQR 11-25) days
Mean duration of diabetes prior to PKT	23.9±5.9 years	24.6±6.3 years
Mean duration of dialysis prior to PKT	29.7±20.7 months [a]	19.7±10.6 months [a]
Mean time since PKT to the survey	4.8±3.5 years [b]	0.7±0.3 years [b]
Mean age at survey completion (last survey for prospective group)	39.5±6.1 years	38.2±5.2 years
Both grafts functioning	106 (84.1%)	18 (90%)
One graft functioning	20 (15.9%)	2 (10%)
- only kidney	19 (15.1%)	2 (10%)
- only pancreas	1 (0.8%)	0 (0%)
Graft function*		
- SCr(mg/dl) / e-GFR (ml/min)	1.14±0.36 / 75.1±24.1	1.12±0.31 / 76.7±21.6
- C-peptide (ng/mL) / HbA1c(%)	3.37±2.21 / 5.29±0.39	3.51±2.08 / 5.21±0.45
CV disease (clinically significant)	22 (17.5%)	3 (15%)
- coronary disease	7 (5.6%)	1 (5%)
- peripheral arterial disease	15 (11.9%)	2 (10%)
Rejection episodes	20 (15.9%)	3 (15%)

*For those with grafts functioning. SCr – serum creatinine. e-GFR – estimated GFR (MDRD)

[a] P<0.01; [b] P<0.001

Table 2 – QoL results in the prospective subgroup

	Before PKT (time 0)	**Before PKT retrospective (time 0 retrospective)**	**P value***	**After PKT (time 1)**	**P value ****
	Total Sample (n=20 PKT patients)				
GIQLI survey					
Core Symptoms (0-40 points)	24.15 ± 6.99	24.5 ± 6.49	0.110	30.30 ± 4.68	<0.001
Physical items (0-24 points)	8.00 ± 3.04	8.35±2.92	0.015	14.80 ± 4.42	<0.001
Psychological items (0-24 points)	10.20 ± 4.31	10.25 ± 4.05	0.577	18.60 ± 3.35	<0.001
Social items (0-16 points)	7.70 ± 2.52	7.80 ± 2.59	0.330	11.15 ± 3.17	<0.001
GI-specific items (0-40 points)	29.25 ± 5.05	29.45 ± 4.73	0.258	33.75 ± 3.34	<0.001
EQ-5D-5L survey					
Mobility (1-5 points)	1.55 ± 0.69	1.55 ± 0.61	1.0	1.15 ± 0.37	0.008
Self-care (1-5 points)	1.45 ± 0.61	1.40 ± 0.50	0.330	1.10 ± 0.31	0.005
Usual activities (1-5 points)	2.90 ± 0.45	2.75 ± 0.44	0.083	1.55 ± 0.69	<0.001
Pain/discomfort (1-5 points)	3.15 ± 0.49	3.10 ± 0.45	0.330	1.60 ± 0.60	<0.001
Anxiety/depression (1-5 points)	3.20 ± 0.70	3.15 ± 0.67	0.330	1.80 ± 0.52	<0.001
VAS scale (%)	40.75 ± 11.04	42.00 ± 8.34	0.234	79.00 ± 8.68	<0.001

Higher scores represent better QoL in the GIQLI questionnaire and worse QoL in the EQ-5D-5L questionnaire. * P value - comparing scores "time 0" to scores "time 0 retrospective"; **P value – comparing the differences between scores "time 0" and "time 1"

Table 3 – Quality of Life Results in the total sample.

	Before PKT	**After PKT**	**P value**	**Effect Size (95% CI)**
	Total Sample (n=126 PKT patients)			
GIQLI survey				
Core Symptoms (0-40 points)	25.10 ± 6.96	29.81 ± 5.48	<0.001	0.76 (-1.53; 0.01)
Physical items (0-24 points)	7.86 ± 4.43	14.83 ± 3.86	<0.001	1.68 (-2.20; -1.17)
Psychological items (0-24 points)	10.36 ± 5.42	18.10 ± 3.96	<0.001	1.64 (-2.22; -1.05)
Social items (0-16 points)	7.24 ± 2.96	11.33 ± 2.72	<0.001	1.45 (-1.70; -1.10)
GI-specific items (0-40 points)	27.98 ± 6.20	32.92 ± 3.80	<0.001	0.96 (-1.60; -0.33)
EQ-5D-5L survey				
Mobility(1-5 points)	1.48 ± 0.68	1.26 ± 0.54	<0.001	0.36 (0.29; 0.44)
Self-care (1-5 points)	1.19 ± 0.41	1.10 ± 0.29	0.001	0.25 (0.21; 0.30)
Usual activities (1-5 points)	2.76 ± 0.66	1.63 ± 0.72	<0.001	1.64 (1.55; 1.72)
Pain/discomfort (1-5 points)	3.13 ± 0.66	1.63 ± 0.58	<0.001	2.43 (2.35; 2.50)
Anxiety/depression (1-5 points)	3.30 ± 0.64	1.79 ± 0.63	<0.001	2.40 (2.32; 2.48)
VAS scale (%)	38.13 ± 16.30	84.17 ± 10.82	<0.001	3.34 (-5.04; -1.64)

Higher scores represent better QoL in the GIQLI questionnaire and worse QoL in the EQ-5D-5L questionnaire. Effect sizes: meaningful but small at 0.20, medium at 0.50, and large at 0.80.

Table 4 - GIQLI scores for each item

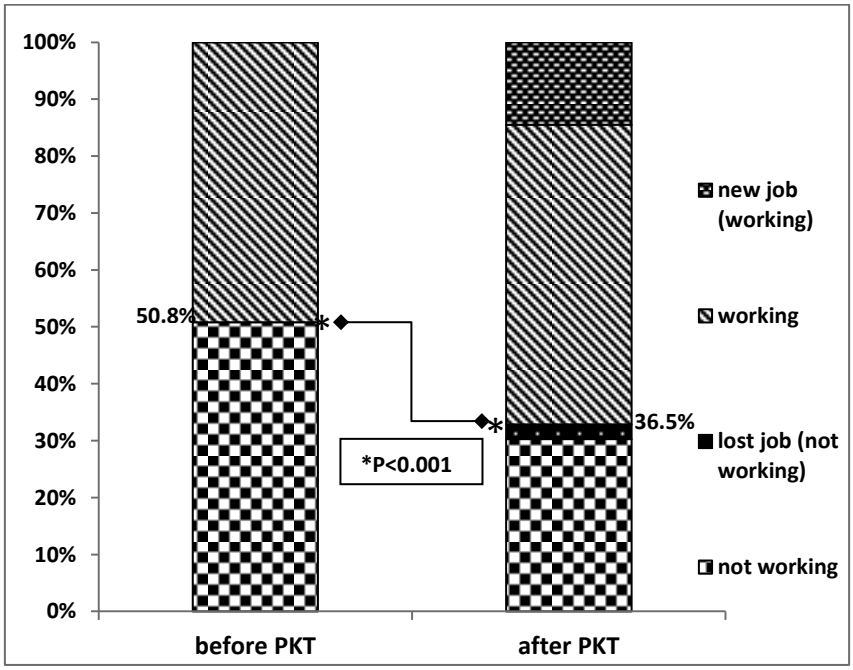
GIQLI item (n=126 patients)		Before PKT	After PKT	P value	Effect size (95% CI)
Core symptoms	Abdominal pain	3.15	3.42	0.007	0.28 (-0.40; -0.17)
	Bloating	2.51	2.89	0.003	0.32 (-0.47; -0.18)
	Epigastric fullness	2.68	3.00	0.004	0.28 (-0.42; -0.13)
	Flatus	2.50	2.59	0.408	0.10 (-0.21; 0.01)
	Belching	2.74	3.00	0.003	0.26 (-0.38; -0.14)
	Bowel frequency	2.71	3.15	<0.001	0.40 (-0.53; -0.26)
	Abdominal noises	2.79	2.71	0.559	-0.07 (-0.08; 0.21)
	Restricted eating	2.27	3.06	<0.001	0.85 (-0.97; -0.74)
	Enjoyment in eating	2.26	3.10	<0.001	0.89 (-1.01; -0.77)
	Fatigue	1.48	2.90	<0.001	1.45 (-1.57; -1.33)
Physical function	Strength	1.32	2.52	<0.001	1.46 (-1.56; -1.36)
	Feeling unwell	1.32	2.92	<0.001	1.88 (-1.99; -1.78)
	Feeling unfit	1.17	2.34	<0.001	1.44 (-1.54; -1.34)
	Endurance	0.99	2.15	<0.001	1.31 (-1.42; -1.20)
	Waking up at night	1.59	2.17	<0.001	0.48 (-0.63; -0.33)
	Appearance	1.48	2.73	<0.001	1.42 (-1.53; -1.31)
Emotional status	Sadness	1.79	3.23	<0.001	1.43 (-1.55; -1.31)
	Nervousness	1.79	2.77	<0.001	0.91 (-1.04; -0.77)
	Frustration	1.83	3.14	<0.001	1.24 (-1.37; -1.11)
	Happiness	1.86	3.09	<0.001	1.21 (-1.34; -1.09)
	Burden of treatment	1.50	3.56	<0.001	2.02 (-2.15; -1.89)
	Coping with stress	1.59	2.31	<0.001	0.73 (-0.85; -0.61)
Social function	Daily activities	1.60	2.90	<0.001	1.43 (-1.54; -1.32)
	Leisure activities	1.61	2.94	<0.001	1.46 (-1.57; -1.34)
	Sexual life	1.68	2.55	<0.001	0.86 (-0.98; -0.73)
	Personal relations	2.35	2.94	<0.001	0.78 (-0.87; -0.68)
GI symptoms	Regurgitation	2.55	3.38	<0.001	0.82 (-0.95; -0.70)
	Dysphagia	3.21	3.68	<0.001	0.57 (-0.67; -0.47)
	Eating speed	2.52	2.82	<0.001	0.37 (-0.47; -0.27)
	Nausea	2.10	3.29	<0.001	1.27 (-1.39; -1.16)
	Diarrhea	2.54	2.91	0.001	0.39 (-0.50; -0.27)
	Bowel urgency	2.63	2.88	0.006	0.27 (-0.38; -0.15)
	Constipation	2.41	2.94	<0.001	0.49 (-0.63; -0.36)
	Blood in stool	3.81	3.96	0.002	0.37 (-0.42; -0.32)
	Heartburn	2.85	3.42	<0.001	0.59 (-0.71; -0.47)
	Uncontrolled stools	3.37	3.63	0.001	0.31 (-0.41; -0.20)

Table 5 – Linear regression multivariable analysis of predictors of posttransplant GIQLI-36 score

Variables	B	95% IC	P value
Recipient gender (M vs F)	0.712	-0.012 – 2.337	0.386
Age at transplantation	0.064	-0.107 – 0.235	0.461
Duration of diabetes prior to PKT	-0.090	-0.250 – 0.071	0.269
Only 1 graft functioning vs 2 grafts	**-5.157**	-9.286 – (-1.028)	**0.015**
Acute rejection	-0.122	-2.453 – 2.210	0.918
CV disease	1.225	-0.847 – 3.297	0.243
Dialysis time	0.021	-0.019 – 0.062	0.294
Duration of PKT hospitalization	-0.037	-0.093 – 0.020	0.198
Time since PKT to the survey	-0.068	-0.311 – 0.176	0.583

Variables included in the model: recipient gender, age at transplantation, duration of diabetes prior to PKT, number of grafts functioning, acute rejection, CV disease, time on dialysis prior PKT, duration of PKT hospitalization and time since PKT to the survey.

