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Anticuerpos antifosfolipídicos de clase IgA como factor de riesgo para morbi-mortalidad cardiovascular en el trasplante de órganos sólidos

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Manuel Serrano Blanco

DIRECTORES

José María Morales Cerdán
Antonio Serrano Hernández

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Anticuerpos antifosfolipídicos de clase IgA como factor de riesgo para morbi-mortalidad cardiovascular en el trasplante de órganos sólidos.

Memoria para optar a la obtención del título de
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Manuel Serrano Blanco

Bajo la dirección de:

Dr. José María Morales Cerdán
Dr. Antonio Serrano Hernández

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D. José María Morales Cerdán, Doctor en Medicina y Cirugía, Investigador Consultor del Instituto de Investigación Biomédica del Hospital 12 de Octubre de Madrid (i+12),

D. Antonio Serrano Hernández, Doctor en Medicina y Cirugía, Medico Adjunto del Servicio de Inmunologia del Hospital 12 de Octubre y Responsable del grupo de Investigación "Ingeniería de Tejidos y Autoinmunidad" Integrado en el área de Enfermedades Crónicas del Instituto de Investigación Biomédica del Hospital 12 de Octubre de Madrid (i+12),

INFORMAN:

Que el trabajo titulado "**Anticuerpos antifosfolipídicos de clase IgA como factor de riesgo para morbi-mortalidad cardiovascular en el trasplante de órganos sólidos"** ha sido realizado por D. Manuel Serrano Blanco, Graduado en Medicina y Cirugía, bajo su dirección en el Instituto de Investigación Biomédica del Hospital 12 de Octubre de Madrid (i+12). Dicho trabajo se presenta para optar al grado de Doctor.

Y para que conste, se expide y firma el presente

en Madrid a veintiocho de Septiembre de dos mil dieciocho.

José María Morales Cerdán, Director

Antonio Serrano Hernández, Director

Manuel Serrano Blanco, Doctorando

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“*La verdad os hará libres*” (Jn 8,32)

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INTRODUCCION

1- DEFINICIÓN DE SÍNDROME ANTIFOSFOLIPÍDICO

El síndrome antifosfolipídico (APS) es un trastorno autoinmune multisistémico caracterizado por la aparición de episodios de trombosis y/o morbilidad gestacional en pacientes que presentan en la sangre anticuerpos antifosfolipídico (aPL) (1). La primera descripción del APS fue hecha por Graham Hughes en 1983 y en ella se definió el síndrome como una tríada compuesta por 1) trombosis 2) morbilidad gestacional (sobretodo pérdidas fetales prematuras y recurrentes y 3) alteraciones hematológicas (trombocitopenia y anemia) (2).

Los aPL son un grupo heterogéneo de autoanticuerpos que reconocen fosfolípidos aislados, fosfolípidos formando complejos con proteínas asociados o a las propias proteínas de unión a fosfolípidos de forma aislada (sin estar asociadas a fosfolípidos). Al contrario que en la mayoría de los procesos autoinmunes asociados a la presencia de los mismos se encuentran fuera del torrente circulatorio, los antígenos reconocidos por los aPL se encuentran fisiológicamente en la sangre humana, ya sea en forma soluble o asociados con otras moléculas situadas en la membrana de células endoteliales, plaquetas y otras células involucradas en la cascada de coagulación (3, 4).

Aunque el APS se conoce como tal desde hace 35 años, la existencia de los aPL y su asociación con patología humana se conoce desde mucho antes (principios del siglo XX) y se describieron tras la introducción de las primeras pruebas serológicas para el diagnóstico de la sífilis.

Wasserman describió un método (basado en la fijación del complemento) que permitía detectar en la sangre de los pacientes con sífilis reactividad frente a extractos antigénicos obtenidos de hígados de fetos fallecidos a causa de la sífilis. Con posterioridad se demostró que esta reactividad también se conseguía si se usaban como antígenos extractos de hígado o corazón sanos, tanto humanos como de otros mamíferos. Según se profundizó en el estudio de esta reactividad se evidenció que la mejor fuente para obtener antígenos era el corazón de buey y que el antígeno concreto que reconocían los anticuerpos era el difosfatidilglicerol (cardiolipina). Este hallazgo permitió perfeccionar el sistema diagnóstico usando cardiolipina (CL) purificada estabilizada con lecitina y colesterol (5).

Ya desde los primeros años en los que se empezó a utilizar el test de Wasserman para diagnóstico de la sífilis se detectó que existían individuos que resultaban falsos positivos para este test. Se trataba de personas que no sólo no tenían la enfermedad sino que jamás habían estado en contacto con el agente causante de la sífilis (*Treponema pallidum) (*6). Moore y Mohr en 1952 realizaron un estudio sistemático en el que definieron que los falsos positivos podían aparecer en dos situaciones: 1) de forma transitoria en la convalecencia de enfermedades infeccionas o tras la administración de vacunas, y 2) de forma persistente en personas que padecían procesos autoinmunes (6, 7). En ese mismo año se publicaron dos casos de pacientes que padecían Lupus Eritematoso Sistémico (LES) y presentaban un tiempo de protrombina alargado pero la adición del plasma de de personas sanas no solo no revertía el test sino que cuando el plasma de los enfermos de LES se agregaba al plasma de personas sanas, se prolongaba también el tiempo de coagulación en los sanos. Esto llevó a considerar que en la sangre de los pacientes con LES existía un factor que se comportaba como anticoagulante *in vitro* el anticoagulante lúpico (AL). Durante muchos años se consideró que la presencia del AL era un hallazgo sin relevancia clínica porque los pacientes con AL solo tenían efecto anticoagulantes en las pruebas de laboratorio y no tenía ninguna repercusión clínica en forma de hemorragias *in vivo*. Esta actitud cambió radicalmente cuando Bowie y col. relacionaron la presencia de AL con la aparición de eventos trombóticos (8) y Nilsson y col. describieron la asociación de la presencia del AL con la aparición de abortos de repetición, dejando definida la asociación patogénica de la presencia del AL que conocemos en la actualidad (6).

La implementación de métodos de detección de la presencia de aPL para uso en pacientes con enfermedades autoinmunes de forma específica comienza en 1983 cuando Harris pone a punto un método de radioinmunoensayo destinado a detectar los anticuerpos anticardiolipina (aCL) no relacionados con la sífilis (9).En este mismo año es cuando Hughes, utilizando el método de Harris, describe el APS como "Síndrome anticardiolipina": un nuevo cuadro clínico en pacientes con Lupus Eritematoso Sistémico. Dos años más tarde se decidió cambiar el nombre de la entidad clinica por "Síndrome antifosfolipídico" (APS) que es como continúa denominándose actualmente (10).

Solo cuatro años después de describirse el APS Charles Mackworth-Young y col. describieron en una pequeña reseña en que el APS podía aparecer en pacientes sin Lupus eritematoso (11).Harris propuso que a esta forma del APS se la llamase síndrome del “Cisne negro”(12) (“Syndrome of the black swan”), denominación que no tuvo éxito por las dobles

interpretaciones de este término en lengua inglesa. En 1989, Asherson, Harris, Gharavi, Hughes y Alarcón-Segovia describieron el “síndrome antifosfolipídico” “primario” (PASP), caracterizado por la tríada descrita por Hughes pero en pacientes que no padecían LES.

Muy poco tiempo después, en 1989, se descubrió un hecho trascendental; Mientras que los pacientes con sífilis reconocen a la cardiolipina en forma aislada, los aPL de los pacientes con cuadros autoinmunes son incapaces de reconocerla a no ser que ésta se presente unida a un cofactor proteico (13, 14). Este descubrimiento permitió el diseño de test específicos para detectar los cuadros autoinmunes y los infecciosos de forma separada. Al año siguiente se identificó al cofactor proteico que debe asociarse con la cardiolipina para ser reconocida por los aCL, se trataba de la Beta-2-glicoproteína I (B2GP1), también conocida como Apolipoproteína H (Apo H) (6).Tras la identificación de los antígenos se realizaron diversos intentos de estandarización de los ensayos diagnósticos, que llevaron a desarrollar los criterios actuales para el diagnóstico del Síndrome Antifosfolipídico (15, 16).

B2GP1 es una proteína de cadena sencilla de 326 aminoácidos, con un peso molecular de aproximadamente 43 kDa que fue identificada por primera vez en 1961 como ApoH.

Se trata de una glicoproteína de unión a fosfolípidos aniónicos (muy conservada evolutivamente) que circula en la sangre a una concentración de aproximadamente 4-5 μM y que se sintetiza fundamentalmente en el hígado aunque también se elabora en riñón, corazón, intestino y placenta.

Pertenece a la superfamilia de proteínas de control del complemento que se organizan en dominios, denominadas dominios "sushi". Estos dominios son secuencias conservadas de cadenas de unos 60 aminoácidos unidas entre sí por dos puentes de disulfuro. Los primeros cuatro dominios tienen cada uno aproximadamente 60 aminoácidos. El quinto dominio tiene 82 aminoácidos, sobre el molde clásico del dominio sushi incorpora una inserción de seis residuos, una extensión C-terminal de 19 aminoácidos y un enlace disulfuro adicional que incluye una cisteína C-terminal.

En el dominio 5 existe una zona con varios aminoácidos lisina 282-287 que al estar cargados positivamente confieren a B2GP1 la afinidad por los fosfolípidos aniónicos y moléculas cargadas negativamente. Adicionalmente existe un bucle hidrofóbico flexible (aminoácidos 311-317), que contiene una secuencia de Trp-Lys que es potencialmente capaz de insertarse en las membranas.

B2GP1 adquiere muchas modificaciones postraduccionales que alteran la estructura y función de la molécula y la exposición de los epítopos crípticos. Las conformaciones intercambiables más frecuentes son tres. La primera conformación se basa en la estructura cristalina de la proteína y tiene forma de "palo de hockey". La segunda conformación, es una formación circular "cerrada" en la que el dominio 1 interacciona con el dominio 5. La tercera conformación, en forma de S, se descubrió utilizando la dispersión de rayos X de ángulo pequeño. Las conformaciones 1 y 2 se visualizaron utilizando microscopía electrónica aunque solo existen datos cristalográficos de la primera conformación (17-19).

2. FORMAS DEL SÍNDROME ANTIFOSFOLIPÍDICO

El síndrome antifosfolipídico se puede manifestar bajo tres formas(20):

1. APS en el contexto de una enfermedad autoinmune sistémica, como el LES (SAD-APS) (21).Fue la primera forma del síndrome que se identificó.

2. APS en forma primaria (PAPS): aparece en pacientes que no presentan ninguna enfermedad autoinmune (22).Cuando se describió el PAPS se consideró que la forma primaria se trataba de un estadio clínico precoz del Lupus eritematoso, que con posterioridad evolucionaría a una sintomatología de lupus (23). La consideración del PAPS como una entidad independiente del LES quedó demostrada con un estudio multicéntrico de donde 70 pacientes con PAPS fueron seguido por 5 años y ninguno de ellos evolucionó a LES (24). Con estos datos, se pudo establecer que el PAPS es una entidad distinta del LES. [15] El PAPS es la forma más prevalente del APS, según algunos autores representaría hasta el 80% de los APS(25) aunque, pese a ello, parece estar infradiagnosticado.

3. APS catastrófico (CAPS): Se trata de una forma de rápido inicio que implica una rápida progresión con aparición de trombosis multiorgánicas que se asocian a una alta mortalidad (26, 27).

Para considerar que un paciente presente un CAPS, se requiere que tenga afectados al menos tres órganos, con síntomas desarrollados en unos pocos días. La afectación más común son las microangiopatías trombóticas que afectan a vasos de pequeño calibre en múltiples órganos (también pueden estar afectados grandes vasos).

Aunque se trata sin duda de la forma de APS con peor pronóstico: el 50% de los pacientes fallecen por fallo multiorgánico (26, 28, 29), afortunadamente es la forma de APS menos prevalente, (se estima un 0,8 de los APS) (25).

3. DIAGNÓSTICO DEL SÍNDROME ANTIFOSFOLIPÍDICO

Desde poco después de ser definido el síndrome, se vio la necesidad de contar con unos criterios consensuados que permitiera identificar a los pacientes que lo padecen. El primer acuerdo para definición del síndrome y la aceptación de los criterios de identificarción para los pacientes fue conseguido en una reunión internacional celebrada en 1998 en Sapporo (Japón). En dicha reunión se acordó establecer unos criterios de clasificación agrupados bajo el epígrafe de criterios clínicos y criterios de laboratorio y se determinó que para reconocer a los pacientes con APS se requería la presencia de al menos un criterio de cada grupo.

Los criterios clínicos consensuados en Sapporo fueron las trombosis vasculares y la morbilidad gestacional. Los criterios de laboratorio consistían en la presencia de aPL (AL y aCL) detectados al menos dos veces separadas por al menos un tiempo de seis semanas (16).

En el año 2004 y ante la existencia de trabajos en los que se describían asociaciones de aPL con otras manifestaciones clínicas y también la presencia de otros aPL en adición a los aCL y AL, se consideró la modificación de los criterios clínicos y de laboratorio definidos en Sapporo dando paso, en la reunión celebrada en Sidney, a unos nuevos criterios que son los que actualmente (2017) todavía continúan en vigor y que se describen en la tabla 1 (16).

En el momento presente no existen criterios diagnósticos para el APS. Los criterios de clasificación del APS se diseñaron con objetivos de investigación y con ellos se trata de seleccionar una población muy homogénea de pacientes con APS, y recoger el menor numero posible de falsos positivos (30-32).

Los criterios de clasificación vigentes también se han utilizado como una aproximación a "criterios diagnósticos". Sin embargo, el hecho de que en su definición se primara la especificidad sobre la sensibilidad implica un bajo rendimiento diagnóstico y una utilidad limitada en la práctica clínica. Basar el diagnóstico en criterios de clasificación tan estancos, no permite abarcar todo el espectro clínico de la enfermedad y dejando un gran número de casos sin diagnóstico o mal diagnosticados (falsos negativos) (33).

Aunque se vienen realizando grandes esfuerzos para establecer unos criterios de diagnóstico, se trata una tarea muy difícil debido a la heterogeneidad en la prevalencia de la enfermedad, la distribución geográfica y el fenotipo clínico. Esto conlleva que la decisión diagnóstica final tenga que quedar bajo el criterio del clínico que estudia a cada paciente (34).

Tabla 1. Criterios de Clasificación del APS establecidos en Sidney.

Tabla confeccionada a partir de los datos de Miyakis et al. 2006. J Thromb Haemost 4: 295–306

Criterios Clínicos	Trombosis vascular	Uno o más episodios clínicos de trombosis arterial, venosa o de pequeños vasos en cualquier tejido u órgano. La trombosis debe ser confirmada de forma inequívoca con criterios objetivos validados como estudios de imagen o histopatología. No debe existir evidencia significativa de inflamación en la pared del vaso.
	Morbilidad del embarazo	Una o más muertes inexplicables de un feto morfológicamente normal a partir de la décima semana de gestación.
		Uno o más nacimientos prematuros de un neonato morfológicamente normal antes de la semana 34 de gestación debido a eclampsia o insuficiencia placentaria
		Tres o más abortos espontáneos consecutivos inexplicados antes de la 10ª semana de gestación, en ausencia de anomalías cromosómicas en los progenitores y de defectos anatómicos o hormonales maternos
Criterios de laboratorio	Anticoagulante lúpico (AL)	Presente en plasma, en dos o más ocasiones detectado de acuerdo con las directrices de la Sociedad Internacional de Trombosis y Hemostasia (Subcomité Científico de AL / anticuerpos dependientes de fosfolípidos).
	Anticuerpos anticardiolipina	De Isotipo IgG y / o IgM. En dos o más ocasiones separadas al menos 12 semanas. A titulo superior al percentil 99 o mayor de 40 GPL o MPL.
	Anticuerpos anti-B2 glicoproteína-I	De Isotipo IgG y / o IgM. En dos o más ocasiones separadas al menos 12 semanas. A titulo superior al percentil 99.

3.1 Criterios clínicos

Trombosis

Es la oclusión parcial o total de un vaso sanguíneo por coágulos generados tras la activación de la cascada de la coagulación y localizada en cualquier zona del árbol vascular, tanto en arterias, venas como capilares. Por ello, el espectro de fenotipos clínicos observables en pacientes con APS puede ser tan amplio (34).

Para poder considerar la trombosis como evento APS (según los criterios de clasificación), ésta debe ser confirmada objetivamente ya sea por técnicas de imagen, ultrasonidos o

histopatología. En los estudios histopatológicos, solo se puede considerar una trombosis como asociada al APS cuando no existen signos de inflamación significativa en la pared vascular. Es importante descartar las características inflamatorias ya que podrían corresponder inflamación a otras causas distintas del APS, como las vasculitis (16).

La etiología de la trombosis es múltiple. Los factores autoinmunes y el APS en particular son responsables de una proporción pequeña de pacientes. La mayoría de trombosis ocurren en el contexto de otras patologías (24, 35, 36) como arteriosclerosis, alteraciones en los factores de coagulación, lesiones del endotelio vascular o válvulas cardiacas, o como fruto de alteraciones de la circulación como es el caso de las arritmias como la fibrilación auricular (37, 38).Por esta razón en muchas ocasiones se llega al diagnóstico de APS por exclusión, después de haber desechado otras etiologías y eso minimiza la visibilidad del APS.

La Alianza APS para Ensayos Clínicos y Redes Internacionales (APS ACTION) publicó en 2013 unas estimaciones de la prevalencia de aPL de consenso en varios grupos clínicos con características clínicas compatibles con APS encontrando que eran positivos para aPL (y por tanto cumplían los criterios de consensos) el 10% de las trombosis venosas profundas, el 11% de los infartos de miocardio y el 13,5% de los Ictus (39, 40). Pese ello se trata de una estimación y son necesarios nuevos estudios prospectivos para definir de forma real cual es el papel del APS en la práctica clínica

Trombosis venosas

La trombosis venosa (TV) es la manifestación clínica más frecuente en los pacientes con APS (55%) y constituye la tercera patología vascular más prevalente (41). Puede afectar a cualquier vaso de cualquier órgano aunque la que más comúnmente se observa es la TV de miembros inferiores. La mayor complicación derivada de las TV es el tromboembolismo pulmonar, (TEP) que puede ocurrir hasta en el 50% de los pacientes sin tratamiento para la TV (24, 35, 36).

Las trombosis venosas diagnosticadas en el APS son trombosis venosas profundas (TVP). Las trombosis venosas superficiales (TVS), tienen una transición más rápida, y no son fáciles de detectar ni confirmar, por esas razones están infradiagnosticadas. Cuando se sospecha una TVS se suele instaurar un tratamiento profiláctico en el mismo acto médico, sin esperar al diagnóstico de confirmación (estudio de imagen o histopatológico). En la mayoría de las situaciones el diagnóstico de confirmación no llega a realizarse por lo que no se tiene certeza de la existencia del trombo y, por tanto, no cumple criterios para clasificarse como

APS. Las TVS suelen aparecer de forma aislada y circunscritas a un territorio concreto. También pueden detectarse formando parte de un estatus trombótico generalizado en la microangiopatía trombótica y en el APS catastrófico (24, 35, 36).

Embolia pulmonar

Los trombos originados en la circulación venosa periférica pueden desprenderse de su lugar de origen y circular a lo largo del territorio venoso, siguiendo el flujo fisiológico de la sangre, hasta impactarse en ramas de las arterias pulmonares, la mayoría de estos émbolos proceden del sistema venoso profundo de las extremidades inferiores desde donde se desprendieron para, acabar en una de las ramificaciones de la arteria pulmonar que tenga menor diámetro que el trombo . Las TEP se consideran complicaciones de una TVP, aunque no siempre se llega al diagnóstico de ambas entidades puesto que entre 40 y 60 % de los pacientes con TVP desarrollan TEP asintomático (42). El 70% de los pacientes que desarrollan una embolia pulmonar (EP) presentan con anterioridad factores de riesgo como trastornos de hipercoagulabilidad, inmovilización prolongada, cirugía reciente, y neoplasias. En el 30% restante no se encuentra ninguna situación predisponerte (41).

Trombosis arterial

En los pacientes con APS, las trombosis arteriales más frecuentes se localizan en el sistema nervioso central (50%), siguen en frecuencia las que afectan al corazón (25%) y el resto afectan al ojo (arteria central de la retina), riñón, hígado y arterias periféricas (24, 35, 36).

Microangiopatía trombótica:

Se entiende como microangiopatía trombótica (MAT) la presencia de micro trombosis localizadas o difusas(43). Aunque se trata de un síndrome que engloba procesos patológicos con etiología diferente, las MAT presenta una seria características clínicas comunes: trombocitopenia, anemia hemolítica microangiopática, y trombosis microvascular, que conducen a la isquemia de órganos y a la disfunción de miocardio.

La microangiopatía trombótica más común y quizás la mejor conocida es el síndrome urémico hemolítico, entidad que se origina en el contexto de una infección por Escherichia coli O157:H7 enterohemorrágica productora de la toxina Shiga, que tiene un elevado tropismo por el receptor Gb3 de las células endoteliales glomerulares, produciendo activación endotelial y síntesis de factor tisular, que deriva en trombosis microvascular, además también afecta a hematíes y plaquetas, de ahí viene la clínica de insuficiencia renal, anemia hemolítica

y trombocitopenia (44). Existe también el síndrome urémico hemolítico atípico, que es causado por mutaciones proteínas reguladoras del complemento, la mutación más conocida se da en el factor H del complemento (45) y también en el contexto de un proceso autoinmune en el que aparecen autoanticuerpos frente al factor H (46).

La MAT puede manifestarse como otras entidades clínicas como el síndrome de HELLP (Gestantes con Anemia hemolítica, elevación de enzimas hepáticas, trombocitopenia y coagulopatía de consumo) (47), el síndrome de coagulación intravascular diseminada, formas inducidas por fármacos, hipertensión maligna, esclerosis sistémica y el propio síndrome antifosfolipídico (48, 49) en donde es especialmente grave en la forma catastrófica del síndrome donde se puede identificar en cerca del 16% de los pacientes (43).

Manifestaciones obstétricas

La morbilidad del embarazo asociada a APS se describe en la tabla 1.

3.2 Características clínicas asociadas al APS pero no incluidas en los criterios de clasificación

Desde la última revisión de los criterios de clasificación en Sidney 2004, numerosos estudios tanto básicos como clínicos sugieren la necesidad de una revisión amplia que incluya nuevas características clínicas y marcadores de laboratorio. Existen entidades clínicas que tienen una fuerte asociación con la presencia de aPL que no se incluyeron en los criterios de clasificación del APS por no existir unanimidad en su aceptación debido a que entonces existían pocos datos o porque estos no presentaban un nivel de evidencia insuficiente (16). En los últimos años diversos estudios han ido aportando información cada vez más completa y han aparecido evidencias científicas de las que anteriormente no se disponía. Por ello muchos autores consideran a estos **criterios no consensuados** como signos altamente sugerentes de APS e incluso algunos de ellos se han incorporado en algoritmos diagnósticos para la toma de decisiones clínicas y terapéuticas.

Estas son las manifestaciones clínicas “extra-criterios” de clasificación más frecuentemente observadas en los pacientes:

Manifestaciones cardiacas:

Se ha descrito hasta un 65% de valvulopatías (disfunción o vegetaciones entre otras) han sido diagnosticadas con síndrome antifosfolipídico sin Lupus Eritematoso Sistémico asociado, sin embargo, el mecanismo fisiopatológico de la lesión valvular provocada por los

aPL aún no se conoce. Éste tipo de valvulopatía en pacientes aPL positivos tiene unas características propias entre las que se incluyen, engrosamiento de >3mm en porción proximal de la valva, nódulos irregulares en cara vascular de la válvula aórtica o en cara auricular de la válvula mitral (50). De hecho, la valvulopatía relacionada con aPL se ha asociado a manifestaciones neurológicas satélite APS como la migraña, isquemia o epilepsia (51).

Manifestaciones neurológicas

Hasta ahora, en los criterios de clasificación solamente se incluyen la apoplejía, accidente isquémico transitorio y la trombosis cerebral. Sin embargo, otras manifestaciones no vasculares asociadas a presencia de aPL entre las que se incluyen una amplia gama de cuadros neurológicos, psicológicos y psiquiátricos como cefalea, migraña, trastorno bipolar, mielitis transversa, demencia, corea, ataques epilépticos, lesiones múltiples similares a la esclerosis , psicosis, deterioro cognitivo, síndrome de Tourette, parkinsonismo, distonía, amnesia global transitoria, trastorno obsesivo compulsivo y leucoencefalopatía, La disfunción cognitiva, ciertos tipos de migraña, mielopatía transversa, epilepsia y ciertos tipos de depresión son otras manifestaciones asociadas a los aPL (16, 52).

En los últimos años se está investigando mucho en la posible implicación de los aPL en el deterioro cognitivo, en un estudio se ha visto que pacientes con aPL en resonancia magnética se ve una sobreactivación cortical frente a los controles sanos, posiblemente debido a un mecanismo compensatorio por pérdida de sustancia blanca (53).

Manifestaciones cutáneas

La manifestación más fuertemente asociada a la persistencia de aPL es la Livedo Reticularis, la cual se presenta principalmente en pacientes con APS secundario a Lupus Eritematoso Sistémico. Consiste en un patrón púrpura moteado y reticulado de venas superficiales. La Livedo Reticularis puede estar asociada a eventos trombóticos, y no solamente en pacientes con LES (54). Otras patologías cutáneas asociadas son: flebitis superficiales, gangrena o úlceras (55).

Manifestaciones renales

El riñón es una de las principales dianas para los aPL, pudiéndose dar trombos en cualquier ubicación del árbol vascular renal así como trombosis de los vasos del trasplante renal (como veremos mas adelante).

Histopatológicamente las lesiones renales en forma de trombos así como la presencia de fenómenos de recanalización focal en el lugar de formación del trombo. Se pueden dividir en agudas, que se suelen dar en forma de microangiopatía trombótica, o bien crónicas, en forma de nefroangiosclerosis, hiperplasia fibrosa de la íntima y atrofia cortical focal. Estas lesiones se han observado tanto en pacientes APS asociado a LES, como en pacientes con APS sin enfermedad autoinmune asociada. Aunque hay que destacar que para poder considerar las lesiones histopatológicas como causa de los aPL, no debe existir inflamación en la pared vascular, ya que en caso de ser así, las lesiones son causa de la nefritis lúpica por depósito de inmunocomplejos (56, 57).