Graphic 1 – Employment status



Discussão e Conclusões

Evolução clínica e metabólica após TRP

Os **trabalhos II.1 e II.2** mostram a evolução clínica dos TRP realizados no Hospital de Santo António, aos 5 e aos 10 anos após o início da atividade desta Unidade. As curvas de sobrevivência do doente e dos enxertos obtidas aos 5 anos (**artigo II.1**), em 42 TRP, revelaram-se similares às dos registos internacionais ([1,2,3]). Foram cautelosamente assumidas como potencialmente sobrevalorizadas, dado tratar-se ainda de um grupo pequeno e com seguimento curto. Os resultados a mais longo prazo (10 anos) e num grupo maior de doentes (111 TRP) vieram consubstanciar os bons resultados iniciais. Em 150 TRP, confirmaram-se esses resultados (**artigo II.2.1**).

Ultrapassada a fase de aprendizagem, melhoraram alguns dos indicadores, como a taxa de reintervenções. A experiência acumulada permitiu-nos avaliar e estratificar melhor o risco clínico inerente a cada situação, e adotar uma atitude mais expectante em alguns casos, ou intervenção mais imediata noutros.

A técnica cirúrgica tem nuances distintas nos diversos centros que realizam TRP ([4]). Também no HSA sofreu evoluções: o implante, no recetor, do *patch* aórtico do dador englobando a artéria esplénica e a mesentérica superior (**artigo II.2**), eliminou a necessidade de reconstruções vasculares e consequentemente traduziu-se na redução de casos de hemorragia ou trombose no pós-operatório.

A inclusão crescente de recetores em lista ativa para TRP também permitiu uma melhor seleção do par dador-recetor, nomeadamente a maximização do *match* HLA entre ambos. A percentagem de doentes TRP com 6 *mismatches* HLA como o dador baixou entre os 5 e os 10 anos de seguimento.

A localização do pâncreas transplantado – intra-celómica, mais profunda e móvel – acrescenta dificuldade técnica e risco na realização da sua biópsia. Por receio das complicações, nos primeiros anos do programa não se fizeram biópsias do enxerto pancreático. O diagnóstico de rejeição aguda era, assim, de presunção, após exclusão de outras potenciais causas de inflamação e/ou disfunção. Durante estes anos, este diagnóstico foi provavelmente sobrevalorizado. A implementação da técnica de biópsia pancreática, através da colaboração da radiologia de intervenção, veio permitir assumir ou excluir com maior segurança o diagnóstico de rejeição do pâncreas. O reflexo deste passo diagnóstico foi a descida da taxa de rejeição aguda global - assumida e tratada - cifrando-se atualmente nos 15%. Os 2 maiores centros de

referência mundial no TRP ([5,6]), reportam igualmente uma descida progressiva das perdas técnicas e uma redução da rejeição aguda – e da perda imunológica – ao longo das suas décadas de experiência.

A principal causa de morte no TRP, após os primeiros 6 a 12 meses, é de origem CV. Os corticóides são usados pela maioria dos centros, como parte da terapêutica imunossupressora do TRP. Têm efeitos laterais vários (cataratas, osteoporose, HTA, obesidade, dislipidemia, insulino-resistência, etc.), alguns dos quais concorrem para o risco CV acrescido dos doentes com DM1 e doença renal crónica. Por este motivo, têm-se vindo a desenvolver estratégias visando a sua eliminação ou minimização da terapêutica imunossupressora de manutenção. Todavia, a retirada de corticóides não é isenta de riscos: uma meta-análise no TR isolado mostrou há 10 anos atrás um aumento da incidência da rejeição aguda após a sua retirada ([7]); que, contudo, não se observou numa outra meta-análise posterior em doentes sob tacrolimus, mantendo-se este risco nos que estavam sob ciclosporina ([8]). Com a utilização de imunossupressão de indução mais potente, nomeadamente a que associa soros anti-linfocitários e manutenção com tacrolimus e ácido micofenólico, algumas unidades encetaram protocolos livres ([9]) ou com suspensão precoce de corticóides ([10]). Um outro estudo ([11]) mostrou benefício na função renal nos TRP que faziam a descontinuação dos corticóides só após os 3 meses.

A suspensão de corticóides, em doentes estáveis e sem intercorrências major – nomeadamente imunológicas -, tem sido também um objetivo da nossa Unidade. A descontinuação, se possível, faz-se entre o 6º e o 12º mês pós-transplante. Num trabalho inicial fomos avaliar os aspetos clínicos e metabólicos, em 77 TRP. Os resultados da nossa experiência (**artigo II.3**), com particular enfoque em 54 casos com TRP com >1 ano de evolução, mostraram que a suspensão foi exequível em 77,8%, sem aumento da rejeição aguda. Estes doentes apresentaram um bom controlo dos fatores de risco CV, ainda que o requerimento de fármacos anti-hipertensores e anti-dislipidémicos não tenha sido diferente nos 2 grupos (com e sem corticóides).

Complicações do TRP com impacto na evolução clínica

A morbi-mortalidade associada ao TRP é substancialmente superior à do TR isolado. A mediana do tempo do 1º internamento no TRP é, na nossa experiência, cerca de 2 vezes superior à do TR isolado (**artigo III.1**). As complicações pós-transplante dividem-se em 2 grandes grupos conforme a sua ocorrência, durante os primeiros 6 a 12 meses, ou posterior. As complicações “técnicas” precoces e associadas ao ato

cirúrgico, como a hemorragia, a trombose e o "*leak"* pancreático com peritonite, são as principais causas de prolongamento do internamento e da permanência em cuidados intensivos. São também a causa primordial de perda do enxerto pancreático, como observados em outros centros ([12,13]). Apesar da tendência progressiva para a sua diminuição nos últimos anos ([5,6]), elas podem ter um impacto significativo na sobrevivência do doente ([12,13]). Na realidade, nos primeiros 3 meses, a mortalidade dos TRP comparados com os que permanecem em lista de espera é superior, tendência que se inverte no final do 1º ano, sendo a partir daí favorável ao TRP ([14]).

Continua por encontrar um bom índice de risco, que faça prever antes do procedimento do TRP, quais os doentes expostos a maior risco de complicações ou de morte. Esta procura mantém-se atualmente ([15]). Avanços neste domínio serão muito úteis, para indicar ou excluir o TRP numa determinada situação, face às caraterísticas do dador e do recetor.

A necessidade de relaparotomias, superior a 30%, é semelhante ao reportado noutras séries de dimensão populacional equivalente ([16]). A elevada taxa de reinternamentos nestes doentes (cerca de ¾), imunossuprimidos e portadores dos 2 enxertos, reflete bem a sua exigência clínica. A maioria dos reinternamentos ocorre no 1º ano e as causas infeciosas prevalecem ([17]). As infeções fúngicas e víricas sistémicas resultaram nos internamentos mais longos, expectável pela sua gravidade. O **artigo III.2** faz uma revisão das principais complicações clínicas com tradução imagiológica encontradas na nossa população de TRP.

Os estudos são praticamente unânimes a reportar melhores resultados na transplantação renal quando esta é realizada *preemptive* - antes do início de diálise crónica - ou nos primeiros 6 meses ([18,19,20,21]). O viés de seleção dos melhores doentes, mais jovens e com menos comorbilidades, para transplante mais precoce não pode ser de todo excluído. No estudo realizado para avaliar o impacto da modalidade dialítica pré-transplante no TRP (**artigo III.3**), encontrámos resultados significativamente inferiores nos doentes que fizeram DP. Não só estes doentes tiveram mais reoperações motivadas por infeção, como perdas pancreáticas também de causa infeciosa e tendencialmente mais tromboses do enxerto renal. A DP foi mesmo um preditor independente de morte do doente, a par da doença CV e da falência de um ou ambos os enxertos.

Alguns estudos prévios documentaram maior taxa de trombose do enxerto renal no TR isolado, em doentes que tinham feito DP pré-transplante ([22,23,24,25]), mas esses

resultados não são consistentes ([26,27]). Uma maior incidência de complicações infeciosas e de sepsis foram também observadas em doentes previamente em DP ([28,29]), mas uma vez mais existem resultados contraditórios ([30]).

No TRP, além dos dados serem mais escassos, existem mais variáveis que podem influenciar o desfecho do transplante. O tempo de hospitalização é mais longo e a imunossupressão mais intensa nos TRP, e estes dois fatores foram encontrados como fatores de risco para infeção mesmo no TR isolado ([29]). Não só na nossa população, mas também noutras séries, a DP associou- se a maior incidência de infeção intra-abdominal ([31,32,33]) e de relaparotomias ([34,35]). A existência prévia do catéter de DP, possivelmente colonizado ([36]); a lesão de fibrose e espessamento peritoneal ([37]); e a ascite residual que permanece muitas vezes nestes doentes em DP ([38]), podem ser fatores condicionantes deste desfecho.

Apesar de ser um tema controverso, sem estudos conclusivos, a DP parece associar-se mais do que a HD a um padrão analítico pró-trombótico ([23]). Num estudo foram mesmo identificados alguns distúrbios nos doentes em DP ([39]), como o aumento da atividade plasmática dos fatores VII, VIII, IX, X, XI e XII da coagulação e da proteína S. A diabetes constitui, ela própria, um risco acrescido para trombose ([23]) e foram encontradas com mais frequência alterações pró-trombóticas nos estudos efetuados em DM1 submetidos a TRP ([40]).

Urge estudar melhor estes doentes que vão ser submetidos a TRP, e em particular os doentes em DP, do ponto de vista hematológico. A estratégia profiláctica anti-trombose poderá ter de ser redesenhada nestes doentes. O estudo dos fatores subjacentes ao maior risco infecioso intra-abdominal nos doentes em DP, poderá aportar os conhecimentos necessários para combater esses fatores de risco, ou indicar até uma mudança na profilaxia anti-infeciosa. É necessário clarificar as razões deste negativo impacto da DP no TRP, e combatê-los, para esta poder continuar a ser uma opção dialítica a sugerir a estes doentes enquanto aguardam pelo TRP

Os dados apresentados nas publicações acima referidos, neste capítulo das complicações, espelham a complexidade do TRP e as dificuldades no seu manuseamento. A abordagem multidisciplinar é essencial nestes doentes.

Doença CV nos TRP

Quisemos saber como evoluíam esses parâmetros metabólicos num grupo maior de TRP, com seguimento mais prolongado, e as repercussões em termos de doença CV.

A **publicação IV** reporta os resultados desse estudo mais alargado. A mortalidade de origem CV nesse estudo era de 1,5%. Em 103 TRP, vivos e com ambos os enxertos funcionantes há mais de 6 meses, registou-se uma incidência de eventos CV não-fatais de 6,8%. Cumpriam critérios para síndrome metabólica (critérios do *National Cholesterol Education Program Adult Treatment Panel 3* – NCEP/ATP3) 8,7% dos doentes, um valor bem abaixo do que se tem observado no TR isolado ([41,42]). Observámos um perfil lipídico mais adverso no subgrupo com corticóides, que também tendiam a ter maior prevalência de HTA. A hipertensão associou-se a um maior valor do IMC; por sua vez os doentes com IMC>25kg/m^2 tinham pior perfil lipídico. Estes resultados mostram uma menor incidência de EAM ([43]) e melhor controlo lipídico ([44]) do que o que tem sido reportado para o TR isolado. Sendo a população em estudo, de TRP, com décadas de evolução de diabetes e com longos anos de insuficiência renal, das de maior risco CV, os resultados obtidos podem ser considerados muito positivos. Conhecidos que são os riscos CV associados aos corticóides, a estratégia seguida na Unidade de suspensão de corticóides da imunossupressão dos TRP, ganha mais argumentos, face a estes resultados.

Evolução do metabolismo mineral e da doença óssea após TRP

A doença óssea renal tem elevada prevalência nos doentes que são submetidos a TRP ([45,46]), sendo as fraturas uma temida complicação ([46,47]), com elevada morbilidade, que chegam a contabilizar-se em 45% dos casos ([47]). Esta população é particularmente suscetível, dado que soma vários fatores que contribuem para a doença óssea, como a diabetes, a doença renal e a utilização dos corticóides ([48]). A adicionar aos 2 últimos, a diabetes é por si só mais um fator predisponente à osteopenia e à baixa remodelação óssea. São conhecidos os efeitos anabólicos da insulina no osso e os efeitos nefastos dos AGE no metabolismo ósseo ([49]).

Existem poucos estudos sobre a doença óssea no TRP, particularmente com mais de 1 ano de seguimento. Nos estudos a 12 meses é normalmente documentada uma perda óssea nos primeiros 6 meses, com estabilização ou recuperação posterior ([46,48]). Esta perda óssea inicial tem sido atribuída a vários fatores, nomeadamente ao uso de corticóides ([50]). Alguns estudos mostraram que esquemas imunossupressores poupadores de corticóides ajudavam a preservar a densidade mineral óssea nos transplantados ([51,52]). A persistência do hiperparatiroidismo após o TR é mais um fator de risco para perda óssea ([52]) e para fraturas ([53]). Foram recentemente publicados os dados do registo norte-americano sobre a osteoporose nos doentes com DM1,

submetidos a TR ou TRP. Verificou-se um menor risco de fratura nos que foram submetidos a TRP, do sexo masculino ([54]).

As normas de orientação clínica KDIGO ([55]) preconizam uma avaliação da densidade mineral óssea nos primeiros 3 meses após TR. Defendem também a utilidade duma avaliação posterior, após estabilização da função renal e redução das terapêuticas concomitantes e da imunossupressão mais intensa, que afeta a remodelação óssea ([55]).

Num primeiro estudo (**artigo V.1**), retrospetivo, fomos estudar a evolução das DMO e dos marcadores bioquímicos de metabolismo ósseo nos nossos TRP. Observámos uma prevalência de osteopenia de 36,8% e de osteoporose de 28% na primeira avaliação peri-transplante. Nas DMO seriadas ao longo do tempo, encontrámos aos 12 meses uma recuperação da densidade mineral óssea na coluna lombar (melhor T-score). Nos doentes com seguimento até aos 4 anos após TRP confirmou-se a melhoria do T-score, sobretudo a nível lombar. Esta recuperação, tal como noutros estudos ([46,47,48]), foi mais notória no osso trabecular (coluna) do que no osso cortical (fémur). A incidência de fraturas pós-TRP foi baixa (8,7%), considerando os valores descritos noutras séries, no TR isolado ([56]) e no TRP ([47,57]). De realçar que 86,7% destes doentes estavam sem corticóides. A estratégia terapêutica adotada na Unidade de suspensão de corticóides, e eventualmente a medicação usada para reduzir a perda óssea (bifosfonatos, cálcio) pode ter estado na base destes resultados animadores.

Fomos então analisar prospetivamente (**artigo V.2**) a evolução do metabolismo ósseo nos TRP. Neste trabalho identificámos 2 preditores independentes da densidade mineral óssea: a FA sérica e o IMC.

A associação entre baixo IMC e piores índices na DMO havia já sido descrita ([58]). A FA elevada no pré-transplante foi descrita como associada a maior mortalidade no pós-TR isolado ([59]). Alguns autores defendem a utilização da FA como marcador do metabolismo ósseo ([60]), uma vez que a correlação entre esta e a histomorfometria óssea será igual ou superior à encontrada com a PTH. Na nossa experiência houve normalização do hiperparatiroidismo. Neste grupo de doentes - mais de 80% sem corticóides – menos de 10% tinham osteoporose na última avaliação. Este trabalho confirmou a melhoria significativa do T-score lombar ao longo das 3 avaliações, bem como melhoria do osso cortical (fémur) entre a 1ª a e 2ª avaliação. Os resultados aqui encontrados mostram uma melhoria da densidade mineral óssea com tempo de

evolução mais longo e consolidam os achados no estudo retrospetivo previamente realizado.

É sabido que não há uma boa correlação entre DMO e a "qualidade" do osso, que só pode ser dada pela histomorfometria óssea. A DMO serve como indicador do volume ósseo. Mesmo assim, observar um ganho no volume ósseo, como foi encontrado nos nossos trabalhos ao longo do seguimento pós-TRP, é algo mais reconfortante do que observar a perda continuada da massa ósseo (através da DMO), reportada noutras séries. O trabalho prospetivo permitiu, ainda, identificar a FA à data do transplante como preditor da osteoporose, quer no osso cortical quer no osso trabecular, o que permite identificar doentes de risco.

A recorrência da autoimunidade pancreática após o transplante

A autoimunidade anti-ilhota pancreática, celular e humoral, como causa da DM1 é um facto comprovado ([61]). A sua recidiva no pâncreas transplantado foi reportada pela primeira vez há 3 décadas atrás, e demonstrada como potencial causa de falência do enxerto ([62,63]). Tem sido pouco valorizada até aos anos mais recentes, estando provavelmente subestimada ([64]). Nos últimos anos tem merecido maior atenção e alguns autores consideram que autoimunidade e aloimunidade podem contribuir em partes iguais para a perda do pâncreas transplantado ([64]). A nossa investigação sublinhou a relevância do seu rastreio e impacto clínico. É necessário o reconhecimento precoce da doença e a suspeição desta etiologia nos diagnósticos diferenciais de causas de disfunção do enxerto.

A recidiva duma doença autoimune após o transplante (como a DM1), com imunossupressão mantida, não é um dado novo. A imunossupressão usada para evitar a rejeição (aloimunidade), provou não ser capaz de controlar a autoimunidade ([64]). Exemplo disso são doenças autoimunes como o *lupus* ou as *vasculites ANCA-positivo*, que podem recidivar nos doentes TR, sem descontinuação da imunossupressão e sem evidência de rejeição. O contacto *de novo* com antigénios que o doente previamente não expressava há muitos anos (insulina endógena, proteínas de superfície das células beta pancreáticas, etc.) pode reativar a resposta imune. Fenómeno semelhante foi verificado, por exemplo, nos doentes com síndrome de Alport. Estes, após TR, podem desenvolver anticorpos anti-membrana basal glomerular contra antigénios do colagénio tipo 4 da membrana (que antes não expressavam), reconhecendo como estranhos esses antigénios expressos no aloenxerto renal "normal".

O diagnóstico de certeza da recidiva da diabetes autoimune no enxerto, faz-se por biópsia pancreática a demonstrar "insulite" isolada ([63]), sem atingimento do tecido pancreático exócrino, normalmente acompanhada de marcadores serológicos, os autoanticorpos pancreáticos. O infiltrado linfocitário atacando especificamente as células beta, e poupando células alfa e delta ([65]) é caraterístico ([63]).

Um grupo norte-americano mostrou que os linfócitos T autoreativos GAD-específicos que apareceram em circulação na recidiva da DM1, desapareceram ou reduziram para níveis muito baixos após terapêuticas com soros anti-linfocitários B ou T. No entanto, reapareceram meses a anos depois, com o mesmo fenótipo, acompanhados de subida dos anticorpos pancreáticos e seguidos de falência total da produção de insulina ([64,66,67]). Estes estudos mostraram que as terapêuticas tentadas não são eficazes e que a recidiva representa a ativação da resposta latente de memória ([64]).

Se os anticorpos têm um papel patogénico direto ou se são apenas marcadores de lesão, não é conhecido. São contudo marcadores indiretos, não-invasivos ([63,68]), com boa correlação com a atividade autoimune ([63,69]). Apesar de não serem o teste definitivo de diagnóstico, a sua fácil exequibilidade e a disponibilidade na maioria das Unidades, levou a que fosse recomendado o seu doseamento periódico como teste de rastreio e monitorização ([64]). Por conseguinte, avaliações mostrando reaparecimento *de novo* ou subida crescente de título, devem funcionar como sinal de alerta para a possível perda autoimune ([63,68,69]). Nem todos os autores verificaram associação entre a subida destes anticorpos e a falência do pâncreas ([70]). Foi reportado um caso, com insulite na biópsia e perda do pâncreas, sem subida dos anticorpos pesquisados ([71]). Contudo, na presença de autoimunidade pancreática *de novo* ou exacerbada, baseada nestes dados serológicos, será recomendável proceder a uma vigilância mais apertada e prosseguir com a estratégia que nos conduza ao diagnóstico definitivo.

Nos estudos realizados na nossa Unidade pudemos confirmar quer a manutenção da negatividade dos autoanticorpos pancreáticos em vários doentes, quer a manutenção da sua positividade, ou mesmo o seu reaparecimento *de novo* noutros doentes TRP (**artigos VI.1 e VI.3**). No primeiro trabalho publicado (**VI.1**), não encontrámos correlação entre a positividade dos anticorpos (em 34% dos doentes) e pior sobrevivência do pâncreas. Embora esse grupo compreendesse um reduzido número de doentes com pior controlo glicémico, não se verificou que na globalidade do grupo houvesse um perfil glicémico significativamente pior.

O estudo seguinte (**artigo VI.3**), com maior número de casos e maior seguimento, teve por objetivo avaliar a evolução dos doentes com autoanticorpos pancreáticos positivos e procurar eventuais fatores de risco. A positividade destes anticorpos à data do transplante (em 35% dos doentes) não se revelou um fator de risco para perda ou pior função do pâncreas. Do mesmo modo, o esquema imunossupressor, a retirada de corticóides, ou a documentação de rejeição aguda prévia, também não foram fatores de risco. No grupo que no final do seguimento apresentava pelo menos um dos autoanticorpos positivos (43,8% dos casos) os anti-GAD eram os anticorpos mais frequentes (31,4%). O maior número de compatibilidades HLA, na análise univariada parecia associar-se a maior probabilidade de anticorpos positivos. Na análise multivariada não se confirmou essa associação com peso significativo. Contudo, confirmou-se uma associação significativa entre HbA1c mais alta e Pept-C mais baixo, com a presença dos anticorpos. Mais ainda, mostrou que a probabilidade de existirem anticorpos pancreáticos positivos era >5 vezes superior nos doentes com HbA1c >5,6%; e que essa probabilidade era significativamente inferior (0,65) nos doentes com níveis de Pept-C mais altos. A função do enxerto renal não diferia entre os dois grupos, desfavorecendo a hipótese da potencial existência dum processo aloimune subjacente (em vez da autoimunidade) como causa deste perfil glicémico mais desfavorável.