La presencia de trombocitopenia se considera de importancia crítica para el manejo de pacientes con APS , además de ser una de las manifestaciones mas frecuentes del APS con porcentajes que oscilan entre el 20 y 50%, algunas series encuentran diferencias significativas en la incidencia de trombopenia entre APS sin enfermedad autoinmune asociada y APS asociado a otras enfermedades autoinmunes, con un 23,1% frente a un 41,9% respectivamente (58), sin embargo en otros estudios no han apreciado estas diferencias entre ambos grupos (36). La trombocitopenia causada por aPL se debe sospechar tras descartar las causas más frecuentes de trombocitopenia, incluso en ausencia de otras manifestaciones relacionadas con aPL. La trombocitopenia relacionada con aPL rara vez es grave, con mayor frecuencia es de leve a moderada y generalmente se asocia con un riesgo mínimo de hemorragia, y la evidencia no respalda su inclusión como una característica clínica central del APS (15-17).

3.3 Criterios de laboratorio

Las primeras determinaciones de la presencia de aPL se realizaron usando técnicas de radioinmunoensayo (RIA)(9). Debido a la complejidad del manejo de reactivos a y niveles de seguridad exigidos, esta técnica se fue abandonando a favor de métodos menos complejos como las técnicas inmunoenzimáticas (ELISA: *enzyme-linked immunosorbent assay*) (59).

El progresivo aumento de la demanda de las peticiones de aPL llevó a la puesta a punto de nuevas técnicas automatizadas que disminuyen el tiempo de procesamiento y el volumen de la muestra. Entre estas técnicas figura el ELISA automatizado (las más usada actualmente) seguido del immunoCAP® y la tecnología Multiplex(9, 60). Las técnicas automatizadas de ELISA y Multiplex fueron las utilizadas en los ensayos de aPL realizados en esta tesis.

La prevalencia de aPL que se han publicado en ensayos sobre poblaciones sanas, (grupos donantes de sangre) se sitúa entre el 1,0% y 5,6%, no obstante la prevalencia real del APS en

la población general es desconocida y se supone que es mayor puesto que muchos pacientes con clínica compatible con APS nunca llegan a ser testados para la presencia de aPL (61).

Anticuerpos anticardiolipina

Los anticuerpos anticardiolipina (aCL) se detectan en el suero o plasma del paciente mediante técnicas que se basan en enfrentar el suero del paciente con un antígeno inmovilizado en fase sólida, el anticuerpo (en este caso aCL) es capturado al unirse al antígeno inmovilizado sobre el substrato. A continuación se detecta la presencia del anticuerpo humano mediante por un anticuerpo anti inmunoglobulina humana (habitualmente de cabra) conjugado con una enzima o un fluorocromo que servirá para su identificación. Tras fijarse el conjugado se enfrenta a un substrato a partir del cual se desprenderá color (ELISA), fluorescencia (immunocap) o luminiscencia (Bioflash) (62, 63). La tecnología Multiplex permite realizar una variedad de bioensayos en la superficie de microesferas de plástico codificadas por fluorescencia por medio de dos láseres que posteriormente son leídas en un analizador por flujo tipo Luminex® (64).

Los sistemas de detección de cardiolipina incorporan como cofactor beta-2-glicoproteína I (B2GPI). Los aCL relacionados con patología autoinmune reconocen son epítopos mixtos formados en la unión de cardiolipina con B2GPI e incluso epítopos de la propia B2GPI (60).

Sólo se aceptan como criterios de consenso de laboratorio los aCL de isotipos IgG e IgM (16) los cuales se cuantifican en unidades internacionales estandarizadas, GPL y MPL respectivamente (60) en relación a una calibración previa (65). GPL y MPL son definidas con 1 µg de IgG o IgM anticardiolipina policlonal purificado, que es distribuido por los laboratorios (59).

Un resultado positivo se define como un valor por encima del percentil 99 en la población general o un valor mayor de 40 GPL o MPL. El consenso establece que la positividad debe persistir durante al menos 12 semanas.

Es posible utilizar sistemas de diagnóstico “home made” siempre que se utilicen los estándares para el diagnóstico analítico(16). Los sistemas modernos vienen estandarizados en Unidades Internacionales utilizando curvas y puntos de cut-off establecidos por el propio fabricante(66) que deben validarse en cada laboratorio. Es importante destacar que en los sistemas de detección de aPL existe un cierto grado de variabilidad en los diferentes lotes tanto inter como intra-laboratorios (65).

Anticuerpos anti-B2GPI

Los anticuerpos anti beta-2 glicoproteína I (aB2GP1) se detectan de modo similar a los aCL. Igualmente sólo los isotipos IgG e IgM contra la B2GPI están incluidos como criterios analíticos para APS (16).

Existe menor variabilidad en los kits para detectar los aB2GP1 de clase IgG/IgM que en los utilizados para valorar aCL (67). Algunas observaciones sugieren que al adherirse la B2GPI a la superficie lipofílica del substrato donde se va a realizar el ensayo cambia de conformación y expone epítopos que podrían unir anticuerpos "no patogénicos" relacionados con infecciones antiguas, lo que implicaría una menor especificidad (68).

Anticoagulante lúpico

El anticoagulante lúpico (AL) fue el primer aPL asociado con clínica de APS. Es un término acuñado por Feinstein y Rapaport en 1972 para designar a un efecto de inhibición de la coagulación que deteriora la activación de protrombinasa a protrombina (PT) y que fue reconocido por primera vez en el plasma de pacientes con lupus eritematoso sistémico (LES)(69). Aun no se conocen cuales son los autoanticuerpos responsables de la actividad del AL. La presencia del AL se detecta por pruebas funcionales de laboratorio donde prolonga el tiempo de coagulación *in Vitro* en ensayos dependientes de fosfolípidos (70, 71). Hay indicios (pero no certeza) a favor de la hipótesis de que el AL es un autoanticuerpo que se dirige contra los fosfolípidos (PL), ya que la preincubación con PL reduce su actividad y el efecto inhibidor es más pronunciado cuando se diluye la adición de PL (72).

En los últimos años se ha intentado, sin éxito, vincular al AL con los anticuerpos contra la B2GPI o la fosfatidilserina/protrombina (aPS/PT) siendo éstos últimos los únicos que han mostrado una moderada correlación(73).

3.4 Anticuerpos asociados al APS no incluidos en los criterios de laboratorio

Existen estudios en los que otros autoanticuerpos antifosfolipídicos distintos de los reconocidos en el consenso son asociados a la clínica del APS. Estos estudios suelen ser controvertidos por estar realizados con pocos pacientes y ser poco reproductibles debido a la utilización de sistemas diagnósticos no estandarizados.

Entre este tipo de aPL destacan los anticuerpos de isotipo IgA de los aCL y aB2GP1. También son importantes los aPT/PS, anti anexina A5 y anti anexina A2 (16). Otros autoanticuerpos frente a lípidos, no relacionados con los aPL clásicos, como los antifosfatidiletanolamina (aPE), antiprotrombina (aPT) son de menor interés por tener escasa relevancia clínica.

4. GENETICA DE B2GP1

El gen que codifica para la B2GP1 tiene un tamaño de 18kb y se localiza en el cromosoma 17, q23-24. Está formado por 8 exones (1,2kb) separados por intrones muy largos (16.2kb) que codifican una proteína de 345 aminoácidos (aa) de la que en el extremo N-terminal se escinden los 19 aa del péptido señal para generar la proteína madura (74).

Los 8 exones del gen de la APoH codifican una proteína formada por cinco dominios tipo Sushi. El exón 1 codifica el péptido señal, el exón 2 el dominio 1, los exones 3 y 4 codifican el dominio 2, y los exones 5, 6 y 7 codifican respectivamente los dominios 3, 4 y 5. La zona final de la proteína y el codón de parada se localizan en el exón 8 (75) (Figura 2).

El promotor del gen B2GPI está localizado muy cerca del origen de transcripción, entre las posiciones 197 y 7. La expresión génica está regulada por diversos factores de transcripción, entre ello se incluye TATA box atípico (TATTA) especifico en hígado que se localiza detrás de la zona de unión del Factor nuclear 1 alfa de hepatocito (HNF1 homeobox A) entre las posiciones 97 y 92 . HNF-1 alfa soluble, activar HNF-1 que a su vez activa el promotor de Apo H. Por tanto HNF1 homeobox A activa la expresión de la Apo H y los niveles de ambos están correlacionados con los niveles de HNF1 homeobox A (77).

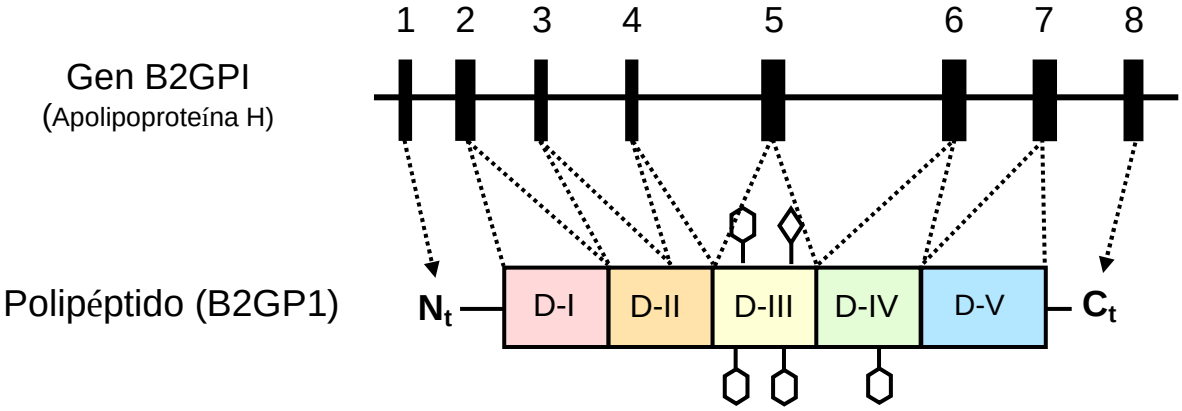

Figura 2. Estructura del gen de la Apolipoproteína H humana (B2GPI). Modificado a partir de Sodin-Semrl & Rozman.(76).

Polimorfismos genéticos

Existen familias en las que varios de sus miembros presentan APS. Esta circunstancia llevó a pensar en la existencia de factores genéticos que contribuyan a desarrollar la enfermedad.

En un estudio de Weber y col. se demostró que alrededor del 40% de los pacientes con APS tenían uno o más familiares con al menos una manifestación clínica de APS (trombosis o pérdida fetal recurrente) (78).

La Apo H presenta variabilidad entre los individuos de diversos grupos étnicos tanto de manera cualitativa (secuencia) como cuantitativa (niveles séricos). Se describió que la mayoría de los pacientes con niveles bajos de B2GP1 era portadores de un alelo nulo (Bg*D), que es co-dominante con respecto al alelo normal (Bg*N)(79). La frecuencia del alelo nulo es baja en la población de origen europeo occidental (0.06) y alta en población africana (0.26, Mozambique) y asiática (Corea: 0.22, Afganistán: 0.12, Iran: 0.11) (80).

Las diferencias estructurales fueron estudiadas por Khambo et al y encontraron cuatro variantes alélicas de la Apo H han sido identificadas: ApoH 1, ApoH 2, ApoH 3 y ApoH 4, esta última esta sólo presente en personas de origen africano(81). El alelo ApoH 2 es considerado como alelo predominante(82). El alelo ApoH 3 se puede dividir en dos subtipos basados en el diferente reconocimiento por un anticuerpos monoclonal: ApoH 3w y ApoH 3b(83).

Dentro de la región codificante de APO H se han descrito 8 polimorfismos de un único nucleótido (SPNs). Los cuatro polimorfismos más importantes se localizan en los codones 88 (exon 3), 247 (exon 7), 306 (exon 7) y 316 (exon 8) (83).

El SPNs del codón 88 (Ser→ Asn) está presente en todos los portadores del alelo ApoH 1, mientras que el SPN en el codón 316 (Trp→Ser) parece estar presente en todos los portadores de ApoH 3w (84).

El SPN más asociado a la producción de anticuerpos anti Apo H (aB2GP1) en pacientes con APS primario, se encuentra dentro del dominio V de la Apo H y consiste en la sustitución en la posición 247 de Val a Leu(85). La presencia de Leu247 se considera como un factor de riesgo genético para el desarrollo de APS. y puede ser importante en la formación de anticuerpos aB2GP1 (85-88).

Las variaciones genéticas en el quinto dominio podrían modificar las propiedades físico-químicas de la proteína, y esto puede afectar la unión a los fosfolípidos (89).

El polimorfismo Val-Leu 247 está situado en el quinto dominio, entre el lado de unión a fosfolípidos y la región que expone el epítopo críptico en el cuarto dominio.

Se han descrito interacciones electrostáticas que mantienen unidos los dominios IV y V por puentes de hidrogeno, se ha especulado con un posible epítopo críptico en el dominio IV si se modificaran dichas interacciones electrostáticas, pero hoy eso no está demostrado (82). El aminoácido 246, posición anterior al polimorfismo 247, sirve para establecer en la estructura terciaria de la Apo H un puente de hidrogeno que une el dominio V con el dominio IV. La sustitución de un aminoácido en la posición adyacente al puente de hidrógeno podría producir un cambio conformacional relevante que esturaría la forma de la Apo H deteriorando su forma de J y aumentando la afinidad por aCL y aB2GP1 (90). La distribución del polimorfismo Trp-Ser 316 fue estudiada en 222 pacientes con LES y fue significativamente diferente entre los pacientes positivos para aCL de los pacientes sin aCL, lo cual sugiere una posible protección del polimorfismo 316(91) para desarrollar estos anticuerpos. Aunque está menos estudiado se ha sugerido que con el polimorfismo 306 Cis →Gly ocurriría una situación similar (82).

5. Tratamiento del APS:

No existe un tratamiento específico para el APS. Los tratamientos que se aplican a los enfermos de APS son fundamentalmente preventivos y basados en interferir en los mecanismos de la coagulación para evitar nuevos eventos trombóticos. Los fármacos más empleados son la heparina no fraccionada (UFH), heparina de bajo peso molecular (LMWH) y los antagonistas de la vitamina K (VKA).

La anticoagulación con VKA es el tratamiento de elección a largo plazo, especialmente en pacientes con tromboembolismo venoso (VTE) e isquemia cerebral embolica (92). La intensidad del tratamiento se mide por el International Normalized Ratio (INR), que debe estar en estos casos entre 2.0-3.0. El mismo tratamiento con o sin aspirina es el indicado para la trombosis arterial(92). UFH o LMWH y aspirina es el indicado para pacientes con morbilidad en el embarazo. El APS catastrófico debe ser tratado con una combinación de VKA y aspirina y/o inmunoglobulinas intravenosas y/o plasma. El rituximab también puede ser una alternativa de tratamiento(92). Resultados de estudios observacionales, han visto un efecto protector de la aspirina en pacientes con LES asintomático (93-96) y en mujeres con APS (97).

En la tabla 2 se indican los fármacos actualmente que se manejan para el tratamiento del APS así como su potencial mecanismo de acción.

Tabla 2. Estrategias de tratamiento en el síndrome antifosfolípidico (APS).
Modificada a partir de Dobrowolski and Erkan.Clin. Immunol. 2018 Mar 3. pii: S1521-6616(18)30119-0.

Fármacos	***Mecanismo***	***Recomendaciones para uso en APS***
Anticoagulantes		
Warfarina	Inhibe de la producción de factores de la coagulación dependientes de vit. K (II, VII, IX y X).	Tratamiento preventivo de trombosis. Mantener el objetivo de INR en 2.5-3 tanto para la trombosis venosa como la arterial
Heparina no fraccionada	Aceleran la inhibición del factor Xa y la trombina por antitrombina III	Uso en pacientes con trombosis aguda
Heparina de bajo peso molecular	Inhiben más al factor Xa que a la trombina (relación de inactivación Xa: trombina de 4:1 a 2:1).	Uso en pacientes con trombosis aguda
Medicacion Basada en la etiopatogenia		
Estatinas	Efectos antiinflamatorios y antitrombóticos pleiotrópicos.	Uso en pacientes con indicación de estatina (hipercolesterolemia).Considerar su uso en APS refractario; no usar en el embarazo
Hidroxicloroquina	Efectos antiinflamatorios y antitrombóticos pleiotrópicos.	Uso en pacientes con indicación de hidroxicloroquina, es decir, lupus; considerar en APS primario refractario.
Rituximab	Anticuerpo monoclonal Anti-CD-20; Agente depleción de células B; inhibición de ICOS / CD4 +.	Puede ser de utilidad para manifestaciones hematológicas y / o microtrombóticas de aPL; Se puede considerar su uso en CAPS.
Belimumab	Inhibición de BAFF / Blys	Se necesitan más datos para formular recomendaciones (a principios de 2018 solo se habían informado dos pacientes).
Eculizumab	Inhibidor del factor 5 del Complemento.	Considere en pacientes con CAPS refractarios; considerar en pacientes con APS y rechazo de aloinjerto renal.
Sirolimus / Everolimus	Inhibición de mTOR.	Se necesitan más datos para formular recomendaciones (solo hay una pequeña cohorte retrospectiva de APS que recibió un trasplante renal)
Defibrotido	Agonista del receptor de adenosina; inhibe actividad de las plaquetas y el tromboxano.	Se necesitan más datos para formular recomendaciones (solo se informó un paciente con CAPS tratado con defibrotido).
Terapia peptídica	Fijación del péptido a B2GP1, dominio I o dominio V para enmascarar epitopos.	No disponible para uso clínico en la actualidad.

6. Aportaciones de nuestro grupo al estudio del APS

Al cumplirse el 30 aniversario de la descripción del Síndrome, Graham Hughes escribió una revisión sobre lo aprendido sobre el síndrome en ese periodo. En ella afirmó que el APS participa en muchas más disciplinas que en el lupus, donde primero fue descrito. También está presente en muchas disciplinas, incluidas la cirugía de trasplante, la gastroenterología, la ortopedia, la psiquiatría y otorrinolaringología donde debemos buscarlo (10).

En este contexto nuestro grupo aceptó el reto lanzado por Hughes y empezó a estudiar también a los pacientes con clínica de APS que se atendían fuera de las Unidades de Reumatología, buscando la presencia de los aPL de consenso y también de los fuera de consenso como la IgA aB2GP1. Los resultados a largo plazo del trasplante renal han mejorado

sustancialmente en las últimas décadas gracias a la introducción de la inmunosupresión basada en inhibidores de calcineurina, mejores metodologías para los test de histocompatibilidad y a los avances en la prevención / tratamiento de infecciones (98). Pese a ello, no se ha modificado el porcentaje pacientes sufren pérdida de injerto en los primeros meses después del trasplante que se mantiene entre 5 y 8% (99).La principal causa de estas pérdidas precoces del injerto es la trombosis del injerto (100, 101), generalmente es atribuida a complicaciones relacionadas con el procedimiento quirúrgico (102).

Se conoce desde hace décadas que la presencia de anticuerpos preformados antes del trasplante contra antígenos situados en la membrana de las células de injerto, principalmente los antígenos HLA, está asociada con el rechazo temprano (agudo e hiperagudo) de injertos renales (103). Asimismo se han observado otros anticuerpos contra antígenos de histocompatibilidad menores y frente a otros autoantígenos pueden ser nocivos para la evolución del aloinjerto renal (104, 105) y su impacto en las complicaciones después del trasplante y la pérdida del injerto se está reconociendo cada vez más (106). En los últimos años, la presencia del APS se ha asociado a muchas situaciones clínicas en las que aparecen eventos trombóticos, incluida la cirugía de trasplante (10).

En este contexto nuestro grupo estudió la presencia de aPL en pacientes con ERC tratados con hemodiálisis y describió que la prevalencia de los autoanticuerpos IgA aB2GP1 es mayor que en la población general (30% frente a 1,5%) (107) y que la presencia de estos anticuerpos se asocia con una mayor incidencia de eventos trombóticos y mortalidad (108, 109). En un estudio prospectivo a 10 años sobre 269 pacientes que recibieron un trasplante renal nuestro grupo, demostró que los pacientes con autoanticuerpos IgA aB2GP1 previos al transplante tienen un mayor riesgo de sufrir pérdida temprana del injerto y un alto riesgo de retraso en la función del injerto. Aunque la principal causa de la pérdida precoz fue la trombosis del injerto no se pudo demostrar que la presencia de estos anticuerpos fuesen un factor de riesgo independiente para la pérdida del injerto debido al pequeño número de eventos trombóticos que no fue suficiente para realizar un análisis multivariable. Además, este estudio se realizó solo con donantes cadavéricos y receptores de baja complejidad inmunitaria, situaciones características a principios del siglo XXI (110). Ante esta situación se planteó la necesidad de realizar un estudio más amplio sobre un gran grupo de pacientes con una mayor variabilidad en el tipo de donante y receptor (donantes vivos, donantes no cardiacos no controlados y donantes extremadamente ancianos) con receptores tratados con hemodiálisis,

diálisis peritoneal y prediálisis. Estos aspectos son los que representan la práctica clínica en España en la segunda década del siglo XXI.

Aunque los anticuerpos anti B2GPI del isotipo IgA no se incluyen en los criterios de laboratorio para APS definidos en 2004 debido a los resultados controvertidos (16), en la misma reunión se alentó a los investigadores a aclarar su papel en el APS (111). En los últimos años, la relevancia clínica de IgA aB2GP1 ha aumentado(112, 113) y en el XIII Congreso Internacional de Anticuerpos Antifosfolipídicos (2010, Galveston, TX), la fuerza de trabajo recomendó la prueba para IgA aB2GP1 en casos negativos para IgG e IgM y aún se sospecha APS (114). Esto ha permitido a esta determinación proporcionar un diagnóstico de excelente utilidad en pacientes con APS sintomatología negativa para aPL de consenso con APS, (114 lupus eritematoso, (115) trombosis en enfermedad renal crónica (ERC), (107, 108) y pérdida temprana de injerto de riñones trasplantados (110).

Si bien la mayoría de los anticuerpos detectados en enfermedades autoinmunes no son la causa directa de la enfermedad, los antifosfolipídicos son directamente patógenos tanto los de clase IgG, IgM(116)como los IgA(113). Sin embargo, el valor predictivo de la presencia de aPL para desarrollar trombosis en un paciente por si mismo es bajo. Para que se desarrollen los eventos clínicos del APS es necesaria la presencia de aPL, pero no suficiente. Para desarrollar la trombosis se necesita también un desencadenante adicional, un segundo golpe que desencadene el proceso (hipótesis del “second hit”, o segundo golpe) (117). Aunque se sabe que la activación de la inmunidad innata, en el contexto de procesos como la infección o la cirugía, desempeña un papel clave en el que lleva al desarrollo del proceso, los mecanismos concretos que inducen el evento trombótico siguen siendo desconocidos (117).

En un estudio prospectivo multicéntrico con 1000 pacientes de APS seguidos durante 10 años, aproximadamente el 15% desarrollaron un evento trombótico en los primeros 5 años (3%/año). En dicho estudio se concluyó que era fundamental buscar nuevos marcadores para detectar los pacientes con riesgo y así prevenir las complicaciones de APS, puesto que a pesar de que los pacientes estaban bajo tratamiento, en algunos de ellos aparecieron trombosis (118).

Para los pacientes con anticuerpos IgA aB2GP1 solo se llevaron a cabo estudios prospectivos en pacientes en hemodiálisis (108, 109), en pacientes trasplantados renales (110) y muy recientemente también en portadores asintomáticos del autoanticuerpos sin ningún antecedente de APS (119). En estos estudios se describió que, solo una minoría de pacientes desarrolló eventos trombóticos, 12%/año en pacientes con trasplante renal durante el primer

año(110), y aproximadamente 25%/año en pacientes en diálisis (108, 109) y 3%/año en los portadores asintomáticos (119). Por lo tanto, la situación es similar con los aPL de isotipo IgA y los de isotipos IgG/IgM y queda patente que se necesitan nuevos biomarcadores para identificar qué pacientes tienen mayor riesgo de trombosis (118, 120).Recientemente nuestro grupo demostró la existencia de inmunocomplejos circulantes (CIC) de B2GPI unida a IgA en pacientes positivos asilados para IgA aB2GP1 (negativos para otros aPL) (121). La presencia de CIC formados por B2GP1 unida a anticuerpos de clase IgG e IgM fue descrita previamente en pacientes con APS(122), aunque la complejidad y la baja sensibilidad del procedimiento de detección no permitió que se estudiasen grandes series de pacientes por lo que no se pudo asociar con la aparición de eventos trombóticos (123). En este contexto nuestro grupo describió un enzimoinmunoensayo de captura que permite la detección de inmunocomplejos de B2GP1 con una alta sensibilidad y baja complejidad lo que permite evaluar fácilmente a grandes series de pacientes (121).

Las tasas de supervivencia progresiva más altas después del trasplante cardiaco han ido aumentando progresivamente en los últimos años (124). Como en el caso del trasplante renal estas mejora de la evolución se debe a la introducción de nuevas técnicas quirúrgicas y la mejora del soporte circulatorio mecánico, estrategias de inmunosupresión y atención postoperatoria. A pesar de estos avances, el fallo primario del injerto (FPI) continúa siendo una de las principales causas de muerte dentro de los primeros 30 días después de TC, seguido de la insuficiencia orgánica múltiple e infección, que representan respectivamente el 38,7%, 18% y 13,1% de las muertes después de TC (124).

El efecto sinérgico de varios factores de riesgo de donantes y receptores se ha asociado con FPI. Sin embargo no se conocen marcadores que se asocien a este fallo y puedan predecirlo de modo que las estrategias preventivas, diagnósticas y terapéuticas centradas en el FPI aún no se han abordado adecuadamente (125). El hecho de de que la mayoría de los pacientes con pérdida temprana de injerto después del trasplante renal fueron positivos en el pretrasplante para los anticuerpos del Isotipo IgA (IgA aB2GP1) hizo plantear la posibilidad de que esta situación pudiese ocurrir también es aquellos órganos, como el corazón, que fisiológicamente también elaboran la proteína B2GP1 (110).

Hipótesis

1. La etiología de la elevada prevalencia de anticuerpos IgA aB2GP1 observada en los pacientes en diálisis aún permanece desconocida. Deben existir factores genéticos y ambientales (como el tratamiento sustitutorio de la función renal, la enfermedad de base o la propia insuficiencia renal terminal) que predispongan a los pacientes a desarrollar los anticuerpos IgA aB2GP1.