O que estes dois estudos apontam é o carácter evolutivo desta patologia: com mais doentes estudados e um seguimento mais longo, confirmou-se a tendência que o estudo inicial indiciava, de glicemias mais altas e Pept-C mais baixo nos doentes com marcadores de autoimunidade pancreática positivos. A continuação da análise prospetiva destes casos permitirá tirar ilações quanto à sua evolução futura e ao impacto na função e sobrevivência do enxerto pancreático.

Os **trabalhos VI.2 e VI.4**, capítulo de um livro e mini-revisão sobre este tema, dão conta não só da nossa experiência, como também do estado da arte atual nesta matéria.

Uma questão pertinente que se coloca de seguida, é que intervenção se poderá realizar no sentido de frenar o processo autoimune, assumindo que ele prejudicará o enxerto a longo prazo. Os esquemas imunossupressores usados na prevenção e tratamento da rejeição de órgãos sólidos, já demonstraram não ser de grande eficácia, pelo menos duradoura ([66,67]).

Até ao momento, mesmo após diagnóstico precoce da DM1, todos os ensaios usando novas drogas (abatacept, etanarcept, teplizumab, ou mesmo o rituximab, já usado na rejeição humoral do TR) falharam na evicção da progressão da destruição autoimune do pâncreas e diabetes declarada ([72]). Já nos anos 80 havia sido tentado o tratamento destes casos com ciclosporina A, sem sucesso ([73]). Apenas o transplante de células precursoras hematopoiéticas mostrou até agora resultados algo promissores ([72]).

Novas armas terapêuticas, imunomoduladoras, estão atualmente em estudo: direcionadas a células T, proinsulina-reativas, CD8+ ([74]); ou terapêuticas anti-CD3 ([75]); ou ainda utilizando células T reguladoras com atividade específica dirigida à autoimunidade ([76]). Um desafio importante destas terapêuticas é que elas não comprometam a atividade imune normal, necessária ao indivíduo.

Esta é, assim, uma área-alvo de intenso interesse e pesquisa atual, que poderá trazer alguma esperança tanto aos diabéticos tipo 1 no início da sua doença, como aos TRP com recidiva da autoimunidade pancreática. Perseveraremos na atenção dispensada a este aspeto da autoimunidade nos nossos doentes transplantados.

A aloimunidade no TRP

Apesar dos inúmeros progressos verificados no TRP nas várias áreas, e também na prevenção da rejeição aguda, esta permanece mais alta do que no TR isolado. Não só o maior volume de massa antigénica, mas também a menor atenção dada à compatibilidade HLA no TRP (por razões várias, nomeadamente menor nº de recetores em lista), poderão explicar esta maior incidência de rejeição aguda ([77]). Tal como descrito para a autoimunidade, também na rejeição (aloimunidade), o diagnóstico de certeza apenas pode ser dado pela biópsia do enxerto. A biópsia do enxerto pancreático, percutânea e guiada por ecografia ou TAC, não é isenta de riscos e a taxa de complicações pode ser superior a 11% ([78]) – o que faz com que muitos centros não a realizem. A indicação para biópsia baseada em critérios clínicos (ex: hiperglicemia) pode ser demasiado tardia; por outro lado a rejeição no TRP pode ser assintomática. A busca por biomarcadores de agressão imume tem sido uma constante nas últimas décadas.

A pesquisa protocolada de anticorpos HLA de novo pós-transplante, particularmente se dador-específicos (DSA), merece atualmente especial atenção e tem sido usada para rastrear reatividade aloimune no recetor. No TR, foi demonstrada em vários estudos a associação entre a presença de DSA e a pior sobrevivência do enxerto

([79,80,81]). No TRP os estudos são muito escassos. Encontrámos apenas 2 trabalhos com um nº de doentes e tempo de seguimento relevantes ([82,83]). Ambos observaram que os DSA se associavam a pior sobrevivência do pâncreas. Cantarovich et al ([82]), encontrou uma maior incidência de rejeição aguda quando existiam anticorpos HLA não específicos para o dador, mas maior ainda e com maior severidade se eram DSA.

Nos TRP realizados na nossa Unidade (**artigo VI.5**), a incidência de DSA de novo foi de 13,2%, o que não é diferente do observado nos estudos prévios ([82,83]), mas o tempo médio para o seu aparecimento foi mais tardio (3,3 anos) do que o reportado por um deles ([82]) – cerca de 1 ano. A indução com globulina anti-timocítica ([84]) e a manutenção com tacrolimus – em comparação com everolimus ([85]), parecem associar-se a menor incidência de DSA de novo. À semelhança de outros estudos no TR, verificámos que a idade mais jovem dos recetores, a sensibilização HLA pré-TRP, o nº de incompatibilidades HLA em DR e a existência de rejeições agudas prévias foram fatores de risco para o desenvolvimento de DSA. Descrevemos pela primeira vez numa população com enxerto pancreático a associação entre rejeição aguda comprovada por biópsia e a formação dos DSA (já antes havia sido descrita, para o enxerto renal); a associação foi especialmente significativa quando a rejeição tinha sido mais grave, com atingimento vascular. Tal como previamente observado para a rejeição aguda no TR, a positividade para DSA teve comprovadamente um impacto negativo na sobrevivência dos dois enxertos. Analisando separadamente o risco de cada um destes eventos, a existência de qualquer um deles - rejeição aguda ou DSA – influenciou a sobrevivência do enxerto renal; os 2 em associação foram determinantes para a falência do pâncreas; e somados tiveram um efeito deletério sinergístico na sobrevivência do transplante duplo.

A presença de DSA quer contra os loci HLA A ou B (classe I) quer contra o locus DR (classe II) influenciou a falência dos enxertos. No TR isolado já havia sido descrita a associação de DSA existentes pré-transplante contra loci HLA classe I e classe II com risco acrescido de falência do enxerto ([86]). A constatação de que o nº de DSA presentes e a sua intensidade (por MFI) foram diretamente proporcionais ao nº de enxertos perdidos, dá relevo à necessidade de análise e valorização dos DSA que sejam detetados. Sabe-se também que em doentes hipersensibilizados a intensidade MFI dos DSA pré-existentes ([87]), ou o valor de pico de MFI ([88]), são dos preditores mais fiáveis de rejeição aguda mediada por anticorpos ([87,88]) e de perda do enxerto ([88]).

Constatámos que rejeição aguda e existência de DSA foram preditores independentes de falência de enxerto. Os DSA influenciarem a perda do enxerto, mesmo na ausência de rejeição clinicamente evidente, pressupondo uma evolução "silenciosa" da rejeição crónica mediada por anticorpo ("humoral crónica") na presença de DSA, que culminará na perda do enxerto. Este fenómeno tinha sido descrito para o TR isolado ([89]), mas segundo o conhecimento atual, não antes descritos para o transplante de pâncreas.

Cremos ser este o primeiro trabalho a reportar estes resultados no TRP: que também para o pâncreas se encontrou uma associação entre rejeição aguda comprovada por biópsia e DSA; e o efeito deletério independente da rejeição aguda e dos DSA na sobrevivência dos enxertos.

Os AGE após TRP

Inúmeras publicações demonstraram a associação dos AGE com a diabetes e a insuficiência renal ([90,91]). Comprovado que está o papel dos AGE nas complicações secundárias da diabetes ([90,91,92,93]), quer na doença microvascular ([94,95,96,97]), quer na doença macrovascular ([98,99,100]), seria interessante apreciar a sua evolução após o TRP. Com ambos os enxertos normofuncionantes, solucionam-se as 2 condições major para a acumulação dos AGE: a hiperglicemia, importante para a sua formação; e a insuficiência renal, importante para a sua excreção. Os AGE têm sido intensivamente estudados durante a evolução da diabetes. Após a transplantação, os estudos são praticamente inexistentes, à exceção de raros trabalhos publicados essencialmente no TR isolado, apenas um deles incluindo alguns doentes com TRP ([101,102]). A população com TRP é por isso um alvo novo de interesse neste domínio, praticamente por explorar. O trabalho publicado no âmbito desta tese (**artigo VII**) é, segundo o nosso conhecimento atual, o que estudou prospetivamente os AGE num maior número de doentes com TRP; e o primeiro a fazer o estudo da evolução dos AGE após o TRP simultaneamente na circulação sanguínea e depositados na pele.

Não existe ainda ainda, atualmente, um método-padrão de avaliação dos AGE. Por outro lado, continua a ser tema de debate qual o melhor AGE a estudar, de entre o heterogéneo grupo dos AGE; ou seja, qual o que melhor se correlaciona com a evolução das complicações da diabetes. Os AGE podem ser medidos no plasma ([103,104,105,106,107,108]), na urina ([103]), ou nos tecidos ([109,110,111,112]). A CML é o AGE mais bem caracterizado ([106]) e o que mais vezes surge nos estudos de avaliação plasmática ([103,104,105,106,107,108]). Níveis mais elevados de CML correlacionaram-se com progressão mais rápida da nefropatia, avaliada pelo espessamento da membrana basal glomerular

([103]); com aumento da rigidez arterial ([104]); com calcificação coronária mais intensa ([105]); e com maior incidência de eventos CV fatais and não-fatais ([107,108]), em doentes diabéticos. Mesmo em idosos não-diabéticos, também se associou a maior incidência de eventos CV ([113]). Os AOPP (advanced-oxidation protein-products) em doentes renais em estadio pré-diálise, também se associaram a maior número de eventos ateroscleróticos ([114]). Os níveis dos AOPP tendem a aumentar após o início da diálise ([115]) e foram mesmo propostos como um marcador fiável do dano proteico induzido pelo processo de oxidação.

A acumulação dos AGE nos tecidos pode ser avaliada directamente no tecido em estudo, por imuno-histoquímica ([109,110,111,112]), ou então por digestão enzimática do colagénio dos tecidos e medição posterior, por processos bioquímicos, dos AGE extraídos ([116,117]). A pele é o órgão mais usado para a sua avaliação por imuno-histoquímica ([109,110,111]), mas também se podem avaliar noutros tecidos, como os vasos ([112]). A expressão cutânea aumentada dos AGE em indivíduos não-diabéticos correlacionou-se com a lesão pela exposição solar ([109,111]). Em diabéticos, correlacionou-se com a desnervação da pele ([110]) e com a progressão da doença microvascular ([116,117]). A sua deposição nos vasos, com a doença macrovascular mais avançada ([112]). A decisão dos produtos a analisar no nosso trabalho (**artigo VII**) teve por base os estudos acima citados. No plasma, estudámos os AGE globalmente e a CML de modo específico, além dos AOPP, usando *kits* comerciais adquiridos para o efeito. Para o estudo imuno-histoquímico dos AGE na pele, adquirimos o anticorpo policlonal anti-AGE. Com estes marcadores e por ambos os métodos, pensámos poder inferir do estado oxidativo global destes doentes.

A determinação plasmática dos AGE refletirá a ligação às proteínas séricas, de maior *turnover*. A sua ligação a proteínas de baixo *turnover*, como o colagénio, será mais duradoura, e por conseguinte mais aproximada do dano tecidular ([118]). Este processo de remoção dos AGE nos tecidos será um processo lento; e a sua tradução na estabilização ou melhoria clínica das complicações secundárias da diabetes, um processo ainda mais lento e moroso, exigindo um mais amplo tempo de seguimento para a sua valorização. A teoria da "memória metabólica" ficou bem patente nos estudos DCCT/EDIC: os benefícios do controlo mais estrito da glicemia (o retardar da doença microvascular) mantinham-se mesmo decorridos mais de 10 anos após o término do estudo ([119,120,121]). Nestes doentes foi ainda estudada a evolução dos AGE no colagénio de biópsias de pele. Sabido previamente que os doentes que haviam sido submetidos a insulina intensiva tinham menor incidência de nefropatia e

retinopatia, este estudo mostrou que os níveis dos AGE estudados (CML e furosina) foram preditores da progressão da nefropatia e retinopatia ([122]). Resultam daqui alguns pressupostos: estes achados são consistentes com o fenómeno da memória metabólica; os AGE pelo seu modo de formação e acumulação e modo de ação, são talvez os agentes mais tradutores deste fenómeno; e, pelo mesmo princípio da memória metabólica, será expectável que estas alterações crónicas microvasculares não sejam facilmente revertidas, mesmo com bom controlo glicémico mantido e função renal normal ([118]). Apesar da limitação do tamanho da amostra (20 doentes), os resultados obtidos no estudo prospectivo ao longo do 1º ano pós-TR (**artigo VII**), permitiram mostrar uma redução dos marcadores – AGE, CML e AOPP – ao longo do seguimento, redução esta estatisticamente significativa para a CML e próxima da significância para os AOPP. A variação dos AGE foi menos marcada e menos consistente, notando-se contudo uma tendência à descida após os primeiros 6 meses. Um seguimento mais alargado poderá eventualmente confirmar a redução destes marcadores plasmáticos ao longo do tempo. A análise imuno-histoquímica da pele com o anticorpo anti-AGE, também denota uma redução da imunorreação ao longo do primeiro ano. Esta avaliação resulta duma apreciação do padrão e da intensidade, que é apenas semi-quantitativa (de 0 a 3+), e por isso mais difícil de traduzir em dados numéricos exatos.

Haverá eventualmente mais variáveis confundidoras na avaliação dos AGE no TRP. Foram já documentados outros eixos de lesão além dos AGE ([123]) - o stress oxidativo, a ativação da via dos polióis, e da PKC, são exemplos. Emergem atualmente estudos sobre mecanismos epigenéticos ([124]) que interferem não só com a evolução da doença microvascular, como podem ser determinantes na sua perpetuação e no fenómeno da memória metabólica. Por outro lado, é sabido que situações de inflamação ou rejeição no doente transplantado, e a própria imunossupressão, podem estimular o stress oxidativo e a formação de AGE – factores que podem explicar o aumento transitório dos AGE, CML e AOPP observado nos primeiros meses pós-TRP, no nosso estudo.

Assim, fará sentido no futuro alargar o seguimento e monitorização dos AGE nestes doentes submetidos a TRP; e a médio-longo prazo, encetar os estudos clínicos para avaliar a correlação entre a expressão dos AGE e a doença microvascular. Esta é uma área nova, muito pouco estudada após o TRP, por conseguinte com potencial interesse investigacional nos próximos anos.

O TRP e a qualidade de vida no doente com DM1 e doença renal crónica

Na avaliação do sucesso do transplante, os indicadores mais usados são as taxas de sobrevivência dos doentes e dos enxertos, de rejeição, e das principais complicações desse transplante. Não menos importantes, mas frequentemente não valorizados do mesmo modo nem avaliados, são os indicadores de qualidade de vida ganha (ou não) com o transplante. Nos anos mais recentes, tem merecido maior ênfase este aspeto da qualidade de vida relacionada com a saúde.

O transplante de pâncreas, sobretudo no pós-operatório precoce, pode trazer grande ansiedade e mal-estar ao doente, dada a morbilidade desse ato operatório e procedimentos associados. O risco de complicações, as consultas frequentes, as análises sanguíneas sucessivas, o receio da rejeição ou disfunção do enxerto, podem influenciar a qualidade de vida. Parece lógico, e será fácil de admitir, que o TRP bem sucedido libertando o doente da diálise e da diabetes, represente um ganho significativo de qualidade de vida. Contudo, para ser de facto vantajoso, os benefícios do TRP terão de sobrepor-se aos constrangimentos do transplante em si, das suas complicações, e até dos efeitos secundários da imunossupressão ([125]) – para além das vantagens que comprovadamente tem na sobrevivência ([126]).

Foram entretanto desenvolvidos inúmeros instrumentos para “medir” a qualidade de vida dos doentes. Os mais usados são os “patient-reported outcome”, questionários respondidos pelo próprio doente. Alguns autores defendem que os inquéritos de qualidade de vida deveriam fazer parte do processo de avaliação e de acreditação de todas as unidades de transplantação ([127]). Analisar a qualidade de vida antes e após o transplante, para além dos indicadores clássicos, permite avaliar o sucesso global desse tipo de transplante ([125]). Os custos com o TRP são elevados ([128]), claramente acima dos custos do TR isolado, sendo necessário mais tempo do que no TR para se reclamar um custo-benefício superior. Também por isso são importantes os estudos de qualidade de vida: um ganho evidente na qualidade de vida dá força aos argumentos a favor da indicação do TRP nos DM1 com doença renal avançada ([129]).

Existem vários questionários, mais genéricos ou mais específicos, ou seja, relacionados com a doença crónica desse grupo de doentes a quem são aplicados. No caso da diabetes, um dos mais usados é o “Diabetes Quality of Life Questionnaire” (DQOL). É dirigido aos doentes com diabetes e com complicações secundárias da doença, bem como às suas limitações, mas após o transplante algumas questões são irrelevantes ou não se aplicam ([125]). Não existem questionários dirigidos

especificamente ao doente diabético após o transplante de pâncreas. Dos inquéritos genéricos usados em várias doenças crónicas e no transplante, destacam-se o "Short-Form 36-item survey" ou SF-36 ([130,131]) e o EQ-5D ([132]). As queixas gastrointestinais do diabético, principalmente devidas à neuropatia autonómica – vómitos, náuseas, diarreia, enfartamento – podem ser debilitantes e até agravadas por alguns dos imunossupressores após o transplante.

No estudo que realizámos nos nossos TRP (**artigo VIII**), em 126 doentes, usámos 2 questionários: o EQ-5D; e um mais pormenorizado, com 36 questões agrupadas em 5 domínios (social; psicológico; físico; sintomas gerais; e sintomas específicos gastrointestinais), o estudo GIQLI. Nesse estudo, incluindo doentes com 1 ou ambos os enxertos funcionantes, foi-lhes solicitado que, para cada item, comparassem o seu estado na última visita com o estado prévio ao transplante. Encontrámos uma significativa melhoria em todos os 5 domínios do EQ-5D e também nos 5 domínios globais do GIQLI. A magnitude das variações encontradas foi claramente significativa. Observámos ainda um aumento significativo do número de doentes que conseguiram voltar a trabalhar ou estudar após o TRP. Das múltiplas variáveis incluídas na análise multivariada, somente a permanência de apenas um enxerto funcionante (em vez dos dois) foi preditora de índices de qualidade de vida inferiores. Quando pedimos que pontuassem o seu estado de saúde de 0 a 100%, obtivemos melhoria do valor médio de 40% pré-TRP, para 79% pós-TRP.

Este trabalho vem documentar um ganho significativo em qualidade de vida com o TRP nos nossos doentes. Dados de outros centros chegam mesmo a equiparar os índices de qualidade de vida após TRP com os da população saudável ([133,134]); e que a perda de um enxerto prejudica claramente a qualidade de vida ([133]). Comparados com os doentes com DM1 que recebem apenas TR isolado, existem resultados díspares sobre se o TRP é superior ao TR ou não ([135]). Estudos prospetivos mostram a superioridade do TRP também neste aspeto da qualidade de vida ([136,137]).

Em suma, os resultados obtidos com o TRP, além de permitirem um melhor controlo metabólico e bons índices de evolução clínica e de sobrevivência, mostram também que o TRP pode melhorar significativamente um aspeto que é tão importante como a qualidade de vida, nos diabéticos tipo 1.

Perspetivas futuras

A pesquisa clínica das variáveis que podem influenciar o sucesso do TRP será um processo contínuo na nossa Unidade. No imediato, o achado do impacto negativo da DP no TRP, terá de ser mais estudado. Seria relevante reproduzir a nossa investigação e validar esses resultados em mais ampla população de doentes com TRP. É importante saber quais as causas que podem condicionar, nestes doentes, a sua sobrevivência e a maior incidência de complicações infeciosas e trombóticas. Se a DP como terapêutica dialítica inicial na doença renal crónica provou ser uma boa opção para muitos dos doentes enquanto aguardam por um transplante; e se em regra estes são doentes com menos tempo de diálise e sem maior número de comorbilidades, é premente conhecer as razões destes resultados. Será realizado um estudo em colaboração com a Hematologia para avaliar o risco trombótico nos doentes candidatos a TRP. Teremos de pesquisar prospetivamente fatores que possam ser determinantes de maior infeção nestes doentes, e encetar esforços para a sua prevenção.

A relevância dos AGE e o contributo do processo de glicosilação avançada para a doença micro e macrovascular nos diabéticos tem sido foco de intensa investigação. Estes estudos têm sido realizados maioritariamente em diabéticos tipo 1 ou tipo 2, à medida que evolui a sua doença, mas pouco após o transplante. Não existe um paralelismo estrito entre o teor dos AGE e as diversas complicações, porque elas dependem de vários e intrincados mecanismos. A medição dos AGE depositados na pele, através de métodos de leitura da autofluorescência emitida, tem recentemente recebido particular destaque. Após o TRP, os estudos são muito escassos. Com um transplante que restabelece a normoglicemia e resolve a uremia, poder-se-ão observar a médio e longo prazo resultados interessantes quanto à evolução dos AGE – por métodos bioquímicos, histológicos ou por autofluorescência. A partir dos estudos que demonstraram a teoria da “memória metabólica”, pode inferir-se que uma involução das complicações secundárias da diabetes após TRP, a ocorrer, só se evidenciará mais tardiamente, após vários anos. Também se sabe que muitos outros fatores distintos, que podem coexistir em paralelo com os AGE, podem interferir neste processo. Ainda assim - e apesar de não ser possível isolar apenas o contributo da glicosilação dos tecidos para a doença microvascular - será interessante tentar correlacionar, a mais longo prazo, a evolução dos AGE com a evolução das complicações secundárias da diabetes, avaliadas por métodos que as tornem mensuráveis.