2. Los aPL de clase IgA, especialmente los aB2GP1, han sido asociados con pérdida precoz del injerto renal y parecen predisponer especialmente a la trombosis del injerto. La presencia de anticuerpos IgA aB2GP1 pretrasplante podría ser un factor de riesgo para pérdida precoz del injerto por trombosis del mismo.

3. Los inmunocomplejos circulantes de IgA unidos a B2GP1, recientemente descritos, podrían estar asociados con la clínica de APS.

4. Aunque la presencia de los IgA aB2GP1 está fuertemente asociada con la incidencia posterior de eventos APS, el valor predictivo positivo es muy bajo, insuficiente como para justificar instaurar tratamientos preventivos a los portadores. Es necesario disponer de nuevos biomarcadores para acotar mejor a la población en riesgo de desarrollar eventos APS.

5. Los pacientes con insuficiencia funcional de otros órganos, como corazón, que también elaboran fisiológicamente B2GP1 también podrían elaborar estos anticuerpos y los portadores de los mismos tendrían complicaciones vasculares graves post trasplante.

Objetivos:

1. Determinar cuales son los factores genéticos y ambientales asociados a la aparición de los anticuerpos IgA aB2GP1 en los pacientes en espera de trasplante renal.

2. Confirmar la observación previa de asociación de los anticuerpos de IgA aB2GP1 en el pretrasplante con la pérdida temprana del injerto renal en unas serie de pacientes con trasplante renal de 12 años consecutivos y en un estudio multicéntrico prospectivo.

3. Comprobar la posible asociación de la presencia de pretrasplante de IgA aB2GP1 en trasplantados renales con la pérdida del injerto por trombosis, así como de otras patologías vasculares.

4. Valorar si la presencia de los inmunocomplejos formados por IgA unida a B2GP1 se asocia con la clínica de APS

5. Evaluar si los pacientes con IgA aB2GP1 que son portadores de inmunocomplejos tienen mayor riesgo de desarrollar eventos trombóticos y vasculares que aquellos que son positivos para el autoanticuerpo y negativos para inmunocomplejos.

6. Comprobar si la prevalencia de los IgA anti B2GPI en pacientes con insuficiencia cardiaca terminal es mayor que en población general.

7. Determinar si en los pacientes con insuficiencia cardiaca que son portadores de IgA aB2GP1 tienen mayor incidencia de eventos vasculares pre y post trasplante y si esos autoanticuerpos tienen influencia en la supervivencia del injerto y del paciente.

Por todo lo anteriormente descrito, nos hemos planteado los siguientes capítulos.

Capítulo 1

Estudio de polimorfismos de la β2-glicoproteína I en pacientes con insuficiencia renal crónica como factor predisponente para el desarrollo de autoanticuerpos anti-β2-glicoproteína I.

FONDO:

En varios trabajos previos del grupo de investigación se demostró que alrededor del 30% de los pacientes con enfermedad renal crónica son positivos para los anticuerpos anti-β2-glicoproteína I (anti B2GP1) de isotipo IgA sin embargo, el origen de estos anticuerpos es desconocido. Asimismo se describió que la presencia de IgA anti B2GP1 se asocian con la aparición de eventos trombóticos, morbilidad cardiovascular y muerte en pacientes en hemodiálisis.

Se especuló que las membranas de diálisis, la edad o la etiología de la enfermedad de la base renal son posibles factores precipitantes para la aparición de estos anticuerpos.

En trabajos previos del grupo se descartó que el tipo de tratamiento de la insuficiencia renal crónica, la edad y el origen de la IRC tuviesen relación con la aparición de los anticuerpos no obstante la influencia de factores genéticos no se estudió.

B2GP1 es una proteína de 326 aminoácidos agrupados en cinco dominios tipo sushi. Se han descrito ocho polimorfismos en B2GP1 de ellos existen dos que tienen influencia en la aparición de anticuerpos a B2GP1: Val / Leu247, que parece predisponer a la producción de anticuerpos contra B2GP1 en pacientes con síndrome antifosfolipídico, y Trp / Ser316, que parece tener producción protectora de anticuerpos de anti B2GP1. El trabajo trata de identificar si los pacientes en hemodiálisis que desarrollan anticuerpos tienen diferente proporción de polimorfismos favorecedores y protectores de la aparición de anticuerpos que la población general y que los pacientes en diálisis negativos para los IgA anti B2GP1

MÉTODOS: Se formaron dos grupos seleccionando de forma aleatoria 46 pacientes dializados que eran positivos para IgA anti B2GP1 (grupo 1) y otros 46 que eran negativos para este autoanticuerpos (grupo 2). Tras la extracción se identificó el grupo y se anonimizaron las muestras para garantizar el anonimato. Se estudiaron . los polimorfismos

Val / Leu247 y el polimorfismo Trp / Ser316 .

RESULTADOS:

Las frecuencias observadas de cada mutación en ambos grupos fueron similares a las descritas en la población general caucásica mediterránea. No se observaron diferencias significativas en los polimorfismos localizados en codones 247 y 316 entre los dos grupos.

CONCLUSIONES:

Los dos grupos de pacientes tienen la misma prevalencia en polimorfismos 247 y 316, y por lo tanto parece que no hay una predisposición genética en nuestra población. Nuevos factores desencadenantes deben ser estudiados.

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Study of β_2–Glycoprotein I Polymorphisms in Patients With Chronic Renal Failure as a Predisposing Factor for the Development of Anti–β_2–Glycoprotein I Auto-Antibodies

M. Serrano[a], O. Cabrera-Marante[a], J.A. Martínez-Flores[a], P. Morales[a], D. Pérez[a], S. Mora[a], F. García[b], E. González[b], E. Paz-Artal[a,c,d], J.M. Morales[a], and A. Serrano[a,d,*]

[a]Department of Immunology and [b]Department of Nephrology, Instituto de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain; [c]Immunology Section, Universidad San Pablo-CEU, Madrid, Spain; and [d]Facultad de Medicina, Universidad Complutense de Madrid, Spain

ABSTRACT

Background. Immunoglobulin (Ig)A anti–β_2–glycoprotein I (aB2GP1) antibodies are associated with thrombotic events, cardiovascular morbidity, and death in dialysis patients. About 30% of patients with chronic renal disease are positive for IgA aB2GP1; however, the origin of these antibodies is unknown. It has been speculated that dialysis membranes, age, or etiology of renal base disease are possible precipitating factors, although these factors do not appear to be the source of antibodies. B2GP1 is a protein of 326 amino acids grouped into five domains. Eight polymorphisms have been described; the most important are Val/Leu^{247}, which appears to predispose aB2GP1 antibody production in patients with anti-phospholipid syndrome, and Trp/Ser^{316}, which appears to have protective antibody production of aB2GP1.

Methods. DNA samples from 92 patients with renal failure on hemodialysis were randomly collected with a 1:1 ratio for the positivity for IgA aB2GP1. Forty-six samples were positive for IgA aB2GP1 (group 1) and 46 negative for IgA aB2GP1 (group 2). All samples were anonymized to study polymorphism Val/Leu^{247} and polymorphism Trp/Ser^{316}.

Results. No significant differences were observed between those who were positive or negative for IgA aB2GP1 in patients with renal failure treated with hemodialysis and the polymorphism located in codons 247 and 316.

Conclusions. The two groups of patients have the same prevalence in polymorphisms 247 and 316, and therefore there appears not to be a genetic predisposition in our population. New trigger factors must be studied.

ANTI-PHOSPHOLIPID (aPL) antibodies belong to a heterogeneous group of auto-antibodies against phospholipids, phospholipid-binding proteins, or both, which are localized on membranes of endothelial cells and others involved in the coagulation cascade [1,2]. Antibodies against phospholipids per se are associated with infectious diseases, whereas auto-antibodies associated with vascular pathology are directed against β_2–glycoprotein I (B2GP1) [3]. B2GP1 is mainly synthesized in the liver [4] but is also produced in small amounts by the bowel and the kidney. Previously, our group described a higher prevalence (33%) of immunoglobulin (Ig)A aB2GP1 antibodies in patients with chronic kidney disease (CKD) treated with hemodialysis [5]. However, the development of these antibodies is still unknown. A recent study demonstrated that the

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*Address correspondence to Antonio Serrano, Immunology Department, Hospital Universitario 12 de Octubre, Avda de Andalucía s/n, 28041 Madrid, Spain. E-mail: aserrano@h12o.es

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presence of these antibodies is not related to the substitutive treatment of renal failure (pre-dialysis, hemodialysis, or peritoneal dialysis) [6]. The genetic predisposition has been postulated as a determining factor, in addition to environmental factors. B2GP1 is a 326–amino acid protein composed of five homologous domains, being the fifth domain of the phospholipid-binding site [7]. B2GP1 gen is located in the 17q23-24 chromosome and has been described as four important polymorphisms in the positions 88, 247, 306, and 316. Genetic variations in the fifth domain could modify the physico-chemical properties of the protein, and this may affect the binding to phospholipids [8].

Polymorphism Val-Leu^{247} is located in fifth domain, between the phospholipid-binding side and the region that exposes the cryptic epitope in the fourth domain. In position 246, a hydrogen bond is established with the fourth domain of the tertiary structure of the B2GP1. For this reason, the substitution of one amino acid in the adjacent position could produce a conformational change that could be relevant to recognize anti-cardiolipin antibodies (aCL) and aB2GP1 antibodies. The polymorphism Val-Leu^{247} has a heterogeneous distribution between the different populations. On the other hand, the Trp-Ser^{316} polymorphism appears to be protective to develop these antibodies [9].

Because of the high percentage of patients with IgA aB2GP1 antibodies in the pre-transplant situation, and, considering the genetic polymorphism predisposition to develop aB2GP1 antibodies, we decided to analyze patients with renal failure in hemodialysis for the most associated polymorphism in the 316 (Trp-Ser) and 247 (Val-Leu) amino acids.

METHODS

Patients

We studied 92 patients with renal failure in hemodialysis at the Hospital 12 de Octubre of Madrid, Spain. Forty-six were positive for IgA aB2GP1 antibodies (group 1) and 46 were negative for IgA aB2GP1 (group 2). All the patients were anonymized in a database, with only the value of IgA aB2GP1 and the underlying renal disease that triggered renal failure. There were no significant differences between the different underlying renal diseases and the presence of IgA aB2GP1 (Table 1). All patients positive for IgA aB2GP1 were white, with the exception of two patients (one South American and one Sub-Saharan). The group with IgA aB2GP1 negative was adjusted with the same proportion.

Table 1. Underlying Diseases in Renal Failure Patients Treated With Hemodialysis

Etiology of End-Stage Renal Disease	IgA aB2GP1 Negative (%)	IgA aB2GP1 Positive (%)	*P*
Glomerulonephritis	4 (8.7)	3 (6.5)	1
Diabetic nephropathy	8 (17.4)	14 (30.4)	.221
Systemic lupus erythematosus	1 (2.2)	0 (0.0)	1
Polycystic kidney disease and undetermined	14 (30.4)	15 (32.6)	1
Nephro-angiosclerosis	5 (10.9)	4 (8.7)	1
Tubulo-interstitial nephritis	2 (4.3)	1 (2.2)	1
Other	12 (26.1)	9 (19.6)	.62

DNA Extraction and Amplification

Genomic DNA from ethylenediamenetetracetate-containing whole blood samples was extracted by use of the MagNA Pure Compact System (Roche, Mannheim, Germany) [10]. Polymerase chain reaction (PCR) was used to amplify genomic regions where Val/Leu^{247} and Trp/Ser^{316} are located.

Primers used for amplification were designed from the available B2PG1 gene sequence: Invitrogen (Life Technologies, Carlsbad, Calif, United States) (5′-TCTCCTTGGTACACCACAGTGGC) and Invitrogen (5′-GTGTAGGTGTACTCATCTACTGTGT) for the Val/Leu^{247} polymorphism and Invitrogen (5′-AACAAGAAACAAGTGTGACATTTTATGTGG) and Invitrogen (5′-TCCCTTAGAATGTTTATCTTTTTCTCCCCC) for the Trp/Ser^{316} polymorphism.

Genomic DNA from each patient was subjected to PCR amplification in a Techne TC-412 thermocycler. The PCR reaction (100 μL) contained 25 pmol/L of each primer with 400 μmol/L of each nucleotide, 5 units of Taq polymerase (Ampli-Taq, Perkin Elmer-Cetus), 7 μmol/L of $MgCl_2$, and 10 μL of a premix PCR buffer (100 mmol/L Tris-Cl pH 8.3, 500 mmol/L KCl). The reaction mixture for both polymorphisms was subjected to an initial denaturation at 94°C for 5 minutes and followed by 25 cycles at 94°C for 20 seconds, 62°C for 15 seconds, 72°C for 40 seconds, and a final extension step at 72°C for 10 minutes. The PCR products for Val/Leu^{247} and Trp/Ser^{316} polymorphisms were 126 and 148 base pairs (bp), respectively.

Digestion With Variation-Specific Restriction Endonuclease

After the PCR amplifications, aliquots (17 μL) of the reaction mixture were digested with 10 units of the restriction endonucleases *Rsa*I or *BstB*I for 3 hours after the protocol recommended by the manufacturer (Promega, Madison, Wis, United States) [11]. The Val/Leu^{247} variation breaks a restriction site for *Rsa*I, whereas Val^{247} DNA is cut into two fragments of 100 and 26 bp. Leu^{247} DNA presents a single undigested fragment of 126 bp. On the other hand, the Trp/Ser^{316} variation creates a restriction site for *BstB*I; therefore, the Trp316 DNA presents an undigested fragment of 148 bp, whereas Ser^{316} DNA is cut into two restriction fragments of 86 and 62 bp. The digestion products were size-resolved in a 2% Nusieve agarose or 6% acrylamide gel and detected by staining with ethidium bromide.

IgA aB2GP1 Determination

IgA aB2GP1 were quantified by means of enzyme-linked immunosorbent assay with the use of QUANTA aB2GP1 Lite (INOVA Diagnostics, Inc, San Diego, Calif, United States) on serum samples. Higher levels of antibodies (20 U/mL) were considered positive, following the manufacturer's instructions.

RESULTS

No significant differences were observed between those positive or negative for IgA aB2GP1 in patients with renal failure treated with hemodialysis and the polymorphism located in 247 and 316 codons (Table 2).

Table 2. Distribution of the Different Polymorphisms at Codons 316 and 247

Polymorphic Site	Genotype/Allele	IgA aB2GP1 Positive (%)	IgA aB2GP1 Negative (%)
Codon 316	Genotype		
	Trp/Trp	45 (97.8)	43 (93.5)
	Trp/Ser	1 (2.2)	3 (6.5)
	Ser/Ser	0 (0.0)	0 (0.0)
	Total	46	46
	Allele		
	Trp	0.989	0.967
	Ser	0.011	0.033
Codon 247	Genotype		
	Val/Val	29 (63.0)	29 (63.0)
	Val/Leu	15 (32.6)	16 (34.8)
	Leu/Leu	2 (4.4)	1 (2.2)
	Total	46	46
	Allele		
	Val	0.794	0.804
	Leu	0.206	0.196

Codon 316

The Trp/Trp genotype was the most prevalent in both groups; in group 1 it was present in 45 patients (97.8%) and in group 2 it was present in 43 (93.5%). The Trp/Ser genotype was found in one patient (2.2%) in group 1 and in three patients (6.5%) in group 2. Nevertheless, the Ser/Ser genotype was not found in either of the two groups.

Codon 247

The genotype Val/Val was most frequently found in both groups. It was detected in 29 patients (63%) in both groups, whereas the genotype Val/Leu was expressed in 15 patients (32.6%) in group 1 and in 16 patients (34.8%) in group 2.

The Leu/Leu genotype was the less frequently found genotype. It was found in two patients (4.4%) in group 1and one patient (2.2%) in group 2.

DISCUSSION

To our knowledge, this is the first study that evaluates the genetic predisposition to develop IgA aB2GP1 antibodies in patients with CKD by analysis of the polymorphisms most related to aPLs. These polymorphisms are located in 247 and 316 amino acids. Our two groups of patients showed the same presence of these polymorphisms.

IgA isotype antibodies are the most common B2GP1 antibodies in patients with terminal renal failure treated with hemodialysis and are related to cardiovascular diseases [5]. Patients treated with peritoneal dialysis and patients not dialyzed show a prevalence similar to that in hemodialysis patients [6]. In anti-phospholipid syndrome (APS), a genetic predisposition to develop B2GP1 antibodies is known, with the expression of the Val/Val^{247} polymorphism [12]. In 1999, a study of Asian patients showed that the presence of the valine allele and the Val/Val^{247} genotype had significant association with aB2GP1 presence ($P = .0018$ and $P = .0003$, respectively) [11]. Atsumi et al [13] reported a correlation between the valine allele and white patients with primary anti-phospholipid syndrome (PAPS) or positive for aCL. That publication also analysed the polymorphisms in the 88 and 316 positions: None were correlated with the disease or with aCL presence. Another important fact from that investigation was the increase of the leucin allele in the group of patients with secondary anti-phospholipid syndrome (SAPS) without aCL [13]. The assay used for aCL detection used in that study was irradiated plates; for this reason, it is possible that in these patients the detection of aPL was not possible. It is important to highlight that Hirose et al [11] did not analyze the patients with APS separately by PAPS and SAPS; the white subjects studied were all from North America, whereas Atsumi et al [13] conducted their study with a cohort of an English population.

In the Mexican mixed population, there was no relation between the polymorphism in position 247 and PAPS; however, the Leu/Leu genotype was significantly increased in SAPS patients [9]. In summary, the data demonstrate that in Asian patients with SAPS and in the white patients with PAPS, the valine allele is related to the generation of antigenic B2GP1. We might consider the valine allele as a risk factor in this population. In the Mexican mixed population, the presence of antibodies against B2GP1 is associated with the Leu/Leu genotype and a protection for the development of these antibodies with the expression of the 316 polymorphism [14]. The Trp-Ser^{316} mutation could affect the phospholipid union. The insertion of one polar amino acid such as serine, in the absence of sequence 313-316 (Leu-Ala-Phe-Trp), may interfere in non-polar reactions between the two molecules [9].

The binding of B2GP1 to anionic phospholipids is essential for the reaction of some aCL present in APS individuals; for this reason, the genetic variation of the B2GP1 precludes the union of the phospholipids with the protein, affecting aCL production. This phenomenon was examined in 222 patients with systemic erythematous lupus, obtaining a distribution of the Trp/Ser^{316} polymorphism with significant differences between the patients positive or negative for aCL. This probably suggests protection of aCL production by the mutated allele in the 316 position. In contrast, the presence of aB2GP1 had no association with any polymorphism in the patients with APS [14].

We did not find differences between patients positive and those negative for IgA aB2GP1 and the polymorphisms studied. In the pursuit of the origin of these antibodies in a population with CKD, we may reject the genetic influence, as we also previously discarded the hypothesis that the hemodialysis membrane promotes a conformational change in the protein, exposing a critical epitope in the physiological conformation [6]. The presence of certain pathogens can induce APS similarities in the sequence with sequences B2GP1 [15]. Therefore, it could be interesting to investigate whether the origin of the IgA aB2GP1 antibodies is secondary to environmental factors such as the infections.

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REFERENCES

[1] Triplett DA. Antiphospholipid antibodies. Arch Pathol Lab Med 2002;126:1424–9.

[2] Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. Semin Nephrol 2007;27:35–46.

[3] Hanly JG. Antiphospholipid syndrome: an overview. CMAJ 2003;168:1675–82.

[4] Averna M, Paravizzini G, Marino G, et al. Liver is not the unique site of synthesis of beta 2-glycoprotein I (apolipoprotein H): evidence for an intestinal localization. Int J Clin Lab Res 1997;27:207–12.

[5] Serrano A, Garcia F, Serrano M, et al. IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. Kidney Int 2012;81:1239–44.

[6] Serrano M, Martinez-Flores JA, Castro MJ, et al. Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with endstage renal disease. J Immunol Res 2014;2014:641962.

[7] Hunt JE, Simpson RJ, Krilis SA. Identification of a region of beta 2-glycoprotein I critical for lipid binding and anti-cardiolipin antibody cofactor activity. Proc Natl Acad Sci U S A 1993;90:2141–5.

[8] McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci U S A 1990;87:4120–4.

[9] Prieto GA, Cabiedes AR. Polimorfismo de la J. Beta 2 glucoproteina, I: Relevancia en el síndrome antifosfolípidos. Rev Esp Reumatol 2002;29:8.

[10] Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.

[11] Hirose N, Williams R, Alberts AR, et al. A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. Arthritis Rheum 1999;42:1655–61.

[12] Lee YH, Choi SJ, Ji JD, Song GG. Association between the valine/leucine247 polymorphism of beta2-glycoprotein I and susceptibility to anti-phospholipid syndrome: a meta-analysis. Lupus 2012;21:865–71.

[13] Atsumi T, Tsutsumi A, Amengual O, et al. Correlation between beta2-glycoprotein I valine/leucine247 polymorphism and anti-beta2-glycoprotein I antibodies in patients with primary antiphospholipid syndrome. Rheumatology 1999;38:721–3.

[14] Kamboh MI, Manzi S, Mehdi H, et al. Genetic variation in apolipoprotein H (beta2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. Lupus 1999;8:742–50.

[15] Harel M, Aron-Maor A, Sherer Y, et al. The infectious etiology of the antiphospholipid syndrome: links between infection and autoimmunity. Immunobiology 2005;210:743–7.

Capitulo 2

La presencia previa al trasplante de anticuerpos antifosfolipídicos IgA anti-β-2-glicoproteína I como predictor de trombosis de injerto después del trasplante renal.

FONDO:
Una de las complicaciones más graves que aparecen en los primeros días y semanas después de un trasplante renal es la trombosis de los vasos que prefunden el injerto.
En trabajos previos del grupo se demostró que en pacientes en hemodiálisis la presencia de anticuerpos anti-β-2 glicoproteína-I de isotipo IgA (IgA anti B2GP1) se han relacionado con eventos trombóticos y la mortalidad. El trabajo trata de demostrar si este riesgo trombótico que se observa en el pretrasplante también sigue influyendo en la aparición de trombosis tras la cirugía de trasplante.
MÉTODOS:
Se realizó un estudio con todos los pacientes trasplantados de riñón en el hospital 12 de Octubre emn un periodo de 12 años de 2000 a 2011 (n = 1375). Los pacientes fueron seguidos por un periodo de dos años tras el trasplante. Los resultados se estudiaron evaluando todos los pacientes en conjunto y también dividiendolos en 3 períodos de cuatro años (cuatrienios) que se compararon.
RESULTADOS:
Se analizaron los sueros pretrasplante de todos los pacientes y se determinó que 401 pacientes fueron positivos para IgA-anti B2GP1 (29.2%, grupo 1). Los 974 pacientes restantes fueron negativos para ese autoanticuerpos (grupo 2).
La pérdida precoz del injerto (en los 6 primeros meses después del trasplante) fue mayor en el grupo 1 (18% frente a 7,2%; P <0,001). La causa más frecuente de pérdida temprana del injerto fue la trombosis, especialmente en el grupo 1 (12,2% frente al 2,6% de los pacientes, p <0,001). De hecho, la trombosis del vaso fue la causa más importante de pérdida de injerto en los 3 períodos de tiempo, independientemente de los cambios demográficos y la

introducción del trasplante con donantes a corazón parado. Llamó poderosamente la atención que IgA anti B2GP1 fue un factor de riesgo independiente para la trombosis del injerto (odds ratio, 5.047; P <0.001). Además, la presencia de IgA anti B2GP1 se asoció con la pérdida temprana del injerto y la función del injerto retrasada (necrosis tubular aguda). La mortalidad a los 24 meses también fue más alta en el grupo 1.

CONCLUSIONES:

La presencia de los anticuerpos IgA anti B2GP1 antes del trasplante fue el principal factor de riesgo para la trombosis del injerto y la pérdida temprana del injerto. Se debe realizar más investigación sobre si la anticoagulación en pacientes con anticuerpos positivos podría mejorar esta complicación catastrófica.

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The Presence of Pretransplant Antiphospholipid Antibodies IgA Anti-β-2-Glycoprotein I as a Predictor of Graft Thrombosis After Renal Transplantation

Jose Maria Morales,[1] Manuel Serrano,[1] Jose Angel Martínez-Flores, PhD,[1] Dolores Pérez,[1] Maria José Castro, PhD,[1] Elena Sánchez,[1] Florencio García, MD, PhD,[2] Alfredo Rodríguez-Antolín, MD,[3] Marina Alonso, MD,[4] Eduardo Gutierrez, MD,[2] Enrique Morales, MD,[2] Manuel Praga, MD, PhD,[2] Esther González, MD, PhD,[2] Amado Andrés, MD, PhD,[2] Estela Paz-Artal, MD, PhD,[1,5,6] Miguel Angel Martínez, MD, PhD,[4] and Antonio Serrano, MD, PhD[1,5]

Background. Vessel thrombosis is a severe complication after renal transplantation. Antibodies anti-β-2 glycoprotein-I of IgA isotype (IgA-aB2GP1) have been linked to thrombotic events and mortality in hemodialysis patients. **Methods.** All kidney transplanted patients from 2000 to 2011 (n = 1375) in our hospital were followed up for 2 years, evaluating 3 time periods. **Results.** At transplantation, 401 patients were positive for IgA-aB2GPI (29.2%, group 1), and the remaining patients were negative (group 2). Graft loss at 6 months posttransplantation was higher in group 1 (18% vs 7.2%; $P < 0.001$). The most frequent cause of early graft loss was vessel thrombosis, especially in group 1 (12.2% vs 2.6% of patients; $P < 0.001$). In fact, vessel thrombosis was the most important cause of graft loss in the 3 time periods, irrespective of demographic changes and introduction of transplantation with asystolic donors. Notably, IgA-aB2GP1 was an independent risk factor for graft thrombosis (odds ratio, 5.047; $P < 0.001$). Furthermore, the presence of IgA-aB2GP1 was associated with early graft loss and delayed graft function. Mortality at 24 months was also higher in group 1. **Conclusions.** In conclusion, pretransplant IgA-aB2GP1 was the main risk factor for graft thrombosis and early graft loss. Further research should be made on whether anticoagulation in antibody-positive patients could ameliorate this catastrophic complication.

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In recent decades, the introduction of calcineurin inhibitor-based immunosuppression, better methodologies in histocompatibility testing, and advances in prevention/treatment of infections have substantially increased short-term and long-term results after renal transplantation.[1] However, 5% to 8% of patients suffer graft loss in the first months after transplantation, a percentage which has remained unchanged in recent decades.[2] Graft thrombosis is the main cause of these early graft losses, generally attributed to surgical procedure related complications, although these may also be due to immune response-mediated conditions, such as acute rejection (AR).[2–4] It is well known that the presence of preformed antibodies against antigens on the membrane of graft cells, mainly HLA antigens, is associated with early rejection of kidney grafts.[5] Other antibodies against minor histocompatibility antigens or autoantigens have also been recognized as harmful for renal

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[1] Department of Immunology, Instituto de Investigación Hospital Universitario, Madrid, Spain.

[2] Department of Nephrology, Instituto de Investigación Hospital Universitario, Madrid, Spain.

[3] Department of Urology, Instituto de Investigación Hospital Universitario, Madrid, Spain.

[4] Department of Pathology, Instituto de Investigación Hospital Universitario, Madrid, Spain.

[5] Immunology Section, Universidad San Pablo-CEU, Madrid, Spain.

[6] Facultad de Medicina, Universidad Complutense, Madrid, Spain.

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J.M.M., M.S., and J.A.M.F. collaborated equally to this work.

A.S. conceived the project. A.S. and J.M.M. designed the research and wrote the article. A.S., M.S., and J.A.M.F. made the antiphospholipid determinations and were responsible for the database and the statistical analysis. J.M.M., F.G., E.M., A.R.A., E.Gu., E.Go., M.P., and A.A. were responsible for the patients care and clinical data collection. M.A.M. and M.A. made the histopathologic studies. M.J.C., E.S., D.P., and E.P.A. were responsible for the histocompatibility data and coordination of the Organ Transplant Waiting List Serum Bank. All authors contributed to the data interpretation and report preparation.

Correspondence: Antonio Serrano, Instituto de Investigación, I + 12 Hospital Universitario, 12 de Octubre Avda. de Andalucía s/n, 28041, Madrid, Spain. (aserrano@h12o.es).

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allograft evolution,[6,7] and their impact on the poor outcome of transplantation is being increasingly recognized.[8]

Antiphospholipid antibodies (aPL) are a group of autoantibodies against phospholipid-binding proteins. The antigens recognized by aPL are found in plasma or in the membranes of endothelial and others cells involved in coagulation cascade.[9] Antiphospholipid syndrome (APS) is a multisystemic autoimmune disorder characterized by recurrent thrombosis and/or gestational morbidity and the presence of aPL.[10] The aPL associated with vascular pathology are directed against apolipoprotein H, also knows as β-2 glycoprotein-I (B2GP1),[11] a protein synthesized mainly in the liver and kidney and found in the serum and membranes of platelets and endothelial cells.[12,13] Only the IgG and IgM isotypes of anti-B2GP1 antibodies were recognized as diagnostic in the international consensus for APS diagnosis (2004, Sidney, Australia).[9] However, over the past few years, much attention has been focused on the diagnostic and pathogenic value of IgA isotype. IgA anti-B2GP1 antibodies (IgA-aB2GP1-ab) have been associated with thrombotic events and conditions consistent with APS in patients negative for other aPL and might have a higher prevalence than IgG and IgM isotypes.[14-16] In fact, testing IgA-aB2GP1-ab is useful in the diagnosis of patients with thrombosis and is recommended in the International Consensus Guidelines (2010, Galveston, TX) in cases in which the IgG and IgM isotypes are negative and APS is still suspected.[17] The prevalence of IgA-aB2GP1-ab in patients with chronic renal failure in hemodialysis is greater than that in the general population (30% vs 1.5%)[18] and is associated with a higher incidence of thrombotic events and mortality.[19,20]

In a recent study based on a 10-year follow-up of 269 patients who received a renal transplant, we demonstrated that patients with pretransplant IgA-aB2GP1-ab have a high risk for early graft failure (EGF) (mainly caused by thrombosis) and a high risk of delayed graft function (DGF). We cannot demonstrate a significant risk for graft thrombosis due to small number of thrombotic events. We concluded that pretransplant IgA-aB2GP1-ab may have a detrimental effect on early clinical outcomes after renal transplantation. This study was performed for a long period with a small cohort of patients transplanted from cadaveric donors and with low immune complexity.[21] In the present work, we have studied the influence of preformed IgA-aB2GP1-ab after renal transplantation focused only on graft thrombosis and early events in a large group of patients having greater variability in the type of donor and recipient (living donors, uncontrolled non–heart-beating donors, and old-for-old transplantation with extremely aged donors) representing the current clinical practice in our hospital and in Spain.

MATERIALS AND METHODS

Study Design

We performed a historical cohort follow-up study that included all patients who had received a kidney transplant in a 12-year period: from January 1, 2000, to December 31, 2011.

The presence of IgA-aB2GP1-ab in pretransplant serum was examined, and 2 cohorts of positive and negative patients were followed-up for 2 years.

First aim: To investigate the possible influence of preformed IgA-aB2GP1-ab on graft thrombosis in all patients and in the 3 different periods of the study in the first 6 months. Main endpoints: graft loss, causes of graft loss and graft survival at 6 months, and differences between time periods of transplant.

Secondary aim: To investigate delayed clinical outcomes (7 to 24 months) in patients positive versus negative for IgA-aB2GP1-ab. Secondary endpoints: graft and patient survival at 2 years causes of graft loss and death and differences between transplant time periods.

Ethical issues: The study was approved by the Hospital "12 de Octubre" Institutional Review Board (References 11/359 and 15/008).

Patients

All patients who received a kidney transplant from 1 January 2000 to 31 December 2011 (N = 1513) were included in the study. Patients who also received a second organ (combined liver-kidney, pancreas-kidney or heart-kidney) were rejected for the study. A total of 138 patients (9.1 %) were excluded: 128 (8.5%) because pretransplant serum samples (1 to 15 days before transplantation) were unavailable, 4 due to a positive hypercoagulability test and 6 because their clinical data were not available (disposition algorithm and outcomes, Figure 1).

There were no significant differences between pretransplant clinical characteristics of the excluded patients and the patients who were finally analyzed (data not shown).

Demographic data of 1375 patients studied (cohort magnum 12 + 12) were: 1365 whites, 2 American Indians, 2 Asians, and 6 East Africans. All patients were followed up for a period of up to 2 years or until graft failure or death. Of these, 269 patients had been previously studied in a 10-year follow-up.[21]

Periods

In the 12-year period, there were important demographic changes and new forms of renal transplantation. Recipient and donor complexity also increased.

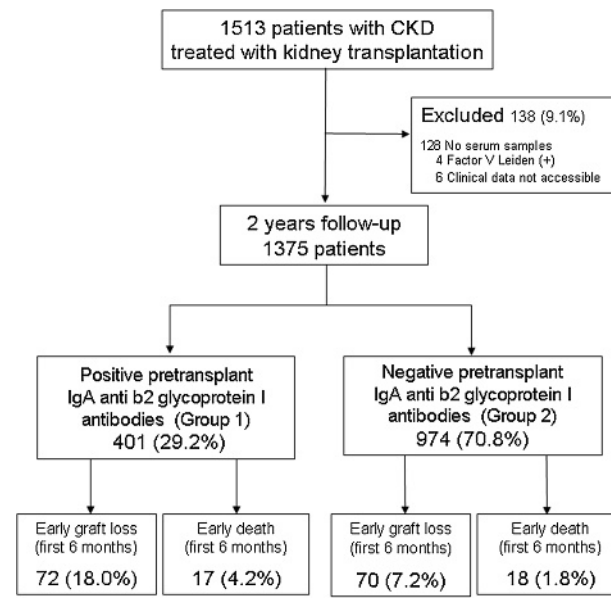

FIGURE 1. Disposition of study and early outcomes.

TABLE 1.

Clinical characteristics of patients with early graft loss versus patients with functioning graft at 6 mo posttransplant

	Early graft loss (n = 142)		Functioning graft (n = 1233)		
Condition	**N/Mean**	**%/SE**	**N/Mean**	**%/SE**	***P***
Sex (men)	85	(59.4%)	731	(59.3%)	0.967
Age, y	**60.9**	**±1.2**	**53.1**	**±0.4**	**<0.001**
Donor age, y	59.5	±1.6	50.6	±0.5	<0.001
Time on dialysis, mo	28.4	±0.9	30.7	±2.8	0.413
Pretransplant clinical characteristics					
Diabetes mellitus	45	(31.7%)	229	(18.6%)	<0.001
Type 1 diabetes	4	(2.8%)	28	(2.3%)	0.909
Type 2 diabetes	**41**	**(28.9%)**	**201**	**(16.3%)**	**<0.001**
Dyslipidemia	51	(35.9%)	418	(33.9%)	0.700
Hypertension	109	(76.8%)	995	(80.7%)	0.315
Tuberculosis	4	(2.8%)	48	(3.9%)	0.686
Hepatitis C	13	(9.2%)	95	(7.7%)	0.657
Hepatitis B	4	(2.8%)	18	(1.5%)	0.386
Patients IgA aB2GP1 positive	**72**	**(50.7%)**	**329**	**(26.7%)**	**<0.001**
Levels of IgA aB2GP1, U/mL	39.7	±5.0	20.8	±0.9	<0.001
Causes CKD					
Chronic glomerulonephritis	12	(8.4%)	250	(20.3%)	0.001
Interstitial kidney disease	14	(9.8%)	104	(8.4%)	0.678
Nephroangiosclerosis	16	(11.2%)	123	(10%)	0.736
Polycystic kidney disease	15	(10.5%)	154	(12.5%)	0.598
Diabetes mellitus	30	(21%)	164	(13.3%)	0.016
Lupus erythematosus	4	(2.8%)	14	(1.1%)	0.201
Vesicoureteral reflux	6	(4.2%)	44	(3.6%)	0.874
Unknown	25	(17.5%)	173	(14%)	0.307
Other	21	(14.7%)	206	(16.7%)	0.643
Transplant-associated factors					
Previous kidney transplant	26	(18.3%)	202	(16.4%)	0.642
PRA at time of transplant >50%	6	(4.2%)	28	(2.3%)	0.256
Historical PRA >50%	12	(8.5%)	102	(8.3%)	0.930
Double renal transplant	4	(2.8%)	35	(2.8%)	0.801
Cold ischemia, h	**20.9**	**±0.5**	**18.8**	**±0.2**	**<0.001**
Delayed graft function	54	(38.0%)	378	(30.7%)	0.073
Donor origin					
Brain death	118	(82.5%)	1021	(80.8%)	0.992
Non–heart-beating	23	(16.1%)	150	(14.1%)	0.230
Living donor	2	(1.4%)	61	(5.1%)	0.087
Patients with pretransplant thrombosis	34	(23.8%)	133	(10.8%)	<0.001
Pretransplant thrombotic events[a]	34		145		<0.001
Venous thrombosis	31		121		<0.001
Arterial thrombosis	1		8		[b]
Pulmonary thromboembolism	2		16		[b]
Stroke	8	(5.6%)	67	(5.4%)	0.924
Acute myocardial infarction	4	(2.8%)	35	(2.8%)	0.801

[a] Several patients had more than 1 type of event.
[b] Nonsignificant.
Variables selected for the multivariate analysis are shown in bold.
CKD, chronic kidney disease.

Therefore, to evaluate the effect of this changing scenario, recipients were separated into 3 subgroups of 4 years each according to the year of transplant: (quadrenniums [QD]): QD-1 (2000-2003) with low-complexity recipients and only brain-dead donors. QD-2 (2004-2007) period that begins transplantation with living and non–heart-beating donors (NHBDs). QD-3 (2008-2011) in which extremely-aged donation, high-complexity recipients and an active NHBD program were fully incorporated into clinical practice.

Database

The recipient pretransplant characteristics include age, original disease, time on dialysis, serology and immunological data, body mass index, arterial hypertension, hyperlipidemia,

TABLE 2.

Pretransplant condition and posttransplant events of patients in group 1 (positive for IgA aB2GP1 antibodies) and in group 2 (negative patients)

	Group 1 (n = 401)		Group 2 (n = 974)		
Condition	**Patients/mean**	**%/SE**	**Patents/mean**	**%/SE**	***P***
Sex (men)	213	(53.1%)	603	(61.9%)	0.003
Age, y	57.9	±0.7	52.2	±0.5	<0.001
Donor age, y	55.4	±09	49.9	±06	<0.001
Time on dialysis, mo	29.3	±1.6	28.6	±1.0	0.585
PRA at transplantation >50%	12	(3%)	22	(2.3%)	0.545
Historical PRA >50%	32	(8%)	82	(8.4%)	0.872
Previous kidney transplant	59	(14.7%)	170	(17.5%)	0.246
Double renal transplant	17	(4.2%)	22	(2.3%)	0.067
Cold ischemia, h	19.6	±0.3	18.8	±0.2	0.031
Induction immunosuppression	302	(75.3%)	695	(71.4%)	0.154
Anticoagulation					
Warfarin (isolated)	18	(4.5%)	36	(3.7%)	0.593
Warfarin associated with aspirin or clopidogrel	4	(1%)	7	(0.7%)	0.846
Aspirin (isolated)	60	(15%)	145	(14.9%)	0.962
Clopidogrel (isolated)	8	(2%)	27	(2.8%)	0.520
Associated pathologies					
Diabetes mellitus	85	(21.2%)	189	(19.4%)	0.495
Type 1 diabetes	6	(1.5%)	26	(2.7%)	0.265
Type 2 diabetes	79	(19.7%)	163	(16.7%)	0.217
Myocardial infarction	10	(2.5%)	29	(3%)	0.750
Stroke	27	(6.7%)	48	(4.9%)	0.227
Patients with pretransplant thrombosis	60	(15%)	107	(11%)	0.046
Causes CKD					
Chronic glomerulonephritis	75	(18.7%)	187	(19.2%)	0.891
Interstitial kidney disease	34	(8.5%)	84	(8.6%)	0.985
Nephroangiosclerosis	39	(9.7%)	100	(10.3%)	0.838
Polycystic kidney disease	43	(10.7%)	126	(12.9%)	0.296
Diabetes mellitus	67	(16.7%)	127	(13%)	0.091
Lupus erythematosus	6	(1.5%)	12	(1.2%)	0.896
Vesicoureteral reflux	10	(2.5%)	40	(4.1%)	0.196
Unknown	64	(16%)	134	(13.8%)	0.331
Other	63	(15.7%)	164	(16.8%)	0.666
Donor origin					
Brain death	343	(85.5%)	796	(81.7%)	0.104
Non–heart-beating (Maastricht type II)	44	(11%)	129	(13.2%)	0.287
Living donor	14	(3.5%)	49	(5%)	0.272
Posttransplant clinical events					
Cardiovascular events	8	(2%)	10	(1%)	0.240
Patients with acute rejection episodes	94	(23.4%)	206	(21.1%)	0.388
Delayed graft function	162	(40.4%)	270	(27.7%)	<0.001
First-month graft loss	50	(12.5%)	49	(5.0%)	<0.001
First-trimester graft loss	61	(15.2%)	59	(6.1%)	<0.001
First semester graft loss	72	(18%)	70	(7.2%)	<0.001
Graft loss first semester					
Acute rejection	5	(1.2%)	8	(0.8%)	0.664
Nonfunctioning graft	8	(2%)	6	(0.6%)	0.043
Death (with a functioning kidney)	2	(0.5%)	11	(1.1%)	0.429
Graft thrombosis	49	(12.2%)	25	(2.6%)	<0.001
Venous thrombosis	17		10		<0.001
Venous and arterial thrombosis	25		11		<0.001
Arterial thrombosis	7		4		0.028
Others	8	(2%)	20	(2.1%)	0.888

Continued next page

TABLE 2. (Continued)

Condition	Group 1 (n = 401) Patients/mean	%/SE	Group 2 (n = 974) Patents/mean	%/SE	*P*
Graft loss from months 7 to 24	11	(2.7%)	17	(1.7%)	0.327
Acute rejection	1		0		[a]
Death (with a functioning kidney)	9		13		[a]
Chronic allograft nephropathy	0		2		[a]
Others	1		2		[a]
Death in 2 y	29	(7.2%)	33	(3.4%)	0.003
Death first semester	17	(4.2%)	18	(1.8%)	0.018
Death from months 7 to 24	12	(3%)	15	(1.5%)	0.121
Death causes					
Cardiovascular diseases	13		10		0.007
Infections	9		15		[a]
Cancer	3		1		[a]
Others	4		7		[a]

All patients treated at pretransplantation with antiplatelet agent or anticoagulation received aspirin or clopidogrel or anticoagulation after transplantation.
[a] Nonsignificant.

diabetes, smoking, and pretransplant cardiovascular disease were specifically recorded in our database. The following were recorded after surgery: type and characteristics of the donor, immunosuppressive treatment at the time of transplantation, incidence of thrombotic and cardiovascular events, neoplasia, ARs episodes and DGF. Graft and patient survival and causes of graft loss and mortality were also recorded. Graft loss data are non-death–censored to better reflect the real situation in the clinical practice. The causes of death in patients who lose the graft by death with a functioning kidney did not differ from those of the general population. In the same way, the mortality data are also not censored for any cause. Only 1 patient (group 2) died by a traffic accident.

Donor age was not used in statistical analysis because it is a recipient-age dependent variable. The policy for donor-recipient selection was based on making an attempt to match both for similar age. The Pearson correlation coefficient between donor and recipient age was 0.79 ($P < 0.001$).

Immunosuppressive Treatment

The most frequently used immunosuppressive protocol was based on tacrolimus associated with prednisone and mycophenolate mofetil (MMF), with or without induction. Patients older than 60 years who received kidneys from older than 60 years donors, immunosuppression regimen was based on cyclosporine A or tacrolimus, prednisone, and MMF with or without induction.[3,5,32]

Induction Treatment

Prednisone, tacrolimus (0.2 mg/kg per day) and MMF (1 g/d) were used. In hyperimmunized patients, thymoglobulin (rabbit antithymocyte globulin) 1.5 mg/kg for 4 to 7 days was also administered. In elderly people (over 60 years), basiliximab (monoclonal antibodies anti-IL2R) 20 mg days 0 and 4) with prednisone, cyclosporine A (10 mg/kg per day) and MMF (2 g/day) were administered until 2003, then tacrolimus (0.1 mg/kg per day) was chosen as a calcineurin inhibitor until the end of this study.

Anticoagulation Treatment

At transplantation, 54 patients (3.9%) were on anticoagulation with warfarin, 205 (15%) with aspirin, 35(2.5%) with Clopidogrel, and 11 (0.8%) with warfarin and an antiplatelet agent.

Patients requiring anticoagulation for venous thromboembolism prophylaxis in the immediate posttransplant period received subcutaneous heparin 5000 units twice daily for a week.

All patients treated at pretransplantation with an antiplatelet agent or anticoagulation received aspirin or clopidogrel or anticoagulation after transplantation.

Definitions

Thrombotic events: were defined following the International consensus statement on the classification criteria for APS[1] as venous thrombosis, arterial thrombosis, pulmonary thromboembolism, graft thrombosis, transient ischemic attack, acute stroke, diagnosed clinically, and confirmed by images techniques and/or by histopathology study.[17] All patients with pretransplant thrombotic events were studied before transplantation by the Hematology Department. Positive cases of hypercoagulability were excluded from the study.

Graft thrombosis was considered only in patients with thrombotic events and negative for AR diagnostic criteria (immunological or histopathological) and complications related to surgery. Dubious graft thrombosis: in 6 cases, there was doubt whether renal thrombosis had taken place in the environment of a possible vascular/technical problem (4) or in the context of an uncertain AR (2). It was decided to not consider them as graft thrombosis and to include them, respectively, in the groups of surgical problems and AR.

Primary nonfunction: this was considered in grafts with good perfusion that never functioned and in which a biopsy study had excluded rejection and other causes of graft failure.

Delayed graft function: this was defined as an initial and reversible graft nonfunction that requires hemodialysis during the first week after surgery, once rejection, vascular complications, and urinary tract obstruction had been excluded.

Cardiovascular event was considered when at least 1 of the following was present: myocardial infarction, angina, coronary revascularization, stroke, heart failure, or peripheral bypass.

Acute rejection was defined as acute deterioration in allograft function with specific histopathologic changes in the

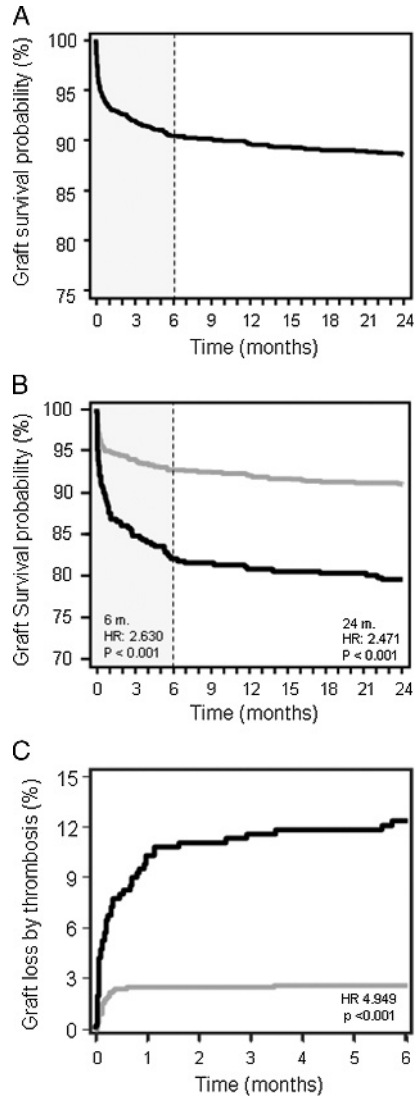

FIGURE 2. Graft-survival and graft-loss by thrombosis. A, Evolution of graft survival on complete follow-up. B, Graft survival in patients on group 1 (positive for IgA aB2GP1 antibodies), dark line, was significantly lower than observed in group 2 (gray line) both at 6 months and in the complete follow-up. HR: Hazard ratio (Kaplan-Meier analysis). Graft survival at 24 months was calculated from the day of transplantation. C, Graft loss by thrombosis in the 6 months after transplantation was significantly higher in patients in group 1 (dark) than patients in group 2 (gray).

graft. Clinically AR is applied to patients with acute worsening in allograft function without histological data.

Laboratory Determinations

Anti-B2GP1 antibodies of IgA isotype were measured in pretransplant serum used for crossmatch or in a serum sample obtained up to 15 days before transplantation. Other aPL antibodies (anti cardiolipin of IgG, IgM and IgA isotypes and aB2GP1 of IgG and IgM isotypes) were evaluated in a sample of 584 patients. Descriptive statistics of pretransplant levels of consensus aPL (IgG and IgM isotypes) is represented as a column bar chart in **Figure S1, SDC,** http://links.lww.com/TP/B263. Prevalence and mean levels were similar to that previously reported[21] and no clinical associations were observed.

Autoantibodies were quantified by enzyme-linked immunosorbent assays with QUANTA Lite (INOVA Diagnostics Inc., San Diego, CA, USA) kit. Following the manufacturer's guidelines, Antibody levels higher than 20 U/ml were considered positive. The cutoff was established following the International Consensus Guidelines on Anticardiolipin and Anti-B2-Glycoprotein I testing[17] with the 99th percentile of a healthy normal population, and it coincided with that recommended by the manufacturer.[15]

Crossmatching between donor and recipient was performed by specific antibody mediated cytolysis in the presence of complement. All transplanted patients were crossmatch-negative.

Panel-reactive antibody (PRA) score is defined as the percentage of the population (%) to which the patient reacts via preformed antibodies. Panel-reactive antibody was studied by specific antibody mediated cytolysis in the presence of complement using pooled lymphocyte panel with at least 35 unrelated, nonselected genotypes. Patients with PRA value of 50% or greater were considered as "sensitized."

Pathology

Most of the patients who suffered renal thrombosis underwent transplactectomy, and the graft tissue was studied in the pathology department immediately after the surgery. Two experienced nephropathologist jointly reevaluated all the cases in the period 2014 to 2015 to confirm the diagnosis.

Statistical Methods

Results are expressed as mean ± standard error, or absolute frequency and percentage. Association between qualitative variables was determined with Pearson χ^2 test or Fisher exact test, when appropriate. Data were expressed as number and percentage.

Comparisons were performed using the Student *t* test in scaled variables with 2 categories. Data were expressed as mean ± standard error.

Graft loss and patients' survival probability were calculated using the Kaplan-Meier method, and differences between the distributions of survival were assessed with the Log-rank test. Graft loss and mortality risk (events that can occur at any time during the study) were also estimated by multivariate analysis using the Cox regression model. Multivariate analysis of the dichotomous outcomes that only appear in the first weeks of the study (graft thrombosis and DGF) was performed using logistic regression model. The relative measure of an effect was expressed as ratio (odds/hazards) and confidence interval. Probabilities less than 0.05 were considered significant.

Data were processed and analyzed using Medcalc for Windows version 15.4 (MedCalc Software, Ostend, Belgium).

RESULTS

Patients With IgA anti B2GP1 Antibodies

IgA-aB2GP1-ab values were above the cutoff (group 1) in 401 (29.2%) of the 1375 patients studied, whereas they were negative in 974 (group 2). The algorithm distribution of patients in each group and outcome distribution are described in Figure 1. Table 1 shows the demographic data of the 1375 patients.

Comparing clinical characteristics of both groups (Table 2), group 1 were older (57.9 ± 0.7 vs 52.2 ± 0.5 years, $P < 0.001$), had higher cold ischemia time (19.6 ± 0.3 vs 18.8 ± 0.2 hours, $P < 0.031$), and suffered more pretransplant thrombotic

TABLE 3.