A relevância da recidiva da autoimunidade anti-ilhota no enxerto pancreático merece mais atenção. Esta é uma área que tem sido descurada, mas que pode refletir-se no futuro numa redução significativa de enxertos perdidos, se vier a ser possível prevenir a sua recidiva ou tratá-la eficazmente. Para tal, é necessário conhecer melhor os mecanismos subjacentes e identificar fatores de risco para a sua recorrência. Há ainda um longo caminho a percorrer neste domínio, e na minha opinião pessoal, esta é uma das áreas mais apelativas de estudo no TRP.

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RESUMO

O TRP constitui a alternativa terapêutica que conduz aos melhores resultados em DM1 com doença renal crónica, tendo-se tornado por isso o tratamento recomendado nesta situação, em doentes selecionados. Trata a insuficiência renal e restaura a normoglicemia, potencialmente revertendo ou estabilizando as complicações secundárias de ambas as doenças, e assim melhorando a sua qualidade de vida.

A complexidade dos doentes, da cirurgia e do pós-operatório, aportam uma morbilidade significativa a este procedimento. As comorbilidades destes doentes que padeceram de diabetes e suas complicações durante décadas, acrescentam dificuldades ao seu manuseamento e à obtenção de resultados a longo prazo.

Reportámos pela primeira vez a experiência de um programa de transplante de rim e pâncreas em Portugal, no decurso de mais de 10 anos de atividade (capítulo II). Descrevemos os critérios de seleção dos candidatos, a técnica cirúrgica, o protocolo imunossupressor utilizado e as principais complicações (artigo II.1). Seguindo as orientações de prática clínica aceites internacionalmente, obtivemos resultados ao 1º e 5º ano semelhantes aos reportados pelo registo internacional de transplante de pâncreas (IPTR).

Aos 10 anos (artigo II.2), verificámos que continuávamos a obter resultados dentro dos padrões de outros centros de referência mundial e do IPTR. O número de doentes transplantados anualmente cresceu progressivamente nesses anos, tendo estabilizado em cerca de 15/ano. Notámos uma redução da incidência de rejeição aguda, que entretanto passámos a poder afirmar ou excluir por biópsia pancreática guiada por imagem, através de um protocolo de colaboração com a Radiologia. Houve modificações na técnica cirúrgica que se traduziram num menor número de complicações. Verificou-se uma ligeira redução do tempo prévio em diálise, reflexo da melhor resposta do programa. Foi possível realizar até essa altura 4 TRP *preemptive*. Num grupo de 150 doentes (artigo II.2.1) sublinhámos os dados de sobrevivência ao 1º e 10º ano: de 97% e 91% para o doente; 93% e 79% para o enxerto renal; e 85% e 69% para o enxerto pancreático, respetivamente.

São sobejamente conhecidos os efeitos deletérios dos corticóides, fármacos que por norma integram os esquemas imunossupressores. A sua suspensão é ambicionada, ainda mais no doente de maior risco CV, como é o caso dos TRP que sofreram de diabetes durante décadas. Contudo, esta suspensão não é isenta de riscos e a

rejeição é temida com esta mudança terapêutica. Reportámos a nossa experiência bem sucedida de retirada de corticóides no TRP, após os primeiros 6 meses (artigo II.3). A seleção criteriosa dos candidatos – sem eventos imunológicos prévios significativos e que podiam manter terapêutica dupla com tacrolimus e micofenolato ou sirolimus – conduziram à retirada segura dos corticóides. Não observámos episódios de rejeição aguda ou agravamento da função renal. Verificámos que a população estudada apresentava uma prevalência baixa de hipertensão e dislipidemia.

A complexidade dos doentes diabéticos submetidos a TRP está patente nos trabalhos apresentados no capítulo III. No artigo III.1 reportámos a frequência (32%) e o motivo das relaparotimias, a duração média do internamento do transplante (maior que no TR, tal como descritos por outros centros), e as principais complicações encontradas durante esse internamento inicial. Tivemos readmissões após alta em 74,2% dos doentes. A causa infeciosa foi a mais frequente, mais elevada no 1º ano pós-TRP; as infeções víricas ou fúngicas sistémicas as que se revestiram de maior gravidade e maior tempo de internamento. Metade dos óbitos até aí registados, foram de causa infeciosa (3 casos).

A articulação com a Radiologia tem sido profícua e tem permitido diagnosticar, ou mesmo tratar, alguns dos problemas que podem ocorrer no TRP. O vasto leque de situações com que o radiologista se pode deparar, face a um doente com este tipo de transplante, foram apresentados no artigo III.2. Salienta-se a importância da familiarização das outras especialidades, como a Radiologia, com o transplante pancreático e com as suas complicações.

A perceção de que haveria maior morbilidade nos doentes submetidos a TRP que previamente tinham feito DP, levou-nos a analisar com mais atenção estes casos (artigo III.3). Confirmámos existir uma maior incidência de infeção intra-abdominal e perda do pâncreas nos doentes em DP; e uma maior incidência de trombose do enxerto renal. Constatámos que a DP foi, a par da doença CV e da perda de um dos enxertos, um preditor independente de morte do doente. As variáveis estudadas não nos permitiram encontrar razões para este desfecho, que os diferenciem dos doentes em HD. Sendo a estratégia de opção por DP como modalidade dialítica inicial, por norma, considerada uma boa opção; e tratando-se neste caso de uma população de doentes jovens e com menos tempo de diálise, é premente estudar mais aturadamente este assunto. Num grupo de doentes mais amplo e com maior seguimento, é necessário esclarecer os motivos destas complicações nos doentes em DP, e preveni-

las ou trata-las. Só assim se poderá continuar a prescrever a DP nos doentes candidatos a TRP.

Para investigar a prevalência da doença CV clinicamente sintomática e o perfil de risco CV associado, fomos estudar uma coorte de 103 TRP com ambos os enxertos funcionantes (artigo IV). A prevalência de síndroma metabólica foi baixa, comparada com o descrito para doentes diabéticos que receberam TR isolado. A HTA foi, de entre os vários critérios para esta síndroma, o mais frequentemente observado (38,5%); a dislipidemia foi menos relevante; e a obesidade foi rara. Os eventos CV pós-TRP ocorreram em 6,8% dos doentes. Pudemos correlacionar a retirada de corticóides com um nível de triglicerídeos mais baixo e menor frequência de HTA. Os doentes com HTA tinham IMC mais alto; estes com IMC mais alto, tinham valores de colesterol total e LDL-colesterol mais elevados. Observámos, assim, um perfil CV mais favorável nos doentes sem corticóides.

A doença mineral óssea em doentes renais crónicos e com diabetes, é relevante. A sua evolução após TRP tem sido pouco estudada. Fomos analisar esta questão nos TRP da nossa Unidade (capítulo V). Num estudo retrospetivo em 57 doentes, maioritariamente do sexo feminino mas em idade pré-menopáusica, verificámos que 28% tinham critérios de osteoporose pré-TRP (artigo V.1). Ao longo de 4 anos, verificou-se uma melhoria claramente significativa nos parâmetros da DMO (T-score) a nível lombar, e menor a nível femoral. A grande maioria dos doentes tinha suspendido corticóides, e na nossa Unidade é prática comum tratar os défices de vitamina D e de cálcio nestes doentes. A incidência de fraturas ósseas foi <10%. Fomos analisar então prospetivamente (artigo V.2) a evolução dos parâmetros bioquímicos de metabolismo ósseo, correlacionando-os com 3 DMO seriadas (intercaladas por um período ≥ 1 ano), e com a evolução do IMC. Neste grupo, 40% tinham osteoporose no colo do fémur e 35% na coluna lombar pré-TRP. Observámos que o T-score não se correlacionou com o sexo, tempo de diabetes ou de diálise, nem com o cálcio ou o fósforo séricos. Confirmámos uma melhoria significativa do T-score lombar ao longo das 3 DMO e também, embora menos constante ao longo do tempo, do T-score femoral. Encontrámos uma correlação entre a FA mais alta e piores índices na DMO; e melhoria na DMO com o aumento do IMC. A FA foi descrita em alguns estudos como tendo melhor correlação com a histomorfometria óssea do que a PTHi. Embora não podendo afirmar que estes doentes evoluem para uma resolução da sua osteodistrofia – porque só a histomorfometria poderá confirmar a natureza e a severidade da doença óssea subjacente - a realidade é que observámos um aumento da massa óssea, o que

é positivo. O índice de fraturas neste grupo foi também <10%. A estratégia de retirada de corticóides e de tratar ativamente os défices de vitamina D e de cálcio pode estar na base destes resultados favoráveis observados nas DMO.

A recidiva ou persistência da autoimunidade anti-ilhota pancreática foi um tema central de estudo nesta tese (capítulo VI). Se é a autoimunidade a causa essencial da destruição das células beta pancreáticas, então é provável que a sua positividade pós-TRP tenha um efeito negativo na função (endócrina) e sobrevivência do enxerto pancreático. Num estudo inicial em 77 doentes (artigo VI.1) detetámos a presença de autoanticorpos pancreáticos em 22 doentes (28,5%). Cerca de ¼ destes tinham positivado após o TRP, e em alguns casos apresentavam título crescente nas medições seriadas. Não pudemos correlacionar esta autoimunidade com a rejeição aguda, a retirada de corticóides, os níveis dos outros imunossupressores, nem com as compatibilidades HLA. Verificámos que era de entre o grupo com estes autoanticorpos positivos, que sobressaíam alguns doentes com perfil glicémico mais adverso, ainda que sem significado estatístico. Levámos então a cabo um novo estudo (artigo VI.3) numa amostra mais alargada de doentes. Confirmámos que os autoanticorpos pancreáticos podiam persistir negativos, manter-se positivos, ou surgir de novo após o TRP. Continuámos sem encontrar correlação com a imunossupressão ou a rejeição, encontrámos sim uma correlação com maior compatibilidade no locus HLA-B - contudo não confirmada na análise multivariada. Os doentes positivos (por persistência ou de novo) tinham uma glicemia em jejum e HbA1c mais elevadas e Pept-C mais baixo. Observámos que a probabilidade de possuírem autoanticorpos era mais de 5 vezes superior nos doentes com HbA1c >5,6%; e 35% menor quando tinham Pept-C mais elevado. A presença destes anticorpos à data do transplante não influenciou o prognóstico do enxerto pancreático. Até ao final do seguimento, a autoimunidade não se refletiu em pior sobrevivência do enxerto pancreático. A avaliação prospetiva destes doentes e de novos doentes, aumentando a dimensão da população estudada, poderá trazer resultados mais consistentes sobre o impacto da autoimunidade persistente ou recidivada no transplante pancreático.

O capítulo do livro (VI.2) e o artigo de revisão (VI.4) resultam duma maturada reflexão sobre este assunto, à luz dos conhecimentos em constante atualização. Abordámos também o problema da falta de eficácia duradoura das estratégias terapêuticas tentadas para prevenir ou tratar a autoimunidade anti-ilhota pancreática.