Multivariate analysis

Factors	Univariate			Multivariate		
(A) Graft loss	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
IgA aB2GP1 positive	2.631	1.896 to 3.650	<0.001	2.284	1.639 to 3.183	<0.001
Age, y	1.036	1.023 to 1.048	<0.001	1.024	1.010 to 1.038	<0.001
Cold ischemia, h	1.055	1.025 to 1.087	<0.001	1.027	0.996 to 1.058	0.095
Type 2 diabetes	1.991	1.387 to 2.856	<0.001	1.493	1.026 to 2.173	0.037
(B) Graft loss by thrombosis	OR	95% CI	*P*	OR	95% CI	*P*
IgA aB2GP1-positive	5.284	3.215 to 8.686	<0.001	5.047	3.048 to 8.357	<0.001
Age, y	1.021	1.005 to 1.038	0.011	1.007	0.989 to 1.025	0.440
Type 2 diabetes	2.079	1.237 to 3.494	0.006	1.847	1.056 to 3.233	0.032
(C) Delayed graft function	OR	95% CI	*P*	OR	95% CI	*P*
IgA aB2GP1-positive	1.767	1.385 to 2.256	<0.001	1.936	1.497 to 2.503	<0.001
Previous kidney transplant	1.501	1.118 to 2.015	0.007	1.381	0.992 to 1.921	0.056
Non–heart-beating donor	3.904	2.810 to 5.426	<0.001	4.487	3.190 to 6.311	<0.001
Dialysis time, mo	1.011	1.008 to 1.015	<0.001	1.011	1.007 to 1.014	<0.001
(D) Global mortality	HR	95% CI	*P*	HR	95% CI	*P*
IgA aB2GP1 antibodies	2.171	1.322 to 3.566	0.002	1.618	0.983 to 2.663	0.060
Age	1.094	1.068 to 1.121	<0.001	1.089	1.062 to 1.117	<0.001
Type 2 diabetes	3.027	1.820 to 5.033	<0.001	1.562	0.931 to 2.621	0.093
(E) Mortality in QD-3.	HR	95% CI	*P*	HR	95% CI	*P*
IgA aB2GP1 antibodies	2.574	1.366 to 4.850	0.004	2.171	1.145 to 4.115	0.018
Age	1.071	1.041 to 1.101	<0.001	1.066	1.035 to 1.098	<0.001
Type 2 diabetes	2.061	1.070 to 3.971	0.032	1.186	0.602 to 2.337	0.624

(A) Cox multivariate analysis ($P < 0.001$) of graft-loss associated variables significant in univariate analysis. (B) Logistic regression multivariate analysis ($P < 0.001$) of variables associated to graft loss by thrombosis. (C) Logistic regression multivariate analysis ($P < 0.001$) of with delayed graft function-associated variables significant in univariate analysis. (D) Multivariate survival analysis all the patients at 24 mo using Cox's Regression Model ($P < 0.001$) of patient-death associated-variables. (E) *Cox's* Multivariate survival analysis of patients transplanted in quadrenium 3 (QD-3).

events (15% vs 11%, $P = 0.046$). Antibody concentration does not correlate with severity of symptomatology (data not shown).

No significant differences were observed in pretransplant anticoagulant treatment and other characteristics except for the lower proportion of men in group 1 (53.1% vs 61.9%, $P = 0.003$).

Graft Failures Are Concentrated in Early Posttransplant Period

During the first 6 months posttransplantation, 142 patients (10.4%) lost their graft (Figure 2A). Main cause of graft loss was thrombosis: 74 patients (52% of losses). Pretransplant differences among patients with EGF and graft-functioning patients were age (60.9 ± 1.2 vs. 53.1 ± 0.4 years; $P < 0.001$), presence of type 2 diabetes mellitus (28.9% vs 16.3%, $P < 0.001$), and positivity of IgA-aB2GP1-ab (50.7% vs 26.7%; $P < 0.001$). Patients with graft loss also had higher cold ischemia time (20.9 ± 0.5 vs 18.8 ± 0.2 hours; $P < 0.001$) and a higher incidence of DGF (39.4% vs 30.7%; $P = 0.042$).

Causes of chronic kidney disease did not differ, except for chronic glomerulonephritis that was less frequent in patients with EGF (8.4% vs 20.3%, $P < 0.001$) and diabetic nephropathy that was more frequent in EGF (21% vs 13.3%, $P = 0.016$). There were no differences in other factors, such as donor type, time on dialysis, or proportion of patients with elevated PRA (Table 1).

Early Kidney Allograft Failure and IgA aB2GP1 Antibodies

Graft loss was significantly higher in group 1 (18.0% vs 7.2%; $P < 0.001$; Table 2). The Kaplan-Meier survival analysis (Figure 2B) showed significantly lower graft survival rates in group 1 both at 6 months (hazard ratio [HR], 2.630; $P < 0.001$) and at 24 months (HR, 2.471; $P < 0.001$).

Regarding causes of graft loss, graft thrombosis was significantly greater in group 1 (Figure 2C): 12.2% versus 2.6% (HR, 4.949, $P < 0.001$). No significant differences were observed in other causes of graft loss (Table 2).

IgA aB2GP1 Antibodies Are an Independent Risk Factor for Early Kidney Allograft Failure

The significant graft loss–associated-factors in the univariate analysis (Table 1) were included in the Cox multivariate analysis. Hazard ratio for the presence of IgA- aB2GP1-ab for graft loss was 2.631 ($P < 0.001$) and after adjusting for other factors, continued to be clearly significant: 2.284; (95%; CI 1.639 to 3.183; $P < 0.001$).

Age and type-2 diabetes also continued to be significant (Table 3A).

IgA aB2GP1 Antibodies Are an Independent Risk Factor for Graft Thrombosis

IgA-aB2GP1-ab, type-2 diabetes and age were identified in univariate analysis as significant mediating factors for graft thrombosis (**Table S1, SDC**, http://links.lww.com/TP/B263). In a logistic regression multivariate analysis type-2 diabetes and particularly IgA-aB2GP1-ab were identified as independent risk factors for graft thrombosis (odds ratio [OR], 5.047; 95% CI, 3.048 to 8.357, $P < 0.001$) (Table 3B).

IgA aB2GP1 Antibodies Are an Independent Risk Factor for DGF

Delayed graft function was more frequent in group 1 (40.4% vs 27.7%, $P < 0.001$). In a multivariate analysis

studying the variables that were significant in a univariate analysis (**Table S2, SDC,** http://links.lww.com/TP/B263), we found that IgA-aB2GP1-ab was an independent risk factor for DGF (OR, 1.936; $P < 0.001$). Non–heart-beating donor, previous transplant and duration of dialysis also were independent risk factors (Table 3C). Other well-known risk factors for DGF,[22] as donor age and cold ischemia time, were not included because they were not significant in the univariate analysis ($P = 0.689$ and 0.316).

Periods

In the first quadrennium (QD-1) most of the recipients were middle-aged patients who received kidneys from deceased donors (99.5%). Patient complexity increased from QD-1 to QD-3. Patients were progressively older (**Figures S2A and S2B, SDC,** http://links.lww.com/TP/B263 and Table 4), had more retransplant (11.7% in QD-1 to 17.5% in QD-3, $P = 0.037$) and with significant increase of pretransplant type-2 diabetes (11.5% in QD-1 to 30% in QD-3, $P < 0.001$). Donors also were progressively older (**Figure S2C, SDC,** http://links.lww.com/TP/B263).

There were no significant differences in the proportion of patients positive for IgA-aB2GP1-ab over the 3 periods. Demographics data and clinical course of patients in each quadrennium are shown in Table 4. Prevalence of posttransplantation clinical events was higher in QD-3 compared with QD-1. Quadrennial Kaplan-Meier graft survival analysis at 6 months (early graft loss) and at complete follow-up showed a progressively lower graft survival probability from QD-1 to QD-3 (Table 4 and Figure 3). Graft survival in each quadrennium, both at 6 months and in the whole follow-up remained significantly lower in group 1 (Table 4 and Figure 4).

Kaplan-Meier graft survival analysis at 6 and 24 months demonstrated that the hazard ratio for group 1 always remained significantly above 2.3, independent of the period studied (**Table S3, SDC,** http://links.lww.com/TP/B263). Graft thrombosis in all 3 periods was significantly higher in patients in group 1 (QD-1: OR, 4.0; $P = 0.015$; QD-2: OR, 8.0, $P < 0.001$; QD-3: OR, 4.6, $P < 0.001$).

Mortality

Mortality was higher in group 1 (7.2% vs 3.4%, $P = 0.003$; Table 2). Causes of death did not differ between the groups except for more frequent cardiovascular disease in group 1 (3.2% vs 1%; $P = 0.007$; Table 2).

Kaplan-Meier analysis showed significantly higher mortality in group 1 (HR, 2.171; 95% CI, 1.253-3.761; $P = 0.002$; **Figure S3, SDC,** http://links.lww.com/TP/B263). In the unvaried analysis of mortality-related factors, we found that age, type-2 diabetes, and presence of IgA-aB2GP1-ab were significantly associated with death (**S 4, SDC,** http://links.lww.com/TP/B263). Multivariate Cox regression analysis showed that only age could be considered an independent factor for death (HR, 1.089; 95% CI, 1.062-1.117; $P < 0.001$). Hazards ratio of the presence of aB2GP1 in the univariate was 2.171 (95% CI, 1.253-3.761; $P = 0.002$) going on behind the multivariate analysis to 1.618 (95% CI, 0.983-2.663; $P = 0.060$) (Table 3D).

Mortality in QD-1 and QD-2 was low: 10 (2.7%) and 14 (3.1%), respectively (Table 4). Because of the low number of events, a Kaplan-Meier analysis ($P = 0.226$ and $P = 0.734$) could not be performed. Because there was a greater number of events (38) in QD-3, we could perform a survival analysis: patients in group 1 had higher mortality (univariate analysis: HR, 2.574; 95% CI, 1.366-4.850; $P = 0.004$). Cox regression multivariate analysis in QD-3 show age (HR, 1.066; 95% CI, 1.035-1.098; $P < 0.001$) and presence of IgA-aB2GP1-ab (HR, 2.171, 95% CI 1.145 to 4.115, $P = 0.018$) as independent risk factors for death (Table 3E).

Late Posttransplant Period

There were no significant clinical differences between both groups in graft and patient survivals from month 7 to the end of the follow-up. Morbidity and causes of graft loss and mortality in this period were also similar in both groups (Table 2). Graft and patients survivals at 2 years (N = 1375), 5 years (N = 957), and 10 years (N = 473) are reported in Table S5 (**SDC,** http://links.lww.com/TP/B263).

DISCUSSION

In the present study, we have demonstrated for the first time in a large series with more that 1300 renal transplants that pretransplant IgA-aB2GP1-ab is an independent risk factor for early graft thrombosis after renal transplantation. Notably, this finding has also been significant in the 3 quadrenniums irrespective of the changes during these 12 years in the characteristics of donors and recipients. Graft and patient survivals at 6 months were significantly lower in patients with pretransplant IgA-B2GP1-ab compared with those having negative antibodies. We previously described that the presence of preformed IgA-aB2GP1-ab is an independent risk factor for graft loss, especially by thrombosis, although we were unable to make a multivariate analysis in the first period after transplantation.[21] We have now been able to corroborate this previous finding and have clearly demonstrated that pretransplant IgA-aB2GP1-ab is an independent risk factor for early graft thrombosis. Therefore, the presence of pretransplant IgA-aB2GP1-ab could be considered a strong predictor of graft thrombosis and consequently EGF and mortality after renal transplantation.

Presently, as immunosuppression becomes more effective, vessel graft thrombosis has become the most important cause of EGF.[23] Several factors may be involved in the pathogenesis of graft thrombosis such as surgical-derived problems and technical errors,[24] compression of the renal artery or vein by fluid collections, and postoperative hypercoagulability.[25] In our study, thrombosis represented 56.3% of the early graft loss after transplantation, the results being similar to those previously reported.[23,26,27] Pretransplant IgA-B2GP1-ab was the main independent risk factor for thrombosis-induced graft loss, only followed by type-2 diabetes. Older patients who received kidneys from older donors had increased prevalence of graft loss and vessel thrombosis compared with the standard cadaveric renal transplantation.[28] In contrast, type-2 diabetes mellitus patients present a hypercoagulability state[29] that increases the risk of vessel thrombosis.[30]

It is well known that the presence of aPL is insufficient to induce thrombosis formation. Patients with aPL for long periods need a "second hit" that involves activation of innate immunity and a proinflammatory microenvironment to trigger thrombotic episodes.[31,32] Surgery is an important risk factor for thrombosis,[23] mainly in uremic patients with severe atherosclerosis. It may also be a second trigger for

TABLE 4.

Demographic and posttransplant events during the 3 quadrenniums

Condition	QD-1 (2000-2003) N = 367		QD-2 (2004-2007) N = 455		QD-3 (2008-20011) N = 553		*P* (trend)
	Patients/Mean	%/SE	Patients/Mean	%/SE	Patients/Mean	%/SE	
Age, y	50.7	±0.4	54.6	±0.7	55.5	±0.7	<0.001
Sex (men)	205	(55.9%)	273	(60%)	338	(61.1%)	0.124
Time on dialysis, mo	28.2	±1.6	29.1	±1.5	28.5	±1.4	0.909
IgA aB2GP1-ab positive	119	(32.4%)	112	(24.6%)	170	(30.7%)	0.811
Older than 60 y	93	(25.3%)	191	(42.0%)	233	(42.1%)	<0.001
Diabetes	43	(11.7%)	92	(25.3%)	139	(33.6%)	<0.001
Type 1 diabetes	6	(1.9%)	11	(3%)	15	(3.6%)	0.366
Type 2 diabetes	37	(10.1%)	81	(17.8%)	124	(22.4%)	<0.001
Previous kidney transplant	43	(11.7%)	88	(19.3%)	97	(17.5%)	0.037
PRA at time of transplant >50%	13	(3.5%)	11	(2.4%)	10	(1.8%)	0.102
Historical PRA >50%	41	(11.2%)	38	(8.4%)	35	(6.3%)	0.009
Donor							
Donor age, y	51.5	±0.5	57.9	±0.7	52.3	±0.5	<0.001
Brain death	46.5	±1.0	53.6	±0.9	58.7	±0.9	<0.001
Non–heart-beating (Maastricht type II)	—	—	39.1	±1.7	43.6	±0.9	<0.001
Living donor	—	—	48.6	±2.0	49.8	±1.5	<0.001
Donor origin							
Brain death	365	(99.5%)	405	(89%)	369	(66.7%)	<0.001
Non–heart-beating	2	(0.5%)	30	(6.6%)	141	(25.5%)	<0.001
Living donor	0	(0%)	20	(4.4%)	43	(7.8%)	<0.001
Causes of CKD							
Chronic glomerulonephritis	85	(23.2%)	86	(18.9%)	91	(16.5%)	0.012
Interstitial kidney disease	51	(13.9%)	30	(6.6%)	37	(6.7%)	<0.001
Nephroangiosclerosis	32	(8.7%)	48	(10.5%)	59	(10.7%)	[a]
Polycystic kidney disease	51	(13.9%)	57	(12.5%)	61	(11%)	[a]
Diabetes	31	(8.4%)	71	(15.6%)	92	(16.6%)	<0.001
Lupus erythematosus	4	(1.1%)	5	(1.1%)	9	(1.6%)	[a]
Vesicoureteral reflux	8	(2.2%)	21	(4.6%)	21	(3.8%)	[a]
Unknown	66	(18%)	68	(14.9%)	62	(11.2%)	[a]
Other	39	(10.6%)	69	(15.2%)	121	(21.9%)	
Post transplant							
Delayed graft function	92	(25.1%)	150	(33%)	192	(34.7%)	0.003
Death in 2 y	10	(2.7%)	14	(3.1%)	38	(6.9%)	0.002
First-month graft loss	15	(4.1%)	31	(6.8%)	53	(9.6%)	0.002
First-trimester graft loss	20	(5.4%)	35	(7.7%)	65	(11.8%)	<0.001
First-semester graft loss	24	(6.5%)	42	(9.2%)	76	(13.7%)	<0.001
Early graft loss (up to 6 mo)							
Acute rejection	4	(1.1%)	2	(0.4%)	7	(1.3%)	[a]
Death	2	(0.5%)	3	(0.7%)	8	(1.5%)	[a]
Graft Thrombosis	15	(4.1%)	25	(5.5%)	34	(6.2%)	[a]
Nonfunctioning graft	1	(0.3%)	2	(0.4%)	11	(2%)	0.013
Others	2	(0.5%)	10	(2.2%)	16	(2.9%)	0.045
Very early graft loss (first 3 months)	20	(5.4%)	35	(7.7%)	65	(11.8%)	<0.001
Graft loss in 2 years	30	(8.2%)	48	(10.5%)	92	(16.6%)	<0.001
Graft loss 7 to 24 month.	6	(1.6%)	6	(1.3%)	15	(2.9%)	0.142
Acute rejection	0		0		1		[a]
Death	5	(83%)	4	(67%)	13	(87%)	[a]
Chronic allograft nephropathy	0		2	(33%)	0		[a]
Others	1		0		1		[a]

[a] Nonsignificant.

thrombosis[13] in patients positive for IgA-aB2GP1-ab in the context of renal transplantation. So, graft thrombosis may be considered more as a latent immune process reactivated by surgery than as a true surgical complication. The potential role of the "second hit" of peritransplant factors, such as infectious agents, inflammatory state, conditioning immunosuppressive treatments and other unidentified factors,[33] require further investigation. It is unknown why patients with chronic renal disease show elevated prevalence of IgA-aB2GP1-ab and how its immune response is generated. It

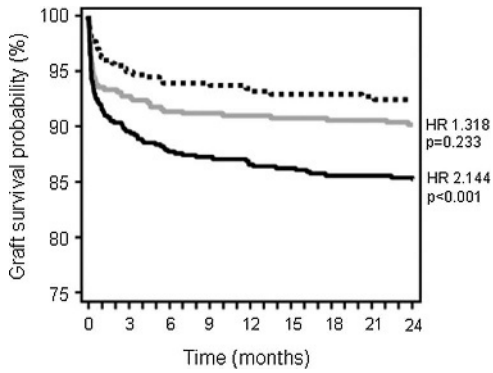

FIGURE 3. Graft survival at 24 months (Kaplan Meier analysis, $P < 0.001$) in the 3 quadrenniums (QD). QD-1 (2000-2003), dotted line. QD-2 (2004-2007), gray line. QD-3 (2008-2011), dark line. HR and *P* values of QD-2 and QD-3 were calculated in reference to QD-1.

has been proposed that misfolded B2GP1[34] produced in an unhealthy kidney may be cross-reacting with antibodies against epitopes detected on mucosal infections by pathogens with molecular mimicry with B2GP1.[18] Therefore, a study on this proposal is recommendable.

The mainstay of preventive treatment for thrombotic APS is antithrombotic prophylaxis, generally using long-term anticoagulation with vitamin K antagonists (VKA).[35] Using VKA is especially difficult in transplanted patients because it increases the risk of hemorrhagic complications and therefore should be used only in selected ones.[36] The next step could be to discover additional biomarkers that would make it possible to identify patients positive for IgA-aB2GP1-ab with higher risk for thrombosis.

Recently, eculizumab has been used in isolated cases of catastrophic APS.[37] It is possible that eculizumab cannot be useful in this special form of APS because the complement does not seem to be activated by IgA.[38] However, to clarify this point, further investigation is required.

It has been recently described that the mammalian target of rapamicine pathway is involved in vascular lesions associated with the APS.[39] In this way, initial immunosuppression based on mTOR inhibitors in renal transplant patients positive for IgA-aB2GP1-ab could be an alternative to the use of VKA to prevent thrombotic events. New prospective studies are necessary to confirm the potential usefulness of these drugs. One important finding of our study is that the presence of pretransplant IgA-aB2GP1-ab is associated with high early mortality in the overall group. In the Cox multivariate analysis of the complete period, only age was found as an independent risk factor. The *P* value of IgA-aB2GP1-ab ($P = 0.060$) is insufficient to consider it an independent risk factor. However, when the multivariate analysis was performed only with the QD-3 (period with higher mortality), type-2 diabetes and IgA-aB2GP1-ab were independent risk factors for death. This aspect should be studied further with a higher number of patients. We previously described the relationship between DGF and IgA-aB2GP1-ab.[21] In recent years, an increase of patients with DGF has been observed due to increased renal transplantation with NHBD[40] and old-for-old transplantation.[41] In this study, the multivariable analysis has identified previous transplantation, donation after cardiac death, time on dialysis, and presence of IgA-aB2GP1-ab as risk factors for DGF, thus making it possible to corroborate this important association. We can hypothesize that some patients with DGF may have mild posttransplant vascular involvement (equivalent to reversible mild microangiopathy) that could be related to IgA-aB2GP1-ab. Therefore, future research in this important area is mandatory.

The most important limitations of our study are that only our center was included, and only a simple pretransplant study of hypercoagulability was performed due to the nature of the present study. Although the patients were evaluated for the V Leiden factor, additional testing, such as prothrombin mutations, activated protein C resistance, antithrombin, protein C and protein S activity, was not systematically performed. However, it must be kept in mind that all transplanted patients were recruited without limitations, representing the actual clinical practice. The factors studied, such as mortality and graft loss, are objective and are not depending on any subjective interpretation.

If this finding is corroborated, prophylactic treatment of pretransplant IgA-B2GP1-ab positive patients could ameliorate this early complication. Furthermore, it may be an outstanding advance to not only avoid graft loss but also for the National Heath Service to decrease the annual economical budget. New multicenter studies are needed to confirm the value of IgA-aB2GP1-ab as an early transplant failure biomarker. Herein, we are describing a possible new form of IgA isotype-based APS, secondary to chronic renal failure

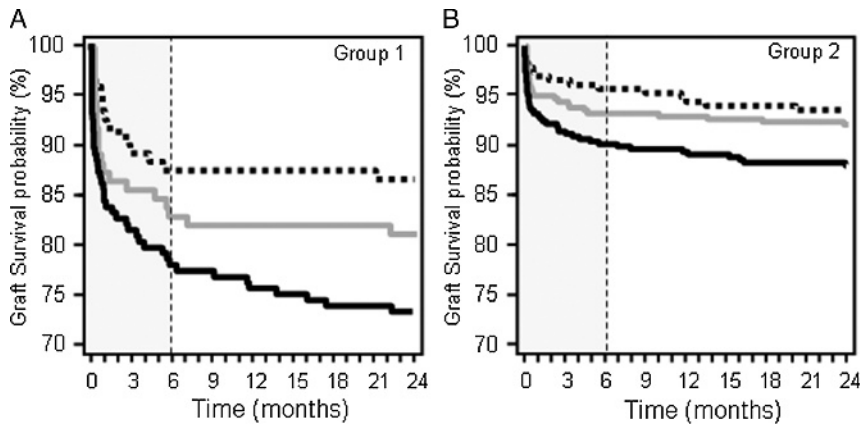

FIGURE 4. Evolution of graft survival in complete follow-up in the 3 time periods (Kaplan-Meier analysis, $P < 0.001$). A, Patients in group 1 (positive for IgA aB2GP1 antibodies). B, Patients in group 2. QD-1 (2000-2003), dotted line. QD-2 (2004-2007), gray line. QD-3 (2008-2011), dark line.

that differs from the renal disease secondary to the classical APS syndrome (IgG-based and IgM-based).[42,43] Finally, this work supports the idea that autoimmunity is relevant after renal transplantation and also suggests that APS, as Grahams Hughes (who first described the APS) proposed, could be involved in many other processes unexplored before, such as renal transplantation.[44]

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REFERENCES

1. Hariharan S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. 2000;342:605–612.
2. Hamed MO, Chen Y, Pasea L, et al. Early graft loss after kidney transplantation: risk factors and consequences. *Am J Transplant*. 2015;15:1632–1643.
3. Ponticelli C, Moia M, Montagnino G. Renal allograft thrombosis. *Nephrol Dial Transplant*. 2009;24:1388–1393.
4. Morales JM, Marcen R, Andres A, et al. Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. *Kidney Int Suppl*. 2008:S94–S99.
5. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med*. 1969;280:735–739.
6. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. *N Engl J Med*. 2010;363:1451–1462.
7. Sánchez-Zapardiel E, Castro-Panete MJ, Castillo-Rama M, et al. Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. *Transplantation*. 2013;96:70–78.
8. Sigdel TK, Sarwal MM. Moving beyond HLA: a review of nHLA antibodies in organ transplantation. *Hum Immunol*. 2013;74:1486–1490.
9. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4:295–306.
10. Gómez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun*. 2014;48–49:20–25.
11. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med*. 2013;368:1033–1044.
12. Ragusa MA, Costa S, Cefalu AB, et al. RT-PCR and in situ hybridization analysis of apolipoprotein H expression in rat normal tissues. *Int J Mol Med*. 2006;18:449–455.
13. Meroni PL, Borghi MO, Raschi E, et al. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat Rev Rheumatol*. 2011;7:330–339.
14. Murthy V, Willis R, Romay-Penabad Z, et al. Value of isolated IgA anti-β2-glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. *Arthritis Rheum*. 2013;65:3186–3193.
15. Ruiz-Garcia R, Serrano M, Angel Martinez-Flores J, et al. Isolated IgA anti-β2 glycoprotein I antibodies in patients with clinical criteria for antiphospholipid syndrome. *J Immunol Res*. 2014;2014:704395.
16. Mehrani T, Petri M. Association of IgA Anti-beta2 glycoprotein I with clinical and laboratory manifestations of systemic lupus erythematosus. *J Rheumatol*. 2011;38:64–68.
17. Lakos G, Favaloro EJ, Harris EN, et al. International consensus guidelines on anticardiolipin and anti-β2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum*. 2012;64:1–10.
18. Serrano M, Martinez-Flores JA, Castro MJ, et al. Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with endstage renal disease. *J Immunol Res*. 2014;2014:641962.
19. Serrano A, García F, Serrano M, et al. IgA antibodies against β2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. *Kidney Int*. 2012;81:1239–1244.
20. Hadhri S, Rejeb MB, Belarbia A, et al. Hemodialysis duration, human platelet antigen HPA-3 and IgA isotype of anti-β2glycoprotein I antibodies are associated with native arteriovenous fistula failure in Tunisian hemodialysis patients. *Thromb Res*. 2013;131:e202–e209.
21. Morales JM, Martinez-Flores JA, Serrano M, et al. Association of early kidney allograft failure with preformed IgA antibodies to β2-glycoprotein I. *J Am Soc Nephrol*. 2015;26:735–745.
22. Perico N, Cattaneo D, Sayegh MH, et al. Delayed graft function in kidney transplantation. *Lancet*. 2004;364:1814–1827.
23. Keller AK, Jorgensen TM, Jespersen B. Identification of risk factors for vascular thrombosis may reduce early renal graft loss: a review of recent literature. *J Transplant*. 2012;2012:793461.
24. Amezquita Y, Mendez C, Fernandez A, et al. Risk factors for early renal graft thrombosis: a case-controlled study in grafts from the same donor. *Transplant Proc*. 2008;40:2891–2893.
25. Sadej P, Feld RI, Frank A. Transplant renal vein thrombosis: role of preoperative and intraoperative Doppler sonography. *Am J Kidney Dis*. 2009;54:1167–1170.
26. Bakir N, Sluiter WJ, Ploeg RJ, et al. Primary renal graft thrombosis. *Nephrol Dial Transplant*. 1996;11:140–147.
27. Phelan PJ, O'Kelly P, Tarazi M, et al. Renal allograft loss in the first postoperative month: causes and consequences. *Clin Transplant*. 2012;26:544–549.
28. Andres A, Morales JM, Herrero JC, et al. Double versus single renal allografts from aged donors. *Transplantation*. 2000;69:2060–2066.
29. Tripodi A, Branchi A, Chantarangkul V, et al. Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay. *J Thromb Thrombolysis*. 2011;31:165–172.
30. Ageno W, Becattini C, Brighton T, et al. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. *Circulation*. 2008;117:93–102.
31. Harris EN, Pierangeli SS. Primary, secondary, and catastrophic antiphospholipid syndrome: what's in a name?*Semin Thromb Hemost*. 2008;34:219–226.
32. Li J, Kim K, Barazia A, et al. Platelet-neutrophil interactions under thromboinflammatory conditions. *Cell Mol Life Sci*. 2015;72:2627–2643.
33. Meroni PL, Shoenfeld Y. Predictive, protective, orphan autoantibodies: the example of anti-phospholipid antibodies. *Autoimmun Rev*. 2008;7:585–587.
34. Tanimura K, Jin H, Suenaga T, et al. β2-Glycoprotein I/HLA class II complexes are novel autoantigens in antiphospholipid syndrome. *Blood*. 2015;125:2835–2844.
35. Erkan D, Aguiar CL, Andrade D, et al. 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. *Autoimmun Rev*. 2014;13:685–696.
36. Pawlicki J, Cierpka L, Król R, et al. Risk factors for early hemorrhagic and thrombotic complications after kidney transplantation. *Transplant Proc*. 2011;43:3013–3017.
37. Lonze BE, Zachary AA, Magro CM, et al. Eculizumab prevents recurrent antiphospholipid antibody syndrome and enables successful renal transplantation. *Am J Transplant*. 2014;14:459–465.
38. Daha NA, Banda NK, Roos A, et al. Complement activation by (auto-) antibodies. *Mol Immunol*. 2011;48:1656–1665.
39. Canaud G, Bienaime F, Tabarin F, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N Engl J Med*. 2014;371:303–312.
40. Sánchez-Fructuoso AI, Marques M, Prats D, et al. Victims of cardiac arrest occurring outside the hospital: a source of transplantable kidneys. *Ann Intern Med*. 2006;145:157–164.
41. Andres A, Polanco N, Cebrian MP, et al. Kidneys from elderly deceased donors discarded for transplantation. *Transplant Proc*. 2009;41:2379–2381.
42. Uthman I, Khamashta M. Antiphospholipid syndrome and the kidneys. *Semin Arthritis Rheum*. 2006;35:360–367.
43. Pons-Estel GJ, Cervera R. Renal involvement in antiphospholipid syndrome. *Curr Rheumatol Rep*. 2014;16:397.
44. Hughes GR. Hughes syndrome/APS. 30 years on, what have we learnt? Opening talk at the 14th International Congress on antiphospholipid antibodies Rio de Janiero, October 2013. *Lupus*. 2014;23:400–406.