Estudámos adicionalmente a importância da aloimunidade no TRP (artigo VI.5). A redução da incidência de rejeição aguda neste transplante é um dado positivo que também registámos. Estudos a correlacionar rejeição aguda e DSA de novo com perdas de cada um ou ambos os enxertos no TRP são escassos. Encontrámos uma incidência de DSA de novo de 13,2%. A idade mais jovem; a sensibilização HLA pré-TRP, quer para classe I quer para classe II; e a rejeição aguda, foram fatores de risco para o aparecimento de DSA. Demonstrámos que a existência de episódios prévios de rejeição aguda, comprovada por biópsia no rim, e pela primeira vez também no enxerto pancreático, se correlacionavam com formação de DSA - especialmente se se tratava de rejeição aguda vascular (grau II ou III de Banff). Verificámos que a presença de DSA foi preditora da perda de cada um dos enxertos independentemente, e de ambos. A intensidade e o número dos DSA aumentaram de modo diretamente proporcional ao número de enxertos perdidos, permitindo porventura identificar os indivíduos de maior risco para perda de enxerto com a monitorização prospetiva dos DSA. Demonstrámos ainda que este impacto dos DSA foi independente da rejeição aguda: os doentes com DSA e sem rejeição aguda também evoluíram mais para perda do enxerto. À data da elaboração deste trabalho, cremos ser este o primeiro a reportar estes resultados no TRP, com base em dados de biópsias pancreáticas.

Os mecanismos de lesão microvascular na diabetes têm sido alvo de intensa investigação. Sabe-se que os AGE, a par de outros complexos processos, são uma das principais vias de lesão microvascular. Contudo, o seu estudo após TRP é muito escasso. Os AGE encontram-se sobretudo ligados às proteínas - e também a aminoácidos, fosfolípidos, ácidos nucleicos e a recetores celulares. Os seus níveis plasmáticos traduzem a sua ligação às proteínas circulantes, de renovação mais rápida. Os seus depósitos tecidulares traduzem a sua ligação a proteínas de longa duração, como o colagénio. A redução ou remoção destes depósitos tecidulares é por conseguinte mais demorada, consistentemente com a teoria da “memória metabólica”. Sabíamos à partida que estes pressupostos poderiam explicar resultados precoces menos relevantes, no estudo que levámos a cabo sobre a evolução dos AGE a nível plasmático e histológico pós-TRP (artigo VII). Um ano de evolução sem uremia e sem diabetes seria certamente um período curto para encontrar variações muito pronunciadas nos níveis dos AGE. Ainda assim, nesse estudo prospetivo, em 20 doentes e ao longo de 12 meses, obervámos uma descida dos AGE, da CML e dos AOPP plasmáticos pelo menos após o 6º mês pós-TRP, significativa para a CML e quase para os AOPP. Após uma subida inicial, possivelmente explicada pela fase

inflamatória mais intensa no pós-transplante imediato, observou-se em regra a descida dos 3 marcadores. Encontrámos ainda, na maioria dos doentes, uma alteração do padrão imunohistoquímico de deposição dos AGE na pele, de citoplasmático difuso para um padrão periférico ou interqueratinocítico; e com frequência uma menor intensidade dessa imunorreação. Estudos a mais longo prazo e num maior grupo de doentes poderão aportar resultados mais esclarecedores, e até permitir correlacionar a evolução dos AGE com a evolução das complicações secundárias da DM1 pós-TRP.

O sucesso global do transplante não se pode avaliar apenas pela análise da sobrevivência, rejeição ou comorbilidades. A qualidade de vida ganha, ou não, com o transplante, é um aspeto muitas vezes negligenciado. Contudo, pode e deve ser avaliada através de questionários desenvolvidos especificamente para quantificar essas mudanças na qualidade de vida. No diabético tipo 1, sob insulinoterapia desde muito jovem e com doença renal crónica em diálise, o TRP pode trazer enormes ganhos na qualidade de vida. O complexo período inicial pós-TRP, com as comorbilidades associadas, pode atrasar esse ganho de qualidade de vida. Porém, ultrapassada essa fase, o ganho tende a ser claramente superior ao obtido com o TR isolado nestes doentes. Os resultados do nosso trabalho sobre a qualidade de vida nos TRP da nossa Unidade (artigo VIII), demonstraram um claro e muito significativo ganho de qualidade de vida após o transplante. Este verificou-se a todos os níveis: físico, psicológico e social. Através do questionário específico para as queixas GI notámos uma melhoria significativa destes sintomas, muitas vezes tão debilitantes para estes doentes. Dos fatores analisados, a perda de um dos enxertos foi o único preditor de pior pontuação nos questionários de qualidade de vida. O incómodo com o tratamento foi marcadamente menor após o TRP do que antes do transplante. Observámos ainda que a devolução destes doentes a uma vida laboral ativa, recuperando o trabalho prévio ou conseguindo um novo trabalho, foi alcançada com maior sucesso após o TRP.

Palavras-chave:

AGE; análise de sobrevivência; anticorpos anti-dador; autoanticorpos pancreáticos; autoimunidade; causas de morte; complicações; diabetes autoimune; diabetes tipo 1; diálise peritoneal; doença metabólica óssea; doença cardiovascular; falência do enxerto; hemodiálise; infeção intra-abdominal; produtos-finais de glicosilação avançada; qualidade de vida; recorrência autoimune; rejeição do enxerto; resultados; síndroma metabólica; transplante pancreático; transplante renal; trombose do enxerto; trombose pancreática; trombose renal.

Lista de artigos publicados, ou em vias de publicação, no âmbito desta tese:

- "***Simultaneous Pancreas-Kidney Transplantation: Five-year results from a single center***". L. Martins, S. Pedroso, A. Henriques, L. Dias, A.M. Sarmento, R. Seca, F. Oliveira, J. Dores, A. Lhamas, T. Coelho, A. Ribeiro, S. Esteves, R. Pereira, R. Almeida, M. Amil, A. Cabrita, M. Teixeira. Transplant Proc 2006; 38:1929-1932.
- **"*One-hundred eleven simultaneous pancreas-kidney transplantations: 10-year experience from a single center in Portugal*"**. L S Martins, A C Henriques, L Dias, S Pedroso, M Almeida, J Santos, J Dores, R Almeida, A Cabrita, M Teixeira. *Transplant Proc* 2011; 43:205-208.
- ***"Pancreas-kidney transplantation: analysis of 150 patients from one centre in Portugal".*** La Salete Martins, I Fonseca, P Aguiar, A Rocha, R Costa, C Santos, J Malheiro, S Pedroso, M Almeida, L Dias, Ac Henriques, A Cabrita, J Davide. *Port J Nephrol Hypert* 2013; 27: 173-178.
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- **"*Cardiovascular risk factors and events in pancreas-kidney transplants*"**. Martins L, Fonseca I, Dias L, Malheiro J, Rocha A, Azevedo P, Silva H, Almeida R, Henriques AC, Davide J, Cabrita A. *Transplant Proc*. 2013 Apr;45(3):1063-5.
- "***Bone mineral density after Kidney-Pancreas Transplantation: four years follow-up in 57 patients***". Autores: S Pereira, S Pedroso, L Martins, P Santos, M Almeida, C Freitas, L Dias, J Dores, R Almeida, A Henriques, M Teixeira. *Transplant Proc* 2010; 42:555-557.
- **"*Changes in bone mineral density following long term simultaneous pancreas-kidney transplantation*"**. Ana Rocha A, La Salete Martins, Jorge Malheirro, Jorge Dores, Clara Santos, Rui Almeida, José Davide, Castro Henriques. (*Submetido*).
- "***Pancreas-kidney transplantation and the evolution of pancreatic autoantibodies***". L Martins, J Malheiro, AC Henriques, L Dias, J Dores, F Oliveira, R Seca, R Almeida, A M Sarmento, A Cabrita, M Teixeira. *Transplant Proc* 2009; 41:913-915.
- Capítulo do Livro: **Manual de Transplante de Pâncreas – "*Recidiva da Diabetes Mellitus pós-Transplante de Pâncreas*",** Autores: Editores: Irene L Noronha; Adriano M Gonzalez; Roberto F Meireles Jr. Editora: Segmento Farma. Edição: Manuais Ano: Set/2011. Páginas: 269-275. Livro indexado: sim – **ISBN 978-85-7900-023-2** (M294 / CDD 617.556).

- **"*Pancreatic autoantibodies after pancreas-kidney transplantation – do they matter?"*** La Salete Martins, Antonio C. Henriques, Isabel M. Fonseca, Anabela S. Rodrigues, José C. Oliverira, Jorge M. Dores, Leonídio S. Dias, Antonio M. Cabrita, José D. Silva, Irene L. Noronha. *Clin Transplant* 2014; 28: 462-469.
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- ***"Detrimental effect of de novo donor-specific HLA antibodies on grafts survival in simultaneous pancreas-kidney transplantation: does it go beyond acute rejection?"*** Jorge Malheiro; La Salete Martins; Sandra Tafulo; Leonídio Dias; Isabel Fonseca; Idalina Beirão; António Castro-Henriques; António Cabrita (*Submetido*)
- ***"AGE levels evolution after pancreas-kidney transplantation: plasmatic and cutaneous assessments".*** La Salete Martins, José C Oliveira, José Ramón Vizcayno, Isabel Fonseca, Carlos Gouveia, Donzília Silva, António C Henriques, Irene L Noronha, Anabela Rodrigues. (*Submetido*)
- ***"Health-related quality of life may improve after transplantation in pancreas-kidney recipients"*.** Authors: Martins LS, Outerelo C, Malheiro J, Fonseca I, Henriques AC, Dias L, Rodrigues A, Cabrita A, Davide J, Noronha I L. (*Submetido*)

SUMMARY

Pancreas-kidney transplantation (PKT) is the therapeutic choice that leads to the best results in type 1 diabetic patients with chronic renal disease. For this reason, it has become the recommended treatment for this situation, in selected patients. It treats renal failure and restores normoglycemia with the possibility of reversing or stabilizing secondary complications of both diseases, thus improving the quality of life of these patients.

The complexity of the patients, of the surgery and of its follow-up brings a significant morbidity to this procedure. The comorbidities of these patients, who have suffered from diabetes and its complications for decades, add difficulties when handling with them and may compromise long term results.

For the first time, we have reported the experience of a kidney-pancreas transplantation program in Portugal, with more than 10 years of activity (chapter II). We have described the candidates' selection criteria, the surgery technique, the immunosuppressive protocol, as well as the main complications (**article II.1**). By following the clinical praxis guidelines internationally accepted, we have obtained results on the 1st and 5th years, similar to the ones reported by the international pancreas transplantation registry (IPTR).

Ten years have elapsed (**article II.2**) and we found that we kept getting results within the standards of other world reference centers and of the IPTR. The number of annual transplanted patients has progressively increased during these years. They are now about 15 a year. We have noticed a decrease on the incidence of the acute rejection. Meanwhile we have been able to confirm or exclude this complication through image-guided pancreatic biopsy, after a collaborative protocol was established with Radiology. There have been changes on the surgical technique that led to fewer complications. We have also reported a slight reduction on the previous time on dialysis as a consequence of a better response of the program. In those 10 years we managed to perform 4 preemptive PKT. In a group of 150 patients (**article II.2.1**) we have highlighted the survival data on the 1st and 10th years: 97% and 91% for the patient; 93% and 79% for the renal graft; and 85% and 69% for the pancreatic graft, respectively.