Capitulo 3

La presencia de inmunocomplejos circulantes en sangre formados por IgA unida a B2 Glicoproteína 1 están fuertemente asociada con la aparición de episodios trombóticos agudos.

OBJETIVO: El síndrome antifosfolipídico (APS) se caracteriza por trombosis recurrente y / o morbilidad gestacional en pacientes con autoanticuerpos antifosfolipídicos (aPL). En los últimos años, los anticuerpos IgA anti-beta2-glicoproteína I (B2GPI) (IgA anti B2GP1) han alcanzado una relevancia clínica similar a los isotipos IgG o IgM. En un trabajo previo del grupo de investigación describimos la existencia de inmunocomplejos formados por IgA unida a B2GPI (B2A-CIC) en la sangre de pacientes con antecedentes de sintomatología de APS y positivos aislados para IgA anti B2GP1 (negativos para isotipos IgG e IgM). Sin embargo, las asociaciones clínicas de la presencia de estos B2A-CIC con eventos trombóticos (TEV) no se conocía.
En el presente trabajo se evalúa la prevalencia de los B2A-CIC en pacientes con trombosis recientes, trombosis antiguas y asintomáticos.

MÉTODOS:
Se estudiaron un total de 145 individuos que fueron positivos aislados para IgA anti B2GP1: 50 controles sin ningún antecedente de APS, 22 pacientes con TEV recientes acaecidas en días anteriores a la extracción (Grupo-1) y 73 pacientes con antecedentes de TEV antiguos (Grupo-2).

RESULTADOS:
Los niveles medios de B2A-CIC en el Grupo-1 fueron 29.6 ± 4.1, significativamente más altos que los del controles (15.1 ±1.9; $p < 0.001$).
La prevalencia de B2A-CIC positivos en el grupo 1 fue significativamente mayor (81.8%, $p<0,001$) que en el grupo control (18%) de pacientes asintomáticos.

En un análisis multivariable, la positividad de B2A-CIC fue una variable independiente para la trombosis aguda con un índice impar de 22.7 (intervalo de confianza 5.1-101.6, 95%, p <0.001). Los niveles de B2A-CIC disminuyeron significativamente dos meses después de la VET. Los pacientes B2A-CIC positivos tenían niveles de plaquetas más bajos que los pacientes B2A-CIC-negativos (p <0,001) y una mayor prevalencia de trombocitopenia (p <0,019). Los niveles de factores del complemento C3 y C4 en los pacientes del grupo 1 de presentaron diferencias significativas en los niveles de en niveles observados en los pacientes de los otros dos grupos.

CONCLUSIÓN:

La presencia de B2A-CIC está fuertemente asociada con la ocurrencia de TEV en fase aguda. Los pacientes que eran B2A-CIC positivos y no desarrollaron trombosis, tenían niveles de plaquetas más bajos que los negativos para B2A-CIC, lo que sugiere un estado de hipercoagulabilidad. El mecanismo de desarrollo de TEV en los pacientes con inmunocomplejos paraste mecanismo no está relacionado con el proceso de activación del complemento. La presencia de B2A-CIC podría utilizarse potencialmente para identificar a los pacientes IgA anti B2GPI positivos con mayor riesgo de desarrollar un evento trombótico.

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Original Article

Circulating Immune Complexes of IgA Bound to Beta 2 Glycoprotein are Strongly Associated with the Occurrence of Acute Thrombotic Events

José A. Martínez-Flores[1], Manuel Serrano[1], Dolores Pérez[1], Gómez de la Cámara A[2], David Lora[2], Luis Morillas[3], Rosa Ayala[4], Estela Paz-Artal[1, 5, 6], José M. Morales[1] and Antonio Serrano[1, 5]

[1]Department of Immunology, Instituto de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain
[2]Department of Epidemiology, Hospital Universitario 12 de Octubre, Madrid, Spain
[3]Department of Rheumatology, Hospital Universitario 12 de Octubre, Madrid, Spain
[4]Department of Hematology, Hospital Universitario 12 de Octubre, Madrid, Spain
[5]Immunology Section, Universidad San Pablo-CEU, Madrid, Spain
[6]Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain

Aim: **Antiphospholipid syndrome (APS) is characterized by recurrent thrombosis and/or gestational morbidity in patients with antiphospholipid autoantibodies (aPL). Over recent years, IgA anti-beta2-glycoprotein I (B2GPI) antibodies (IgA aB2GPI) have reached similar clinical relevance as IgG or IgM isotypes. We recently described the presence of immune complexes of IgA bounded to B2GPI (B2A-CIC) in the blood of patients with antecedents of APS symptomalology. However, B2A-CIC's clinical associations with thrombotic events (TEV) have not been described yet.**

Methods: **A total of 145 individuals who were isolate positive for IgA aB2GPI were studied: 50 controls without any APS antecedent, 22 patients with recent TEV (Group-1), and 73 patients with antecedents of old TEV (Group-2).**

Results: **Mean B2A-CIC levels and prevalence in Group-1 were 29.6±4.1 AU and 81.8%, respectively, and were significantly higher than those of Group-2 and controls ($p<0.001$). In a multivariable analysis, positivity of B2A-CIC was an independent variable for acute thrombosis with a 22.7 odd ratio (confidence interval 5.1−101.6, 95%, $p<0.001$). Levels of B2A-CIC dropped significantly two months after the TEV. B2A-CIC positive patients had lower platelet levels than B2A-CIC-negative patients ($p<0.001$) and more prevalence of thrombocytopenia ($p<0.019$). Group-1 had no significant differences in C3 and C4 levels compared with other groups.**

Conclusion: **B2A-CIC is strongly associated with acute TEV. Patients who did not develop thrombosis and were B2A-CIC positive had lower platelet levels, which suggest a hypercoagulable state. This mechanism is unrelated to complement-fixing aPL. B2A-CIC could potentially select IgA aB2GPI-positive patients at risk of developing a thrombotic event.**

Key words: Immunocomplex, Immune complex, Autoimmunity, Autoantibodies, Antiphospholipid syndrome, Seronegative antiphospholipid syndrome, APL, B2GPI, Cardiolipin, IgA

Introduction

Antiphospholipid syndrome (APS), also known as Hughes syndrome, is a multisystemic autoimmune disorder characterized by recurrent thrombosis and/or gestational morbidity and the presence of antiphospholipid antibodies (aPL)[1].

Diagnosis of APS is based on strict guidelines and requires clinical and laboratory criteria. Thrombotic events in patients with APS may be arterial, venous, or small vessel thrombosis of any organ, which must be diagnosed by objective methods such as imaging techniques or histopathology[1]. Gestational morbidity includes unexplained spontaneous abortions or

Address for correspondence: Antonio Serrano, Immunology Department, Hospital Universitario 12 de Octubre, Avda. de Andalucía s/n, 28041 Madrid, Spain
E-mail: aserrano@h12o.es
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deaths of a normal fetus and premature births due to eclampsia and pre-eclampsia of placental insufficiency[2, 3]. There are three different APS disease entities: primary (P-APS, without other concurrent morbidity), secondary to a pre-existing systemic autoimmune disease (S-APS), and catastrophic, consisting of multiple organ thrombosis with simultaneous multiorgan failure and a mortality rate close to 50%[4].

The aPL are a heterogeneous antibody group that can be directed against phospholipids, phospholipids–plasma proteins complexes or, mainly, phospholipid binding proteins. Antigens recognized by aPL can be located in plasma or associated with anionic phospholipids on plasma membranes of endothelial cells, platelets and other cells related with the coagulation system[5, 6]. International consensus accepted aPL for APS diagnosis are lupus anticoagulant (LA), anticardiolipin antibodies (aCL) of IgG and IgM isotypes, and anti-beta-2-glycoprotein I antibodies (aB2GPI) of IgG and IgM isotypes[2, 5].

Although anti-B2GPI antibodies of IgA isotype are not included in the laboratory criteria for APS defined in 2004 due to controversial results[2], in the same meeting researchers were encouraged to clarify its role in the APS[6]. In the last few years, the clinical relevance of IgA aB2GPI has increased[7, 8] and at the 13th International Congress on Antiphospholipid Antibodies (2010, Galveston, TX), the task force recommended testing for IgA aB2GPI in cases negative for IgG and IgM where APS is still suspected[9]. This determination has allowed for great diagnostic utility in patients with APS symptomatology negative for consensus aPL (APS-like patients)[9], lupus erythematosus[10], thrombosis in chronic kidney disease[11, 12], or early graft loss of transplanted kidneys[13].

While most of the antibodies detected in autoimmune diseases are not the direct cause of disease, aPL of IgG, IgM[14], and IgA[8] isotype are directly pathogenic. However, the presence of aPL is necessary, but not sufficient, to produce an APS event, so an additional trigger is needed to develop thrombosis[15]. The predictive value of the presence of aPL in developing thrombosis in a patient is low, and there are few prospective studies in APS. A 10 year follow-up multicenter prospective study of 1000 APS patients was conducted, and about 15% of patients developed a thrombotic event in the first 5 years. The study concluded that it was necessary to search for new markers to prevent the complications of APS, since even though the patients were under treatment, some of them continued to develop thrombosis[16]. For patients positive for IgA aB2GPI only, prospective studies were conducted in patients in hemodialysis[11, 17] and in renal transplant patients[13]. However, only a minority of patients developed thrombotic events-- 12% in renal transplant patients during the first year[13] and approximately 50% in patients on dialysis within two years[11, 17]. Therefore, new biomarkers are needed to identify which patients have a higher risk of thrombosis[16, 18].

The presence of circulating immune complexes (CIC) of B2GPI and antibodies (IgG and IgM) in APS patients has been reported[19] although they were not associated with the occurrence of thrombotic events[20]. We recently described a new method to detect specific CIC of IgA bounded to B2GPI (B2A-CIC) demonstrating its presence in patients positive only for IgA aB2GPI antibodies[21]. In the present study, we show for the first time that the presence of B2A-CIC identifies a subgroup of patients prone to develop thrombosis.

Patients and Methods

Study Design.

1. A cross-sectional study conducted to determine the presence of IgA-B2GPI immune complexes and their association with recent and older thrombotic events in patients positive only for IgA anti-B2GPI (negative for other aPL).

2. A prospective study of B2A-CIC short-term evolution in patients with recent thrombotic events.

The study complies with the Spanish legislation and European Community directives.

Patients

All patients positive only for IgA aB2GPI and negative for other aPL: aCL, IgG, IgA or IgM, and aB2GPI, IgG or IgM were recruited during one year (ending on November 30, 2014) from those referred by their physicians for aPL study to the Hospital 12 de Octubre Immunology Department. These patients were separated in two groups:

Group-1. Twenty-two patients positive for IgA aB2GPI-ab with recent thrombotic events consistent with APS clinical characteristics (**supplementary Table 1**) in the 30 days before aPL determination. Sera were evaluated for presence of aPL immediately after the event (mean 9.8 ± 2.2 days). All the serum samples were evaluated in the first month.

The mean age was 68.5 ± 2.4 years; 11 (50%) were male. One patient (4.5%) had an autoimmune disease and is consistent with secondary APS (S-APS). The rest of the patients were patients consistent with a diagnosis of primary APS (P-APS). Clinical characteristics are described on **Table 1**. 21 (95.4%) were Caucasians and 1 (4.6%) was east-African.

Group-2. Seventy-three patients positive for IgA

Table 1. Characteristics of patients on Group-1 and control group.

	Control Group ($n=50$)		Group 1 ($n=22$)		p
	Mean/n	SE/ %	Mean/n	SE/ %	
Age (y)	59.1	± 2.3	68.5	± 2.4	0.006
Sex (m)	10	20.0%	11	50.0%	0.020
Diabetes mellitus type 2	12	24.0%	7	31.8%	n.s.
Arterial hypertension (controlled)	20	40.0%	11	50.0%	n.s.
Dyslipidemia	11	22.0%	7	31.8%	n.s.
Atrial fibrillation	0	0.0%	0	0.0%	n.s.
No autoimmune underlying pathologies	32	64.0%	21	95.5%	0.007
Underlying autoimmune pathologies	18	36.0%	1	4.5%	0.007
Systemic Lupus Erythematosus	13	26.0%	1	4.5%	n.s.
Rheumatoid arthritis	4	8.0%	0	0.0%	n.s.
Systemic Sclerosis	1	2.0%	0	0.0%	n.s.

SE: standard error of the mean.

aB2GPI-ab with old thrombosis. Thrombotic events (**supplementary Table 1**) must have happened more than six months before the date of blood extraction (mean 782 ± 105 days). Eight patients (8.2%) had an autoimmune disease and are consistent with secondary APS (S-APS). The rest of patients were patients consistent with a diagnosis of primary APS (P-APS). The mean age was 59.1 ± 2.3 years; 40 (54.8%) were male. 70 (95.9%) were Caucasians and 3 (4.1%) were east-African.

Pretreatment: There were no significant differences among patients receiving treatment with immunomodulators, anticoagulants, antiplatelet or antimalarial treatment.

Asymptomatic Control Group (Control group). 50 patients positive only for IgA aB2PPI without APS-symptomatology (any thrombotic or APS-related obstetric antecedents) were recruited. The mean age was 59.1 ± 2.3 years; 10 (20%) were male. All patients were confirmed positive and remained free of thrombotic events from the time of diagnosis. The mean of time free of thrombosis from diagnosis of the presence of autoantibodies was 56.1 ± 4.5 months and the number of determinations of IgA aB2GPI that were made during the follow up period was 7.8 ± 1.4. All of the determinations were positive. Clinical characteristics are described on **Table 1**.

Patients with prothrombotic conditions secondary to other factors such as sepsis, homocystinemia, and genetic defects of coagulation factors (thrombin mutations, factor V Leiden, antithrombin deficiency, etc.) were not included. Data of the patients and controls were collected in an anonymized database.

Definitions

Thrombotic events: Venous and arterial thrombosis diagnosed following Sydney consensus of APS criteria[2].

Current-thrombosis (CT): Thrombotic event that occurred within the 30 days prior to blood collection.

Old-thrombosis (OT): When previous thrombosis occurred from 1 to 36 months before blood collection.

Thrombocytopenia: platelets levels below 140×10^3/uL

Laboratory determinations. aCL and aBGPI antibodies (IgG and IgM) were measured using the BioPLex 2200 multiplex immunoassay system (Bio-Rad, Hercules CA, USA). Antibody levels higher than 18 U/mL were considered positive following the manufacturer's guidelines.

IgA aCL and aB2GPI antibodies were quantified by enzyme-linked immunosorbent assays (ELISA) using IgA-aCL and IgA-aB2GPI QUANTA Lite (INOVA Diagnostics Inc., San Diego, CA, USA). Antibody levels higher than 20 U/mL were considered positive following the manufacturer's guidelines and the 99th percentile of a healthy population in our hospital[7].

Complement factors C3 and C4 levels were measured using Beckman Coulter IMMAGE Immunochemistry System (Beckman Coulter Inc. Pasadena, CA, USA).

Quantification of B2A-CIC levels was performed as previously described[21]. Sera with values of B2A-CIC higher than 21 AU were considered positive. All the procedures were performed in a Triturus® Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain).

Lupus Anticoagulant

Lupus anticoagulant (LA) is not routinely performed in all patients with a first thrombotic event. They are only done in special coagulation studies in patients with repeated thrombosis or elevated thrombotic risk. In patients who have a first thrombosis, LA is only ordered when the hematologist considers it is appropriate due to clinical characteristics. LA activity was detected by coagulation assays following the guidelines of the International Society on Thrombosis and Hemostasis (ISTH)[22]. We used the HemosIL dRVVT Screen, HemosIL dRVVT Confirm and HemosIL Silica Clotting Time assays (Instrumentation Laboratory SpA, Milano, Italy).

Statistical Methods

Results were expressed as mean ± standard error or absolute frequency and percentage. In scaled variables with two categories, comparisons were performed using the Student's *t*-test. Association between qualitative variables was determined with Pearson's Chi-square test incorporating Yates Continuity Correction. *P* values less than 0.05 were considered significant.

A box-and-whisker plot represents the values from the lower to upper quartile (25 to 75 percentile) in the central box. The median is represented as the middle line into the box. Data were processed and analyzed using Medcalc for Windows version 15.4 (MedCalc Software, Ostend, Belgium).

Results

Presence of B2A-CIC

No significant differences in aB2GPI and aCL antibodies levels (IgG, IgM and IgA) between Group-1, Group-2 and control patients were observed (**Supplementary Table 2**). Levels of B2A-CIC were significantly higher in Group-1 (29.6 ± 4.1 AU) than in the control group (15.1 ± 1.9 AU; $p=0.003$; **Fig. 1A**). 81.8% of patients in Group-1 (18/22) were positive for B2A-CIC. This percentage was significantly higher ($p<0.001$) than the 18% (9/50) observed in the control group of patients positive for IgA aB2GPI and without any thrombotic antecedent (**Fig. 1B**).

Prospective Study

Group-1 patients were followed-up. Twenty patients were reevaluated to quantify B2A-CIC between the second and sixth month post-thrombotic event (four of them were reevaluated a second time in this period). Two patients were unavailable because they did not attend the scheduled follow-up visit. IgA aB2GPI mean levels at the moment of thrombosis did not differ from the levels in the scheduled follow-up visit and all of them were positive (data not shown). In the 20 patients who completed the follow-up, B2A-CIC levels decreased after the thrombotic event (**Fig. 2A**): mean B2A-CIC at the time of thrombosis was 35.3 ± 3.8 AU and in the reevaluation, it was 20.3 ± 2.7 AU ($p=0.002$). The percentage of B2A-CIC positive patients two months after the thrombotic event was 35.0% (7/20), similar to the control group ($p=0.224$). In the four patients in Group-1 who were negative for B2A-CIC in the first sample (mean 3.8 ± 1.8 AU), significant differences were not observed in the second sample at the time of the follow-up visit (mean 3.1 ± 1.0 AU).

A case example of evolution of B2A-CIC in a patient in Group-1 who had a second thrombotic event is shown in **Fig. 2B**. The patient had suffered a portal vein thrombosis and had elevated levels of B2A-CIC the 2nd day after the thrombosis. B2A-CIC levels dropped by 50% in the 2nd month clinical reevaluation, and while the patient was asymptomatic, they were still positive for B2A-CIC. However, 180 days after the first thrombosis, the patient developed another thrombotic event (deep venous thrombosis) and levels of B2A-CIC increased by 50% compared to the 2nd month post-thrombosis. This was the only patient with recurrent thrombosis during the follow-up.

B2A-CIC in Patients with Thrombotic Antecedents

Mean levels of B2A-CIC in sera of patients on Group-2 (patients who had a thrombotic event more than 6 months before blood extraction) was 14.8 ± 1.8 AU-- significantly lower ($p<0.001$) than patients on Group-1 (first sample) but without significant differences to Group-1 reevaluation samples (2−6 month after the thrombotic event) or to the control group ($p=0.143$ and $p=0.908$ respectively).

Group-2 and the control group showed similar percentages of B2A-CIC positive patients: 17.8% (13/73) vs 18.0% (9/50), $p=0.832$. Also, Group-2 did not differ from the percentage of B2A-CIC positive patients in Group-1 reevaluation after the thrombotic event ($p=0.177$).

Multivariate Study

The variables associated with patients with recent thrombosis that were significant in the univariate analysis (**Table 1**) were included in a multivariate analysis. The odds ratio for B2A-CIC in the multivariate study was 22.7 for recent thrombosis ($p<0.001$), appearing as a risk factor for thrombosis. The odds ratio for the presence of an autoimmune disease was 0.08 OR ($p=0.046$, **Table 2**). Sex and age were not significant.

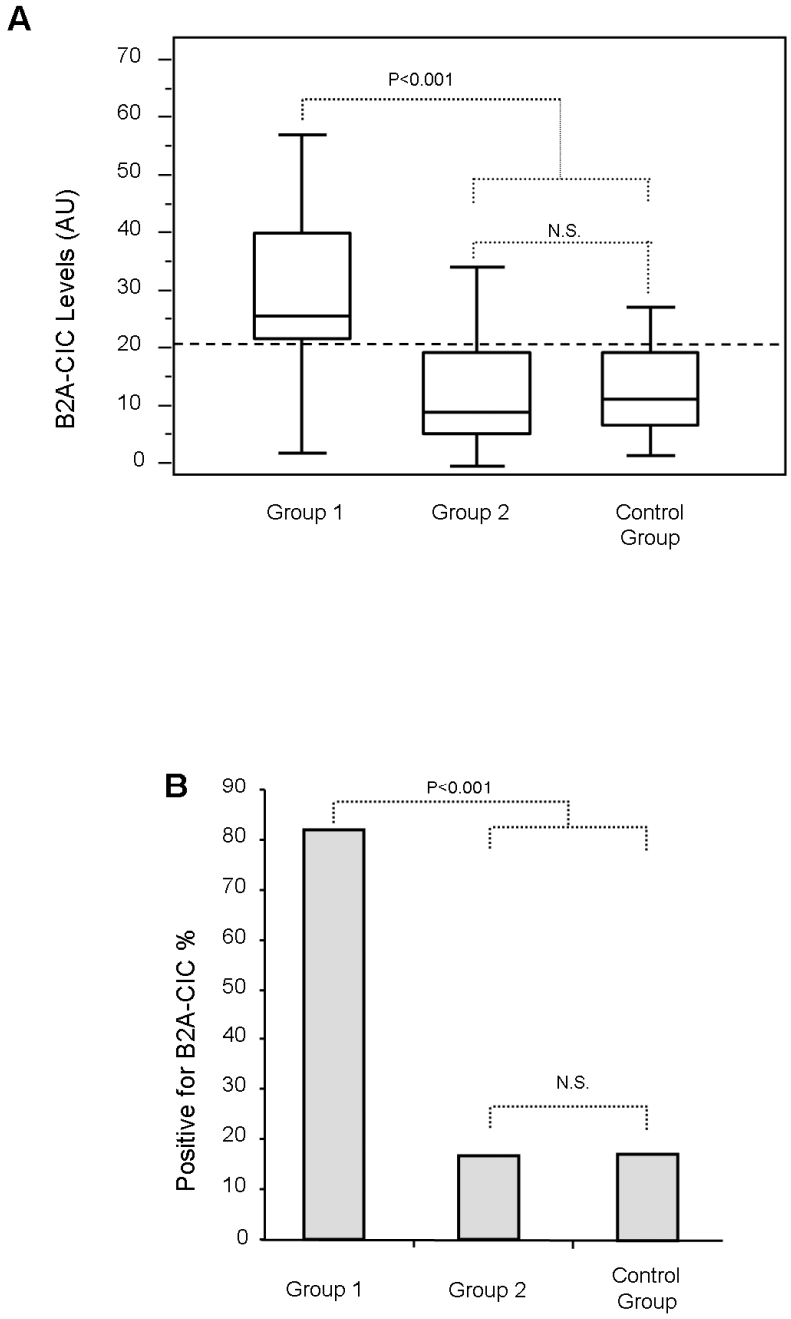

Fig. 1. Levels (A) of immune complexes of IgA bounded to B2GPI (B2A-CIC) and percentage of positives (B) in Group-1 and controls. Dotted line is the cutoff.

Lupus Anticoagulant

52 patients were analyzed for LA according to hematologist criteria: 10 in Group-1 (all were negative) and 42 in Group-2. Six patients in Group-2 (11%) were positive and all of them were negative for B2A-CIC.

Platelet Levels

The Group-1 mean platelet levels were 182.7 ± 14.6 × 1000/uL, which was lower than those in Group-2 (229.2 ± 9.0 × 1000/uL; $p = 0.010$) or in the control group (225.2 ± 8.9 × 1000/uL; $p = 0.017$). However, no significant differences were observed between the control group and Group-2 (**Fig. 3a**).

When IgA aB2GPI positive patients were ana-

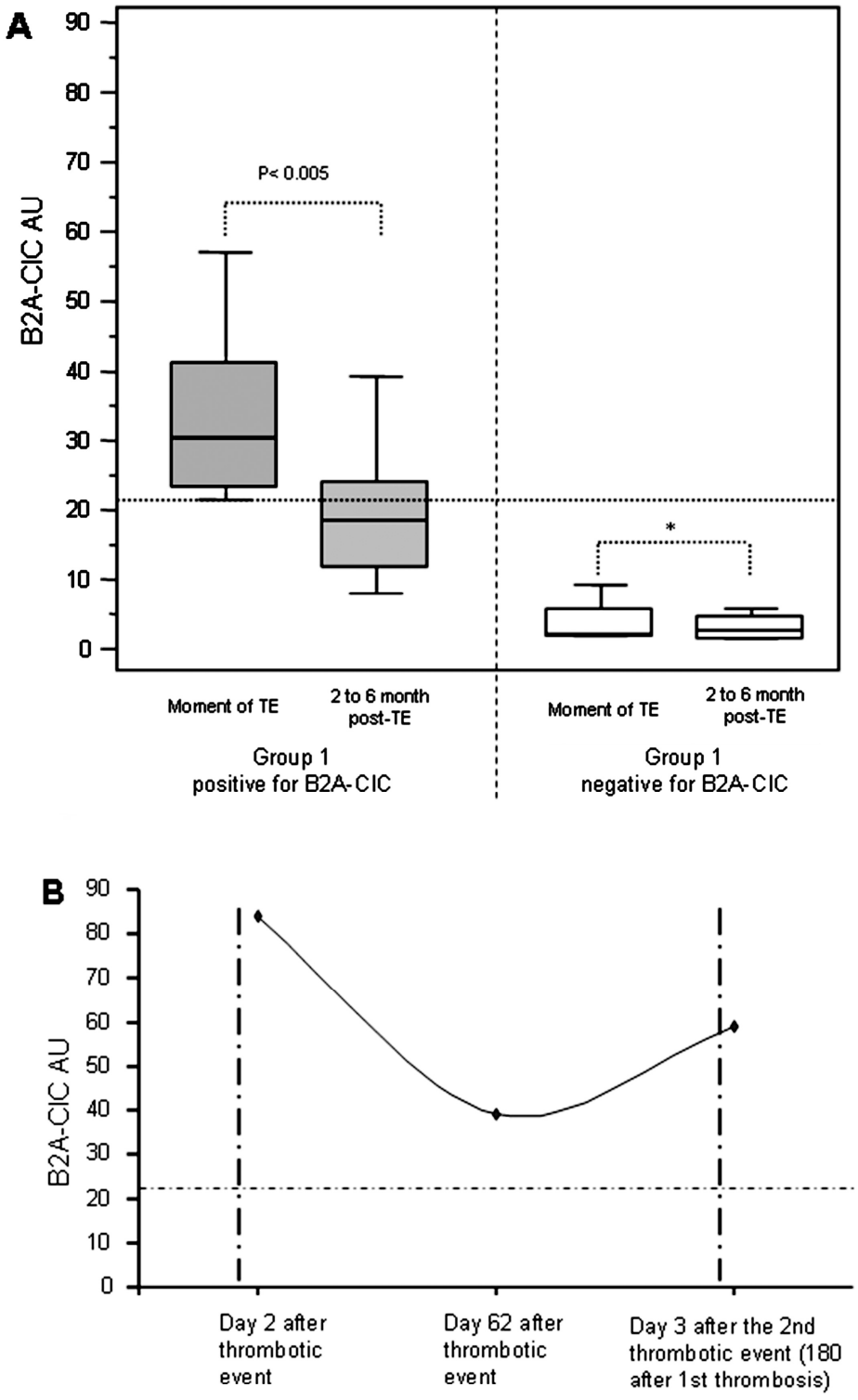

Fig. 2. Evolution of immune complexes of IgA bounded to B2GPI (B2A-CIC) levels in patients with current thrombosis (Group-1) positive and negative for B2A-CIC (A) and a case example of a Group-1 patient positive for B2A-CIC who redeveloped a thrombotic event (B). The horizontal dotted line shows the cutoff. The vertical dotted line shows the moment of thrombosis

* = non-significant.

Table 2. Multivariate analysis ($p<0.001$) of factors associated with recent thrombosis.

Variable	Odds Ratio	95% CI	*P*
B2A-CIC positive	22.7	5.055 to 101.571	<0.001
Age	1.0	0.969 to 1.095	0.335
Sex	2.8	0.648 to 12.502	0.166
Presence of autoimmune disease	0.08	0.007 to 0.954	0.046

Area under the ROC curve: 0.915; 95% CI: 0.823 to 0.968.

lyzed according to whether they were B2A-CIC positive or negative, independently of whether they had a current or past thrombosis, or did not have thrombosis, we obtained mean platelet levels of 185.5 ± 9.4 × 1000/uL for positive B2A-CIC and 234.8 ± 6.9 × 1000/uL for negative B2A-CIC ($p=0.0001$) (**Fig. 3b**).

In Group-1, 27.3% of patients (6/22) had thrombocytopenia. This proportion was not significantly higher ($p=0.07$) than that observed in the control group, 10.0% (5/50), or in Group-2, 12.3% (9/73; $p=0.18$). Nevertheless, thrombocytopenia in B2A-CIC positive patients (in all groups) was 22.5%, significantly higher ($p=0.019$) than the 7.6% observed in B2A-CIC negative patients in the same groups.

Complement Levels

Group-1 patients had 123.7 ± 7.1 mg/dl and 26.0 ± 2.5 mg/dl C3 and C4 mean levels, respectively, Group-2 patients had 121.1 ± 4.6 mg/dl and 23.7 ± 1.3 mg/dl C3 and C4 mean levels, respectively, and the control group had 113.4 ± 3.9 mg/dl and 22.8 ± 1.0 mg/dl C3 and C4 mean levels, respectively. Mean levels of Complement C3 and C4 factors in all patients groups and subgroups were within the normal range (88−252 mg/ dl for C3 levels and 12−75 mg/ dl for C4 levels) and were not significant. Only 1 patient with recent thrombosis had low levels of C4 (**Fig. 4**).

Discussion

In this work, for the first time, we have described a high prevalence of B2A-CIC in patients with recent thrombosis and positive isolated for IgA aB2GPI antibodies compared with patients who presented old thrombosis and those without thrombotic antecedents. IgA aB2GPI antibodies are directly thrombogenic but the mechanisms of antibody-mediated thrombosis are unknown[8]. Although the presence of aB2GPI antibodies is a necessary condition, only a small group of patients positive for these antibodies develop thrombotic complications. It has been proposed that the presence of antibodies would generate a prothrombotic microenvironment. Thrombus formation would require additional prothrombotic factors ("second hit"), which are related to immune responses and are still not well known[15]. Therefore, as demanded in recent studies, it is necessary to search for new markers that permit the screening of patients who are really at risk of suffering a thrombotic event[16].

The aCL assay is mainly for the detection of B2GPI-dependent antibodies; however, our patients presented an isolated IgA positivity for B2GPI (negative for IgA aCL). There are several studies showing that positivity for IgA aCL and IgA aB2GPI are independent[7, 8, 23]. The epitopes recognized by the IgA aB2GPI are mainly located in the domains 4−5 of the B2GPI protein, this region being the phospholipid binding area. When cardiolipin is incorporated to B2GPI, IgA-binding epitopes are not accessible and patients only present isolated IgA aB2GPI antibodies[24, 25].

We have found that patients with acute thrombosis (Group-1) have a higher prevalence of B2A-CIC positive and higher levels of B2A-CIC than in patients with antecedents of thrombosis (Group-2) or patients in the control group. Both prevalence and levels decrease after the thrombotic event. Group-1 patients were reevaluated between two and six months after TEV showing B2A-CIC levels and positive prevalence similar to those in Group-2 and the control group.

The presence of B2A-CIC is not exclusive to patients with a history of thrombotic events. It also appears, but with lower prevalence, in asymptomatic patients. This would suggest that the presence of B2A-CIC would not be directly thrombogenic but would rather behave as an additional risk factor that favors the prothrombotic microenvironment, thus increasing the probability of occurrence of the thrombotic event.

Multivariable analysis shows positivity for B2A-CIC as an independent factor for recent thrombosis and that patients without autoimmune diseases had more thrombosis. This makes sense because of the presence of IgA aB2GPI is more frequent in patients with P-APS than in S-APS[7]. By performing a follow-up study on patients with recent thrombosis, we have been able to detect a drop in B2A-CIC levels two months after the thrombotic event. In spite of the

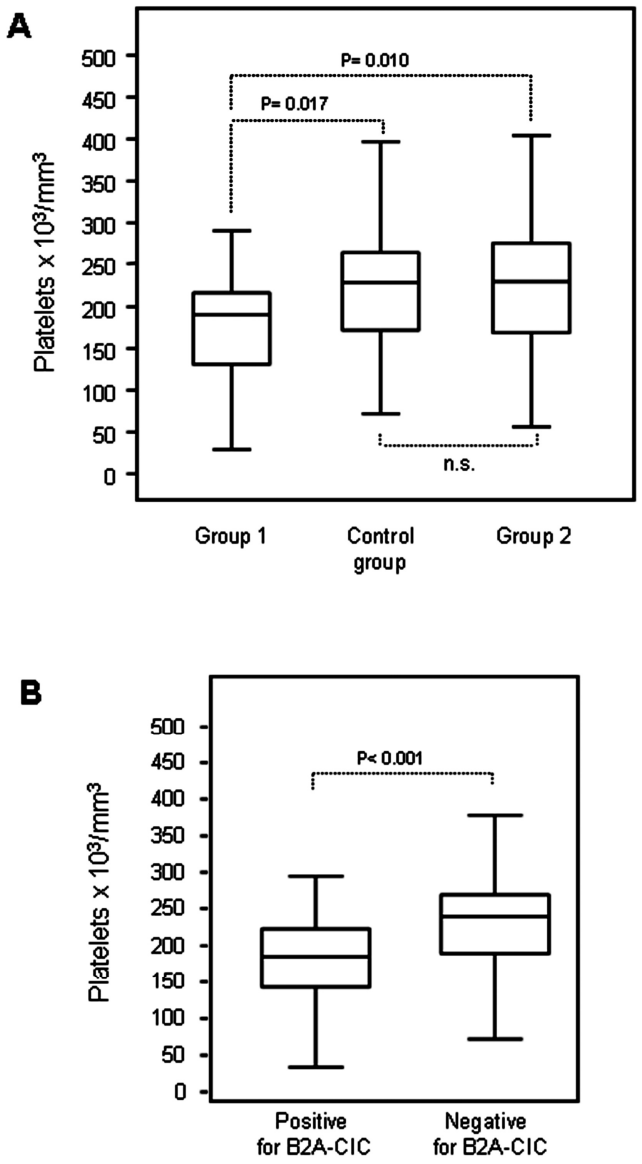

Fig. 3. Platelet levels in the groups (A) and in positive and negative for immune complexes of IgA bounded to B2GPI (B2A-CIC) (B).

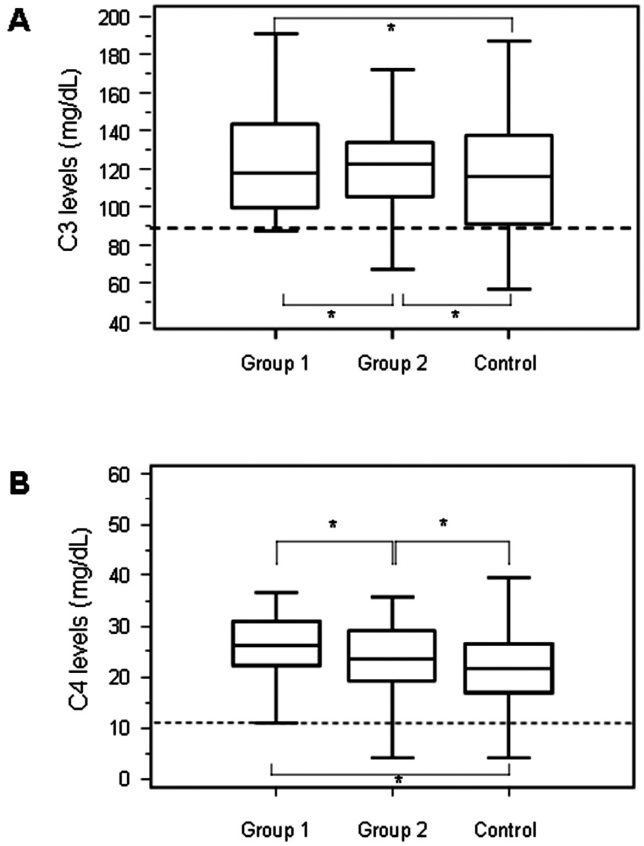

Fig. 4. C3 (A) and C4 (B) complement levels in groups.
* = non-significant.

small number of patients, this decrease is significant and suggests a formation of B2A-CIC during the thrombotic event.

In the analysis of platelets, we observed that the mean levels of Group-1 are lower than in the control group. This may be because they would have been consumed during the thrombotic event. Nevertheless, patients with elevated B2A-CIC levels also have significantly lower means than those in the non-elevated B2A-CIC levels. This may suggest that B2A-CIC would induce a certain degree of platelet activation/aggregation and might be able to activate platelets to produce platelet-consumption and even to produce thrombotic events in some patients. Complement activation plays an important role in the pathogenesis of aPL-induced thrombosis. Therefore, hypocomplementaemia is common in APS patients (aPL of IgG or IgM isotypes), reflecting complement activation and consumption[26, 27], and blockade of the complement system has been proposed as an effective therapy for complex forms of APS[28]. Our finding of normal C3 and C4 levels in patients with anti-B2GPI antibodies of IgA isotype and APS-events is perfectly consistent with the results obtained because IgA does not fix complement[29].

The mechanism by which the aB2GPI antibodies produce pathology is unclear. Some studies suggest that B2GPI changes its conformation after binding to the plasma membrane of platelets and endothelial cells. This would enable B2GPI antibody binding, thus producing endothelial activation[30], platelet activation[31] and an altered coagulation state[32, 33], which could, in turn, trigger a proinflammatory and procoagulant state. The presence of the B2A-CIC could help make this activation process more effectively by

increasing the probability of triggering thrombotic events in a complement-unrelated mechanism. Currently, there is no cure for APS and the treatment should be individualized and adapted to the characteristics of each patient[34]. The risk of thrombosis in aPL positive asymptomatic individuals is low but increases with the concurrence of other risk factors such as smoking, use of estrogens, prolonged immobilization, infections, or surgical procedures[35, 36]. In spite of this, at least 50% of patients who develop thrombosis do not have any other risk factor at the time when the event occurs[37]. Asymptomatic individuals with positive blood tests for aPL without other prothrombotic factors do not require treatment[38]. APS patients with thrombotic antecedents are usually treated to reduce the risk of recurrent thromboembolism[34]. The mainstay of the treatment is thromboprophylaxis, usually using Vitamin K antagonists. However, there is no consensus regarding the patient screening criteria and treatment duration because anticoagulant drugs are among the most common medications that cause adverse events[39]. Therefore, in order to select which patients should receive thromboprophylaxis, new biomarkers are needed that would make it possible to identify patients with a pro-thrombotic state and at high risk of clinical events[40]. B2A-CIC identification could be a new biomarker to define the population to be treated at risk of thrombotic events.

Limitations of the study: we have selected patients positive only for IgA aB2GPI and deliberately excluded seronegative and positive patients for other isotypes because they could have been a confounding factor. This makes essential the inclusion of this population in future studies. Also, our population is slightly older because although we have selected all patients with thrombosis during a year, the population of the hospital area is older, as is the population of Spain. Despite this, age was not significant in the multivariate analysis for the development of thrombosis. Another major limitation was that blood samples at the moment of thrombosis were not available.

In summary, IgA aB2GPI antibodies are, per se, a risk factor but they are not sufficient to discriminate the population potentially at risk of thrombosis. We have described for the first time an association among patients with elevated levels of B2A-CIC and acute thrombosis. Notably, B2A-CIC levels become negative 2−6 months after the thrombotic event. Furthermore, patients B2A-CIC positive present less platelet levels, suggesting a hypercoagulability state by platelet activation. This mechanism seems to be independent of complement. The study of the B2A-CIC may help us to better understand the process of a prothrombotic state prior to the development of an APS event. From a clinical point of view, if these are corroborated, it may be useful for the diagnosis of seronegative-APS and may help in the decision of whether treatment with thromboprophylaxis would be useful or not. Due to the difficulty of predicting a thrombotic event, this hypothesis needs to be confirmed in prospective studies with an elevated number of patients to determine the B2A-CIC levels pre-thrombosis in more patients and the potentially role of B2A-CIC as a predictive marker.

Authors' Contribution

José Ángel Martínez-Flores and Manuel Serrano collaborated equally to this work

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Conflict of Interest

The authors declare no conflicts of interest in this study.

References

1) Gomez-Puerta JA and Cervera R: Diagnosis and classification of the antiphospholipid syndrome. J Autoimmun, 2014; 48-49: 20-25
2) Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, PG DEG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG and Krilis SA: International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost, 2006; 4: 295-306
3) Devreese K and Hoylaerts MF: Challenges in the diagnosis of the antiphospholipid syndrome. Clin Chem, 2010; 56: 930-940
4) Asherson RA, Cervera R, Piette JC, Font J, Lie JT, Burcoglu A, Lim K, Munoz-Rodriguez FJ, Levy RA, Boue F, Rossert J and Ingelmo M: Catastrophic antiphospholipid syndrome. Clinical and laboratory features of 50 patients. Medicine (Baltimore), 1998; 77: 195-207
5) Willis R, Harris EN and Pierangeli SS: Pathogenesis of

the antiphospholipid syndrome. Semin Thromb Hemost, 2012; 38: 305-321
6) Branch DW: Summary of the 11th International Congress on antiphospholipid autoantibodies, Australia, November 2004. J Reprod Immunol, 2005; 66: 85-90
7) Ruiz-Garcia R, Serrano M, Angel Martinez-Flores J, Mora S, Morillas L, Martin-Mola MA, Morales JM, Paz-Artal E and Serrano A: Isolated IgA Anti- beta 2 Glycoprotein I Antibodies in Patients with Clinical Criteria for Antiphospholipid Syndrome. J Immunol Res, 2014; 2014: 704395
8) Murthy V, Willis R, Romay-Penabad Z, Ruiz-Limon P, Martinez-Martinez LA, Jatwani S, Jajoria P, Seif A, Alarcon GS, Papalardo E, Liu J, Vila LM, McGwin G, Jr., McNearney TA, Maganti R, Sunkureddi P, Parekh T, Tarantino M, Akhter E, Fang H, Gonzalez EB, Binder WR, Norman GL, Shums Z, Teodorescu M, Reveille JD, Petri M and Pierangeli SS: Value of isolated IgA anti-beta2 -glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. Arthritis Rheum, 2013; 65: 3186-3193
9) Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC and Pierangeli SS: International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum, 2012; 64: 1-10
10) Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sanchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth VP, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta MA, Jacobsen S, Buyon JP, Maddison P, Dooley MA, van Vollenhoven RF, Ginzler E, Stoll T, Peschken C, Jorizzo JL, Callen JP, Lim SS, Fessler BJ, Inanc M, Kamen DL, Rahman A, Steinsson K, Franks AG, Jr., Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman MH, McGwin G, Jr. and Magder LS: Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum, 2012; 64: 2677-2686
11) Serrano A, Garcia F, Serrano M, Ramirez E, Alfaro FJ, Lora D, de la Camara AG, Paz-Artal E, Praga M and Morales JM: IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. Kidney Int, 2012; 81: 1239-1244
12) Serrano M, Martinez-Flores JA, Castro MJ, Garcia F, Lora D, Perez D, Gonzalez E, Paz-Artal E, Morales JM and Serrano A: Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with end-stage renal disease. J Immunol Res, 2014; 2014: 641962
13) Morales JM, Martinez-Flores JA, Serrano M, Castro MJ, Alfaro FJ, Garcia F, Martinez MA, Andres A, Gonzalez E, Praga M, Paz-Artal E and Serrano A: Association of Early Kidney Allograft Failure with Preformed IgA Antibodies to beta2-Glycoprotein I. J Am Soc Nephrol, 2015; 26: 735-745
14) Giannakopoulos B and Krilis SA: The pathogenesis of the antiphospholipid syndrome. N Engl J Med, 2013; 368: 1033-1044
15) Meroni PL, Borghi MO, Raschi E and Tedesco F: Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol, 2011; 7: 330-339
16) Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramon E, Buonaiuto V, Jacobsen S, Zeher MM, Tarr T, Tincani A, Taglietti M, Theodossiades G, Nomikou E, Galeazzi M, Bellisai F, Meroni PL, Derksen RH, de Groot PG, Baleva M, Mosca M, Bombardieri S, Houssiau F, Gris JC, Quere I, Hachulla E, Vasconcelos C, Fernandez-Nebro A, Haro M, Amoura Z, Miyara M, Tektonidou M, Espinosa G, Bertolaccini ML and Khamashta MA: Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis, 2015; 74: 1011-1018
17) Hadhri S, Rejeb MB, Belarbia A, Achour A and Skouri H: Hemodialysis duration, Human platelet antigen HPA-3 and IgA Isotype of anti-beta2glycoprotein I antibodies are associated with native arteriovenous fistula failure in Tunisian hemodialysis patients. Thromb Res, 2013; 131: e202-209
18) Devreese KM: Antiphospholipid antibodies: evaluation of the thrombotic risk. Thromb Res, 2012; 130 Suppl 1: S37-40
19) Banzato A, Frasson R, Acquasaliente L, Bison E, Bracco A, Denas G, Cuffaro S, Hoxha A, Ruffatti A, Iliceto S, De Filippis V and Pengo V: Circulating beta2 glycoprotein I-IgG anti-beta2 glycoprotein I immunocomplexes in patients with definite antiphospholipid syndrome. Lupus, 2012; 21: 784-786
20) Biasiolo A, Rampazzo P, Brocco T, Barbero F, Rosato A and Pengo V: [Anti-beta2 glycoprotein I-beta2 glycoprotein I] immune complexes in patients with antiphospholipid syndrome and other autoimmune diseases. Lupus, 1999; 8: 121-126
21) Martinez-Flores JA, Serrano M, Perez D, Lora D, Paz-Artal E, Morales JM and Serrano A: Detection of circulating immune complexes of human IgA and beta 2 Glycoprotein I in patients with antiphospholipid syndrome symptomatology. J Immunol Methods, 2015;
22) Brandt JT, Triplett DA, Alving B and Scharrer I: Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Thromb Haemost, 1995; 74: 1185-1190
23) Mattia E, Ruffatti A, Tonello M, Meneghel L, Robecchi B, Pittoni M, Gallo N, Salvan E, Teghil V, Punzi L and Plebani M: IgA anticardiolipin and IgA anti-beta2 glycoprotein I antibody positivity determined by fluorescence enzyme immunoassay in primary antiphospholipid syndrome. Clin Chem Lab Med, 2014; 52: 1329-1333
24) Sweiss NJ, Bo R, Kapadia R, Manst D, Mahmood F, Adhikari T, Volkov S, Badaracco M, Smaron M, Chang A, Baron J and Levine JS: IgA anti-beta2-glycoprotein I autoantibodies are associated with an increased risk of thromboembolic events in patients with systemic lupus erythematosus. PLoS One, 2010; 5: e12280
25) Andreoli L, Chighizola CB, Nalli C, Gerosa M, Borghi MO, Pregnolato F, Grossi C, Zanola A, Allegri F, Norman GL, Mahler M, Meroni PL and Angela T: Antiphospholipid Antibody Profiling: The Detection of IgG Antibodies Against beta2glycoprotein I Domain 1 and 4/5 Offers

Better Clinical Characterization: The ratio between anti-D1 and anti-D4/5 as a new useful biomarker for APS. Arthritis Rheumatol, 2015;
26) Oku K, Atsumi T, Bohgaki M, Amengual O, Kataoka H, Horita T, Yasuda S and Koike T: Complement activation in patients with primary antiphospholipid syndrome. Ann Rheum Dis, 2009; 68: 1030-1035
27) Lim W: Complement and the antiphospholipid syndrome. Curr Opin Hematol, 2011; 18: 361-365
28) Erkan D, Aguiar CL, Andrade D, Cohen H, Cuadrado MJ, Danowski A, Levy RA, Ortel TL, Rahman A, Salmon JE, Tektonidou MG, Willis R and Lockshin MD: 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. Autoimmun Rev, 2014; 13: 685-696
29) Daha NA, Banda NK, Roos A, Beurskens FJ, Bakker JM, Daha MR and Trouw LA: Complement activation by (auto-) antibodies. Mol Immunol, 2011; 48: 1656-1665
30) Raschi E, Testoni C, Borghi MO, Fineschi S and Meroni PL: Endothelium activation in the anti-phospholipid syndrome. Biomed Pharmacother, 2003; 57: 282-286
31) Lutters BC, Derksen RH, Tekelenburg WL, Lenting PJ, Arnout J and de Groot PG: Dimers of beta 2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2'. J Biol Chem, 2003; 278: 33831-33838
32) Levine JS, Branch DW and Rauch J: The antiphospholipid syndrome. N Engl J Med, 2002; 346: 752-763
33) Matsuura E, Shen L, Matsunami Y, Quan N, Makarova M, Geske FJ, Boisen M, Yasuda S, Kobayashi K and Lopez LR: Pathophysiology of beta2-glycoprotein I in antiphospholipid syndrome. Lupus, 2010; 19: 379-384
34) Punnialingam S and Khamashta MA: Duration of anticoagulation treatment for thrombosis in APS: is it ever safe to stop? Curr Rheumatol Rep, 2013; 15: 318
35) Erkan D, Yazici Y, Peterson MG, Sammaritano L and Lockshin MD: A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. Rheumatology (Oxford), 2002; 41: 924-929
36) Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, Bossi F, Ziller F, Sblattero D, Meroni P and Tedesco F: Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. Blood, 2005; 106: 2340-2346
37) Barbhaiya M and Erkan D: Primary thrombosis prophylaxis in antiphospholipid antibody-positive patients: where do we stand? Curr Rheumatol Rep, 2011; 13: 59-69
38) Giannakopoulos B and Krilis SA: How I treat the antiphospholipid syndrome. Blood, 2009; 114: 2020-2030
39) Piazza G, Nguyen TN, Cios D, Labreche M, Hohlfelder B, Fanikos J, Fiumara K and Goldhaber SZ: Anticoagulation-associated adverse drug events. Am J Med, 2011; 124: 1136-1142
40) Bertero MT: Primary prevention in antiphospholipid antibody carriers. Lupus, 2012; 21: 751-754

Supplementary Table 1. Antiphospholipid Syndrome (APS) clinical inclusion criteria.