The harmful effects of the steroids are quite well-known. They are drugs that normally integrate immunosuppressive regimens. Steroid withdrawal is a pursued strategy,

moreover in patients at higher cardiovascular (CV) risk, as it is the case of PKT patients who have been suffering from diabetes for decades. However, this withdrawal is not risk-free and rejection is feared from such therapeutic change. We have reported our well-succeeded experience of steroid withdrawal in PKT patients, more than 6 months (**article II.3**) after the surgery. Careful selection of candidates – without significant previous immune events and that could keep double therapy with tacrolimus and mycophenolate or sirolimus – led to the safe withdrawal of steroids. Episodes of acute rejection or renal function deterioration have not been observed. We have seen that the studied population showed a low prevalence of hypertension and of dyslipidemia.

The complexity of diabetic patients that have been submitted to a PKT is shown in studies presented in chapter III. In **article III.1** we have reported the frequency (32%) and the reason for relaparotomies, the average length of the transplantation hospitalization (longer than in the kidney-alone transplantation, as described by other centers), and the main complications found during that first hospitalization. We had readmissions after discharge from hospital in 74.2% of the patients. Infectious cause was the most frequent, at a higher rate in the 1st year after PKT; systemic viral or fungal infections were the most severe and the ones that required longer hospitalization. Half of the deaths registered by that time were infection-related (3 cases).

The collaboration with Radiology has been quite fruitful and has allowed the diagnosis or even the treatment of some of the problems which may occur after PKT. The wide range of situations the radiologist might face, in a patient with this transplant were put forward in **article III.2**. We pointed out the importance of the familiarization of other medical specialties, like Radiology, with pancreatic transplantation and its complications.

The perception that there was a higher morbidity within patients undergoing PKT, who had previously been on peritoneal dialysis (PD), led us to study these cases more carefully (**article III.3**). We have confirmed the existence of a higher intraabdominal infection incidence and loss of the pancreas in patients undergoing PD; and a higher renal graft thrombosis incidence. We have noticed that PD was, together with the CV disease and the loss of one of the grafts, an independent predictor of the patients' death. The studied variables have not allowed us to find reasons for this outcome that differentiate these from hemodialysis patients. The strategy of choosing PD as the initial dialytic method being usually considered a good option; and dealing, in this case,

with a young patient population with less time on dialysis, implies that we urgently need to study this matter thoroughly. In a wider group of patients and with longer follow-up, it is necessary to clarify the reasons for these complications in PD patients and to prevent or efficiently treat them. This is essential to keep going on prescribing PD to patients who may be candidates to a PKT.

To investigate the prevalence of the clinically symptomatic CV disease and the associated CV risk profile, we have studied a cohort of 103 PKT patients with both grafts functioning (**article IV**). The metabolic syndrome prevalence was low when compared with what was described for diabetic patients who have received an isolated kidney transplant. Among several criteria for this syndrome, hypertension was the most frequently observed (38.5%); dyslipidemia was less relevant; and obesity was rare. Posttransplant CV events have occurred in 6.8% of patients. We could correlate steroid withdrawal with a lower level of triglycerides as well as a lower frequency of hypertension. Hypertensive patients had higher body mass index; these had higher total cholesterol and LDL-cholesterol values. Thus, we have reported a more favorable CV profile in patients not taking steroids.

The bone mineral disease is relevant in chronic renal patients with diabetes. Its progress after PKT has not been enough studied yet. We have examined this issue in PKT of our Unit (chapter V). In a retrospective study with 57 patients, mostly female at pre-menopausal age, we have reported that 28% had revealed pretransplant osteoporosis criteria (**article V.1**). For 4 years there has been a clearly significant improvement in bone mineral density (BMD) parameters at lumbar level and, not so high, at femoral level. The majority of patients had suspended steroids and in our Unit it is common practice to treat vitamin D and calcium deficiency in these patients. The incidence of bone fractures was <10%. We decided, then, to analyze prospectively the evolution of bone metabolism biochemical parameters (**article V.2**), correlating them with the 3 consecutive BMD (at least 1 year apart one from each other) and with the evolution of the body mass index. Within this group, 40% had femoral neck osteoporosis and 35% on the lumbar spine, before PKT. We have observed that the T-score didn't correlate neither with the gender, duration of the diabetes or the dialysis, nor with the serum calcium or phosphate. We have ratified a significant improvement of the lumbar T-score over the course of the 3 BMD as well as of the femoral T-score, this one less constant over time, though. We have found a correlation between the highest alkaline phosphatase levels and the worst scores in the BMD; and an improvement of the BMD with the increase of the body mass index. In some studies, alkaline

phosphatase was described as having a better correlation with bone histomorphometry than the parathyroid hormone. Although we can't say that these patients evolve towards their osteodystrophy resolution – because only the histomorphometry can confirm the nature and the severity of the underlying bone disease – the fact is that we have registered an increase in bone mass, which is positive. The rate of fractures in this group was also under 10%. The strategy of steroid withdrawal and the active treatment of vitamin D and calcium deficiency may have contributed for these BMD favorable results.

The relapse or persistence of pancreatic autoimmunity has been a main issue of study in this thesis (chapter VI). If autoimmunity is the main cause for the destruction of pancreatic islet beta-cells, then, it is probable that this positive autoimmunity after PKT has a negative effect on the endocrine function and survival of the pancreatic graft. On the first study with 77 patients (**article VI.1**) we have detected the presence of pancreatic autoantibodies in 22 of them (28.5%). About ¼ of these patients turned positive after PKT and in some cases showed increasing levels during follow-up. We could not correlate this autoimmunity either with the acute rejection, steroid withdrawal, the levels of other immunosuppressants, or with HLA compatibilities. However, within the group with positive pancreatic autoantibodies we have seen some patients with a more adverse glycemic profile, although without statistical significance. We have performed a new study (**article VI.3**) with a wider sample of patients (135 patients). In this study, we have confirmed that the pancreatic autoantibodies could remain negative or positive, or could recur after PKT. We kept on not finding any correlation with the immunosuppression or with rejection episodes. We have initially observed that patients positive for these autoantibodies had higher compatibility on the HLA-B locus, but this association was not confirmed on multivariate analysis. The positive patients (due to persistence or recurrence) had higher fasting blood glucose levels, higher HbA1c and lower C-peptide. The probability of having autoantibodies was more than 5 times higher in patients with HbA1c > 5.6%; and 35% lower when they had higher C-peptide levels. The presence of these antibodies at the time of the transplantation has not influenced the pancreatic graft prognosis. Until the end of the follow-up, autoimmunity could not be associated with worse pancreatic graft survival. The prospective evaluation of these and of new patients, increasing the follow-up and the size of the sample population studied, may bring more consistent results about the impact of persistent or relapsed autoimmunity on the pancreatic transplant.

The chapter of the book (**VI.2**) and the review article (**VI.4**) are the result of a mature

reflection on this matter, having in mind constantly updated knowledge. We have also discussed the lack of lasting efficiency of the therapeutic strategies tried so far to prevent or to treat the pancreatic autoimmunity

Additionally, we have studied the importance of the alloimmunity in PKT (**article VI.5**). The reduction of acute rejection incidence in this transplant is a positive achievement that we have also registered. Studies correlating the acute rejection and the presence of donor-specific antibodies (DSA) with losses of each or both grafts in PKT patients are scarce. We have found an incidence of *de novo* DSA in 13.2% of our patients. Recipient younger age; pretransplant HLA sensitization, for class I or for class II; and acute rejection, were risk factors for the appearance of DSA. We have shown that the existence of previous acute rejection episodes, proved by kidney biopsy, and for the first time also by pancreatic graft biopsy, correlated with *de novo* DSA appearance – especially if it was a vascular acute rejection (grade II or III of Banff). We have seen that the presence of DSA predicted the loss of each graft independently, and also both grafts loss. The intensity and the number of DSA increased proportionally to the number of lost grafts, eventually allowing the identification of individuals with the highest risk of losing the graft with the prospective monitoring of DSA. We have also shown that this impact of DSA was independent from the acute rejection: patients with DSA and without acute rejection have also evolved more towards graft loss. At the time of this study, we believe it is the first one to report such results in PKT based on pancreas graft biopsies.

The mechanisms of diabetic microvascular disease have been intensely investigated. It is known that AGE, together with other complex processes, is one of the most important pathways of microvascular injury. However, its study after PKT is scarce. AGE are mainly linked to proteins – and also to amino acids, phospholipids, nucleic acids and to cellular receptors. Their plasmatic levels reflect their connection to circulating proteins, with higher turnover. Their tissue deposits represent their linkage to long lasting proteins, like collagen. Consequently, reduction or removal of these tissue deposits lasts longer, accordingly to the “metabolic memory” theory.

Before starting, we knew these facts would eventually explain less relevant results in the study conducted to evaluate plasmatic and histological AGE evolution after PKT (**article VII**). One year without uremia and without diabetes is certainly too short a period to find quite marked variations at the AGE levels. Even so, in these 20 patients with only a 12-months prospective evaluation, we have observed a decrease in the

mean AGE, CML and AOPP levels, at least after the 6th month post-PKT, being this significant for CML – and near significant for AOPP. After an initial transitory increase, possibly due to the intense inflamatory state in the early period after PKT, we have registered a consistent decrease of the plasmatic mean values of the 3 markers. We have also found, in the majority of patients, a change in the AGE immunoreaction pattern, from a diffuse cytoplasmic to a peripheral or interkeratinocytic pattern; and frequently a less marked immunoreaction intensity. Long term studies, in a larger sample, may bring more enlightening results or even allow the correlation between AGE changes and the evolution of secondary complications of diabetes after PKT.

The global success of a transplant cannot be evaluated only by the analysis of the survival, rejection or comorbidity data. The quality of life that people may or may not gain with the transplant is quite often neglected. However, it can and should be evaluated through surveys specifically held to quantify these changes in the quality of life. In type 1 diabetic patients, under insulin therapy from a young age and with chronic renal disease undergoing dialysis, PKT might bring about great gains in the quality of life. The complex posttransplant initial period, with the associated comorbidities, may delay this life quality gain. However, when people overcome this period, the gain tends to be considerably higher than the one gained with an isolated kidney transplant in these patients. Results of the study on the quality of life in the PKT of our Unit (**article VIII**) have shown a clear and quite significant gain in the quality of life after transplantation. It has been observed at all levels: physical, psychological and social. Through the survey that specifically evaluated the gastrointestinal complaints, we have reported a significant improvement of these symptoms, which are frequently so disabling for these patients. From the analyzed factors, the loss of one of the grafts was the only predictor of worst quality of life scores. The burden of the medical treatment was considerably inferior after PKT than before the transplant. We have also noticed that the returning of these patients to an active working life, recovering their previous job or finding a new one, was most successfully reached after the PKT.

Key-words:

Advanced glycation end-products; AGE; autoimmune diabetes; autoimmune recurrence; autoimmunity; cardiovascular disease; causes of death; complications; donor-specific antibodies; graft failure; graft rejection; graft thrombosis; hemodialysis; intra-abdominal infection; metabolic bone disease; metabolic syndrome; outcomes; pancreas transplantation; pancreatic autoantibodies; pancreatic thrombosis; pancreatic transplantation; peritoneal dialysis; quality of life; renal thrombosis; renal transplantation; survival analysis; type 1 diabetes.

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