APS Clinical criteria	Group-1 (n=22)		Group-2 (n=73)	
Venous thrombosis	17	77.3%	65	89.0%
Deep venous thrombosis	7	31.8%	31	42.5%
Deep venous thrombosis and pulmonary embolism	2	9.1%	19	26.0%
Portal vein thrombosis	2	9.1%	3	4.1%
Portal vein thrombosis and pulmonary embolism	1	4.5%	0	0.0%
Pulmonary embolism	5	22.7%	13	17.8%
Arterial thrombosis	5	22.7%	8	11.0%
Mesenteric thrombosis	1	4.5%	2	2.7%
Pulmonary thrombosis	0	0.0%	1	1.4%
Central artery of the retina	1	4.5%	1	1.4%
Carotid thrombosis	0	0.0%	1	1.4%
Cerebral thrombosis	2	9.1%	2	2.7%
Coronary thrombosis	1	4.5%	1	1.4%

Supplementary Table 2. Antiphospholipid antibodies levels (IU/ml) in the three groups.

	Group-1		Group-2		Control group	
	Mean	SE	Mean	SE	mean	SE
aCL IgG	4.7	1.4	5.6	1.8	3.1	0.4
aCL IgM	4.3	0.9	4.6	1.2	4.3	0.8
aCL IgA	4.2	0.5	4.1	0.5	3.3	0.3
aB2GPI IgG	4.7	1.5	5.7	1.9	3.2	0.6
aB2GPI IgM	2.7	0.4	2.6	0.5	3.5	0.7
aB2GPI IgA	66.1	5.8	68.8	7.2	68.2	7.7

SE: standard error of the mean. aCL: Anti-cardiolipin. aB2GPI: anti-Beta 2 Glycoprotein I.

Capitulo 4

Complejos inmunes de β2-glicoproteína I e IgA: un marcador para predecir la trombosis después del trasplante renal en pacientes con anticuerpos antifosfolipídicos.

FONDO:
El valor predictivo de la presencia de aPL es bajo par la aparición de los eventos trombóticos es bajo: Solo una minoria de los pacientes aPL positivos llegará a desarrollar el evento, por ello no es ético ni práctico instaurar tratamientos preventivos sobre todos ellos. Se necesitan nuevos marcadores para identificar cuales son los portadores de aPL con riesgo de sufrir eventos trombóticos y tomar medidas preventivas exclusivamente sobre ellos.
En unos trabajos previos del grupo de investigación se describió la existencia de inmunocomplejos circulantes de IgA unida a la β2-glicoproteína I (B2A-CIC) en la sangre de algunos pacientes con aPL de clase IgA. La presencia de estos B2A-CIC se ha asociado con la aparición de eventos trombóticos agudos (capitulo 3).
En este trabajo se estudia el posible valor predictivo de la presencia de B2A-CIC para determinar el riesgo de aparición de eventos trombóticos agudos en pacientes que van a someterse a cirugía de trasplante, un conocido desencadenante (“second hit”) de eventos trombóticos agudos en pacientes que son portadores de aPL.

MÉTODOS:
Se realizó un estudio de seguimiento basado en la cohorte Magnum 12 + 12 de pacientes que recibieron un trasplante de riñón (n = 1339). Se establecieron tres grupos: pacientes del grupo 1 que fueron positivos para la IgA anti-β2-glicoproteína y B2A-CIC (n = 125); pacientes del grupo 2 que solo dieron positivo para IgA anti B2GP1 (n = 240); y grupo control, pacientes que fueron negativos para IgA anti B2GP1 (n = 974).
Los niveles de autoanticuerpos y B2A-CIC se cuantificaron inmediatamente antes de la cirugía de trasplante y los pacientes fueron seguidos durante 6 meses.
RESULTADOS:
En el grupo 1 (pacientes positivos para B2A-CIC), el 46,4% de los pacientes experimentó algún tipo de trombosis durante los seis meses de seguimiento, una cifra significativamente

mayor que el 10,4% observado en el grupo 2 (P <0,001) y el 8,6% en el grupo control (P <0,001). La incidencia de trombosis del injerto en el grupo 1 (31.2%) fue significativamente mayor que la observada en el grupo 2 (3.3%, P <0.001) y el grupo control (2.6%, P <0.001). En un análisis multivariable, la presencia de B2A-CIC se comportó como una variable independiente para el riesgo de experimentar cualquier tipo de trombosis postrasplante (Hazard ratio: 6.72, intervalo de confianza del 95%, 4.81-9.37) y, especialmente, para el riesgo de desarrollar una trombosis del injerto (Hazard ratio: 14.75; Intervalo de confianza del 95%, 9.11-23.89). No se encontraron diferencias significativas entre los pacientes del grupo 2 (B2A-CIC-negativos) y el grupo de control.

CONCLUSIONES:

La presencia de B2A-CIC es un predictor de eventos trombóticos agudos. Los pacientes que positivos para IgA anti B2GP1 solo están en riesgo de experimentar trombosis si son B2A-CIC positivos. Si estos pacientes son pacientes B2A-CIC-negativos, tienen el mismo riesgo de desarrollar eventos trombóticos que el grupo de control. Los tratamientos para prevenir eventos trombóticos agudos deben enfocarse en pacientes B2A-CIC-positivos.

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β_2-Glycoprotein I/IgA Immune Complexes

A Marker to Predict Thrombosis After Renal Transplantation in Patients With Antiphospholipid Antibodies

BACKGROUND: Antiphospholipid syndrome is characterized by recurrent thrombosis and gestational morbidity in patients with antiphospholipid autoantibodies (aPLs). Predictive value of the presence of aPLs is low, and new markers are necessary to identify aPL carriers at higher risk and take preventive measures on them. The presence of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC) has been associated with occurrence of acute thrombotic events. In this work we study its possible predictive value for the appearance of acute thrombotic events in patients who are going to undergo transplant surgery, a well-known trigger of acute thrombotic events in aPL carriers.

METHODS: We performed a follow-up study based on the Magnum 12+12 Cohort of patients who received a kidney transplant (n=1339). Three groups were established: group 1 patients who were positive for IgA anti-β_2-glycoprotein I (aB2GP1) and B2A-CIC (n=125); group 2 patients who were positive only for IgA aB2GP1 (n=240); and control group, patients who were negative for IgA aB2GP1 (n=974). Levels of autoantibodies and B2A-CIC were quantified immediately before the transplant surgery and patients were followed up for 6 months.

RESULTS: In group 1, 46.4% of patients experienced any type of thrombosis versus 10.4% in group 2 ($P<0.001$) and 8.6% in the control group ($P<0.001$). The incidence of graft thrombosis in group 1 (31.2%) was significantly higher than that observed in group 2 (3.3%, $P<0.001$) and the control group (2.6%, $P<0.001$). In a multivariate analysis, the presence of B2A-CIC was an independent variable to experience any type of posttransplant thrombosis (hazard ratio, 6.72; 95% confidence interval, 4.81–9.37) and, prominently, for graft thrombosis (hazard ratio, 14.75; 95% confidence interval, 9.11–23.89). No significant differences were found between B2A-CIC–negative and control group patients.

CONCLUSIONS: The presence of B2A-CIC is a predictor of acute thrombotic events. Patients who were positive for IgA aB2GP1 only are at risk of experiencing thrombosis if they are B2A-CIC positive. If they are B2A-CIC–negative patients, they have the same risk as the control group. Treatments to prevent acute thrombotic events should focus on B2A-CIC–positive patients.

Manuel Serrano, MD*
José A. Martínez-Flores, PhD*
Dolores Pérez, BS
Florencio García, MD, PhD
Oscar Cabrera, MD
Daniel Pleguezuelo, MD
Estela Paz-Artal, MD, PhD
José M. Morales, MD, PhD
Esther González, MD, PhD
Antonio Serrano, MD, PhD

*Drs M. Serrano and Martínez-Flores contributed equally.

Correspondence to: Antonio Serrano, MD, PhD, Department of Immunology, Instituto de Investigación, Hospital Universitario 12 de Octubre, Avda. de Andalucía s/n, 28041 Madrid, Spain. E-mail aserrano@h12o.es

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Clinical Perspective

What Is New?

- The presence of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC) has been associated with acute thrombotic events in patients with IgA isotype antiphospholipid antibodies.
- The presence of B2A-CIC at pretransplant is the main independent risk factor to experience any type of posttransplant thrombosis in the first 6 months after kidney transplantation.
- The worst complications in the first weeks post-transplant, ie, graft thrombosis–mediated graft loss, were much more frequent in patients who were B2A-CIC positive.
- Immune complexes emerge as a biomarker that explores a new pathophysiological pathway of antiphospholipid syndrome.

What Are the Clinical Implications?

- Determination of immune complexes helps to identify which patients with antiphospholipid antibodies have high risk of developing thrombosis.
- Patients who were positive for IgA anti-β_2-glycoprotein I have a much higher risk of developing thrombotic events if they are B2A-CIC positive.
- B2A-CIC–negative patients have the same thrombosis risk as the control population.
- Treatment to prevent thrombosis should focus mainly on B2A-CIC–positive patients.

Antiphospholipid antibodies (aPLs) are a group of autoantibodies directed mainly against phospholipid-binding plasmatic proteins that can also be localized on membranes of endothelial cells and platelets.[1,2] The aPLs most frequently associated with vascular pathology are directed against β_2-glycoprotein 1 (B2GP1),[3] a plasma protein synthesized mainly in the liver, heart, and kidney.[4]

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thrombosis or obstetric complications in patients having aPL.[5,6] Diagnosis of APS requires both clinical and laboratory criteria established in 2005. Clinical criteria include thrombosis (in arteries, veins, or small vessels) and miscarriages or fetal loss that must be diagnosed by objective methods such as imaging techniques or histopathology.[1,7] The presence of at least one of the following aPLs is considered by international consensus as a laboratory criterion for APS diagnosis: lupus anticoagulant, antibodies of IgG or IgM isotypes anticardiolipin, or anti-β_2-glycoprotein I (aB2GP1).[1,5]

The clinical relevance of antibodies aB2GP1 of IgA isotype (IgA-aB2GP1) has been increasing in recent years, especially in patients who are negative for other isotypes (IgA isolated-positive).[8–11] Therefore, in the 13th International Congress on Antiphospholipid Antibodies (2010, Galveston, TX), the task force recommended testing for the IgA-aB2GPI in cases in which other aPLs were negative (IgG and IgM isotypes) and APS was still suspected.[12]

The presence of aPLs is necessary, but not sufficient, to promote an APS event. Additional factors are needed to trigger thrombosis (second-hit hypothesis). Although it is known that the activation of the immune response and innate immunity, in the context of processes such as infection or surgery, play a key role and behave as second hits, the mechanisms that induce the thrombotic event remain unknown.[2]

A multicenter prospective study of 1000 patients with APS followed up for 10 years shows that ≈15% of patients developed a thrombotic event in the first 5 years. The predictive value of the presence of aPLs is insufficient to clearly identify the group having the highest risk of events, and new biomarkers are needed to identify which patients have a higher risk of thrombosis.[13]

We described the existence of circulating immune complexes (CICs) of IgA bound to B2GP1 (B2A-CIC) in the blood of patients who were positive for IgA-aB2GPI with a history of APS symptomatology.[14] In a recent work, we described that the presence of B2A-CIC is strongly associated with the occurrence of acute thrombotic events (odds ratio, 22.7; $P<0.001$).[15] This association was established through the quantification of B2A-CIC in serum samples obtained immediately after the occurrence of an acute thrombotic event because no serum samples before the occurrence of thrombotic events were available. Therefore, the possible predictive value of the presence of the B2A-CIC cannot be demonstrated.

In recent years, APS has been associated with many clinical situations including transplant surgery.[16] Graft thrombosis is the main cause of graft loss in the first weeks after kidney transplantation.[17,18] Our group recently described the association of pretransplant presence of IgA aB2GP1 antibodies with graft thrombosis and graft loss in the first weeks after kidney transplantation.[19] Although most patients who have experienced thrombosis have had IgA-aB2GP1, the predictive value of the presence of IgA-aB2GP1 is insufficient to clearly identify the group having the highest risk of events, and additional markers are needed to better identify patients who require preventive treatment.[20]

In this work, we analyze the presence of B2A-CIC in pretransplant serum from a group of patients who are going to undergo a known trigger for APS events, transplant surgery, and its relationship with the occurrence of thrombotic events and graft loss by thrombosis in the first 6 months after transplantation.

METHODS

Study Design

We performed a historical-cohort follow-up study based on the Magnum 12+12 Cohort that included all patients who had received a kidney transplant in the Hospital "12 de Octubre" (Madrid, Spain) in a 12-year period from January 1, 2000 to December 31, 2011.[20] Sera used for the analysis were collected in the 24 hours before the transplant surgery.

Aim

This study aimed to determine the pretransplant prevalence of B2A-CIC in patients who are positive for IgA-aB2GP1 and investigate their possible association with thrombosis, graft thrombosis, and graft loss in the first 6 months after transplant. The main end points were thrombosis, graft loss, causes of graft loss and graft survival at 6 months, the period of greater risk of posttransplant thrombotic events.[21,22]

Ethical Issues

The study was submitted to the Ethics Committee for Clinical Research of Hospital 12 de Octubre and received a favorable report (Reference Number CEIC-15/008). No informed consent was required.

Patients

All patients in the Magnum 12+12 Cohort (N=1375) were included in the study and were studied as long as they maintained a functional graft and up to the end of the observation time. Patients left the study when they lost the graft or died.

Two subcohorts were formed: the control cohort that includes the patients who were negative for IgA-aB2GP1 (n=974) and the positive cohort that includes all the patients who were positive for IgA-aB2GP1. A total of 36 patients who were positive for IgA-aB2GP1 (9%) were excluded from the positive cohort because their pretransplant serum samples were unavailable. There were no significant differences between the pretransplant clinical characteristics and posttransplant evolution of the 36 excluded patients and the patients of the original complete group of patients who were positive for IgA-aB2GP1 (online-only Data Supplement Table I).

Patients in the positive cohort (n=365) were divided into 2 groups: group 1, positive for the presence of B2A-CIC; and group 2, negative for the presence of B2A-CIC.

Immunosuppressive Treatment

The most used immunosuppressive protocol was based on tacrolimus associated with steroids and mycophenolate mofetil. Immunosuppression regimen in patients >60 years, who received kidneys from >60-year-old donors, was based on cyclosporine A (until 2003) or tacrolimus, steroids, and mycophenolate mofetil with or without induction. Only 15 patients were treated with sirolimus/everolimus associated with cyclosporine A or tacrolimus.

Thymoglobulin (rabbit antithymocyte globulin) 1.5 mg/kg for 4 to 7 days was also administered in hyperimmunized patients and patients with a transplantation from non–heart-bearing donors. In elderly people (>60 years of age), basiliximab (monoclonal antibodies anti-IL2R) (20 mg day 0 and 4) with steroids, cyclosporine A (10 $mg \cdot kg^{-1} \cdot d^{-1}$) and mycophenolate mofetil (2 g/d) were administered until 2003. After that and until the end of this study, tacrolimus (0.1 $mg \cdot kg^{-1} \cdot d^{-1}$) was chosen as a calcineurin inhibitor.

Preventive Antithrombotic Treatment

Preventive treatment with antithrombotic agents was administered to patients with vascular morbidity and with risk for thrombosis during the pretransplant period. Treatment was interrupted in all the patients immediately before their surgery, and those treated with anticoagulation received subcutaneous heparin 5000 U twice daily during 1 week in the immediate posttransplant period. All patients who had been treated pretransplantation with anticoagulation or an antiplatelet agent received the same preventive treatment after transplantation.

Definitions

Thrombotic Events

Thrombotic events were defined in accordance with the International Consensus Statement for Antiphospholipid Syndrome[1] as venous thrombosis, arterial thrombosis, pulmonary thromboembolism, and graft thrombosis diagnosed clinically and confirmed by imaging techniques or histopathology study.[17]

Graft Loss by Thrombosis

Graft loss by thrombosis was considered in patients who had lost their graft and presented graft vein/arterial thrombosis. Graft thrombosis was considered only in patients without surgery-related complications or acute rejection diagnostic criteria.

Patients With Thrombosis Not Related to Graft Loss

This included patients who had experienced at least 1 episode of any type of thrombosis, excluding graft loss–related thrombosis.

Patients With Any Type of Thrombosis

This included the patients who have had at least 1 episode of any type of thrombosis, including graft loss by thrombosis.

Transplant-Related Mortality

This was defined as mortality attributable to any cause within 6 months of transplantation.

Long-Term Outputs

These were defined as those that occur beyond the first transplant semester.

Cytomegalovirus Infection

This was defined in accordance with the evidence of cytomegalovirus replication determined with a polymerase chain reaction–based quantitative nucleic acid amplification test.

APS Events

These include thrombotic events, myocardial infarction, and stroke according to the International Consensus Statement.[1,23] Gestational morbidity was not considered in this work.

Acute Rejection

Acute rejection was defined as acute deterioration in allograft function with acute rejection–specific histopathologic changes in the graft.

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Nonfunctioning Graft

This was defined as a graft that never functioned, after having ruled out infections, surgical complications, accelerated or hyperacute rejection, clear signs of massive thrombosis, vascular complications, and urinary tract obstruction.

Laboratory Determinations

IgA-aB2GPIs were quantified by enzyme-linked immunosorbent assays using QUANTA Lite β_2 GPI IgA (INOVA Diagnostics Inc). Cutoff was established at 20 U/mL with the 99th percentile of a healthy normal population[8,12] that coincided with the manufacturer's recommendation.

B2A-CIC levels were quantified as previously described. Sera with values of B2A-CIC higher than 20.9 AU were considered positive.[21] All the immunoassays were performed in a Triturus Analyzer (Diagnostics Grifols, S.A.).

Statistical Methods

Results are expressed as absolute frequency and percentage or medians with interquartile ranges. Association between qualitative variables was determined with the Pearson χ^2 test (or Fisher exact test, when appropriate). Data were expressed as number and percentage. The Mann-Whitney *U* test was used for comparisons in scaled variables with 2 categories.

Graft loss by thrombosis, graft-survival probabilities, and patient-survival probabilities were calculated using the Kaplan-Meier method and differences between the survival distributions were assessed with the log-rank test. The relative measure of a condition on survival was expressed as hazard ratio (HR).

Multivariate analyses were performed using Cox regression (proportional-hazards model). The relative measure of an effect was expressed as hazard ratio.

Receiver operating characteristic (ROC) curve analysis was performed to determine the cutoff value of the assays. The optimum cutoff point was established using the maximum value of the Youden index (J value).

Probabilities <0.05 were considered significant. For multiple comparisons *P* values were adjusted with the Bonferroni correction.

Donor age was not evaluated in the statistical analysis because it is a recipient age–dependent variable. (When selecting the donor recipients, an attempt was made to match up their ages as much as possible.)

Data were processed and analyzed using Medcalc for Windows version 16.8 (MedCalc Software). The adjustment of *P* values for multiple comparisons were obtained by the Bonferroni method using the p.adjust function of the "R" program language (R Foundation for Statistical Computing).

RESULTS

A total of 125 (34%) of the 365 patients who were IgA-aB2GP1 positive had B2A-CIC values above the cutoff (group 1), whereas 240 were negative (group 2). Table 1 shows the pretransplant characteristics of the 1339 patients studied: group 1, group 2, and the control group. Levels and distribution of positive patients for each group of other aPLs (IgG and IgM) are described in online-only Data Supplement Table II.

In comparison of the pretransplant clinical characteristics (Table 1) of patients in group 1 versus the control group, group 1 patients were older (median, age 62 versus 52 years; *P*<0.001), had higher cold ischemia time (median, 22 versus 20 hours; *P*<0.001), and experienced more pretransplant thrombotic events (21.6% versus 11.0%, *P*<0.001). On analyzing the clinical characteristics of the group 2 patients, it was found that, in comparison with the control group, they were older (median age, 60 versus 52 years; *P*<0.001) and the proportion of men was significantly lower (47.9% versus 61.9%; *P*<0.001).

Graft Loss Caused by Graft Thrombosis

A total of 72 patients had graft loss caused by graft thrombosis (GL-GT) representing the main cause of graft loss: 51.4% of the 140 patients who had lost their graft in the first semester.

The percentage of patients with GL-GT for the control group was 2.6% (n=25), 31.2% (n= 39) for group 1 and 3.3% (n=8) for group 2. Most of the cases of graft thrombosis (54.2%) occurred in the patients in group 1. The percentage of patients who were positive for B2A-CIC was 82.9% in patients with GL-GT who were positive for IgA-aB2GP1 (39/47, Figure 1).

The relative risk for GL-GT for group 1 patients (B2A-CIC positive) versus the control group was 12.16 (95% confidence interval [CI], 5.94–29.39; *P*<0.001). The relative risk for the incidence of GL-GT in patients in group 1 versus group 2 was 9.36 (95% CI, 4.51–19.41; *P*<0.001). No significant differences in GL-GT were observed between patients in group 2 and the control group (Table 2).

The Kaplan-Meier survival analysis (Figure 2A) showed significantly higher GL-GT rates in group 1 than in the control group (HR, 14.6; 95% CI, 6.0–32.0; *P*<0.001) and in comparison with group 2 (HR, 10.9; 95% CI, 4.1–28.8; *P*<0.001). Differences between group 2 and the control group were not significant.

When patients with GL-GT were compared with the rest of the patients, they were older (median age, 61.5 versus 54 years; *P*=0.010), they had a higher proportion of type 2 diabetes mellitus (30.6% versus 16.8%; *P*=0.005), and most were positive for IgA-aB2GP1 (65.3% versus 25.1%; *P*<0.001). Furthermore, patients with GL-GT had significantly higher levels of B2A-CIC than patients without graft thrombosis (median, 35.1 versus 13.6 AU/mL; *P*<0.001; Figure 2B) and most were positive for B2A-CIC (54.2% versus 6.8%; *P*<0.001; online-only Data Supplement Table III).

Graft Survival at 6 Months Was Significantly Lower in Group 1 Patients

The patients in group 1 also had the highest number of cases of graft loss (for all the causes) in the first 6 months after transplantation.

Table 1. Pretransplant Characteristics of Patients in the 3 Groups

Condition	B2A-CIC (+) n=125		B2A-CIC (–) n=240		Control Group n=974		*P* Value*		
	Number or median	% or IQR	Number or median	% or IQR	Number or median	% or IQR	B2A-CIC (+) vs B2A-CIC (–)	B2A-CIC (+) vs Control Group	B2A-CIC (–) vs Control Group
Male sex	75	60	115	47.9	603	61.9	0.111	1.000	<0.001
Age, y	62	50–71	60	46.5–69	52	40–64	0.360	<0.001	<0.001
Time on dialysis, mo	17.5	8.2–32.4	17.5	89-36.1	17.5	8.7–34.5	1.000	1.000	1.000
Panel reactive antibody at time of transplant >50%	3	2.4	4	1.7	22	2.3	1.000	1.000	1.000
Cold ischemia	22	18–24.1	21	15–24	20	16–23	0.177	<0.001	0.618
Previous kidney transplant	16	12.8	28	11.6	170	17.5	1.000	0.714	0.114
Associated pathologies									
Diabetes mellitus	32	25.6	46	19.1	188	19.3	0.594	0.372	1.000
Type 1	3	2.4	2	0.8	26	2.7	1.000	1.000	0.435
Type 2	28	22.4	44	18.3	163	16.6	1.000	0.444	1.000
Myocardial infarction	4	3.2	6	2.5	29	3	1.000	1.000	1.000
Stroke	9	7.2	15	6.2	48	4.9	1.000	1.000	1.000
Thrombosis antecedents (patients)†	27	21.6	30	12.4	107	11	0.102	<0.001	1.000
Arterial thrombosis	1	0.8	5	2.1	2	0.2	1.000	0.912	0.012
Pulmonary thromboembolism	1	0.8	4	1.7	13	1.3	1.000	1.000	1.000
Venous thrombosis	26	20.8	24	10	99	10.2	0.021	<0.001	1.000
Causes end-stage renal disease									
Chronic glomerulonephritis	13	10.4	28	11.6	140	14.4	1.000	0.852	0.975
Interstitial kidney disease	10	8	21	8.7	84	8.6	1.000	1.000	1.000
IgA nephropathy	9	7.2	16	6.6	47	4.8	1.000	1.000	0.969
Polycystic kidney disease	12	9.6	27	11.2	126	12.9	1.000	1.000	1.000
Nephroangiosclerosis	11	8.8	24	10	100	10.3	1.000	1.000	1.000
Diabetes mellitus	26	20.8	36	15	127	13	0.630	0.078	1.000
Lupus erythematosus	2	1.6	4	1.7	12	1.2	1.000	1.000	1.000
Vesicoureteral reflux	5	4	4	1.7	40	4.1	0.939	1.000	0.318
Unknown	21	16.8	40	16.6	134	13.8	1.000	1.000	0.882
Other	16	12.8	40	16.6	164	16.8	1.000	0.924	1.000
Donor origin									
Brain death	113	90.4	202	83.5	796	81.7	0.414	0.066	1.000
Living donor	2	1.6	9	3.7	49	5	1.000	0.408	1.000
Non–heart-beating	10	8	30	12.4	129	13.2	0.777	0.387	1.000
Double renal transplant	5	4	9	3.7	22	2.3	1.000	1.000	0.837
Preventive antithrombotic treatment									
Coumarin (isolated)	9	(7.2)	8	(3.3)	36	(3.7)	0.289	0.188	1.000
Coumarin (comb)	2	(1.6)	2	(0.8)	7	(0.7)	1.000	0.910	1.000
Low-dose aspirin	29	(23.2)	28	(11.7)	145	(14.9)	0.012	0.050	0.603
Clopidogrel (isolated)	4	(3.2)	4	(1.7)	27	(2.8)	1.000	1.000	0.993

B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I; and IQR, interquartile range.

**P* values were adjusted by the Bonferroni method for multiple comparisons.

†A patient may have >1 thrombotic event.

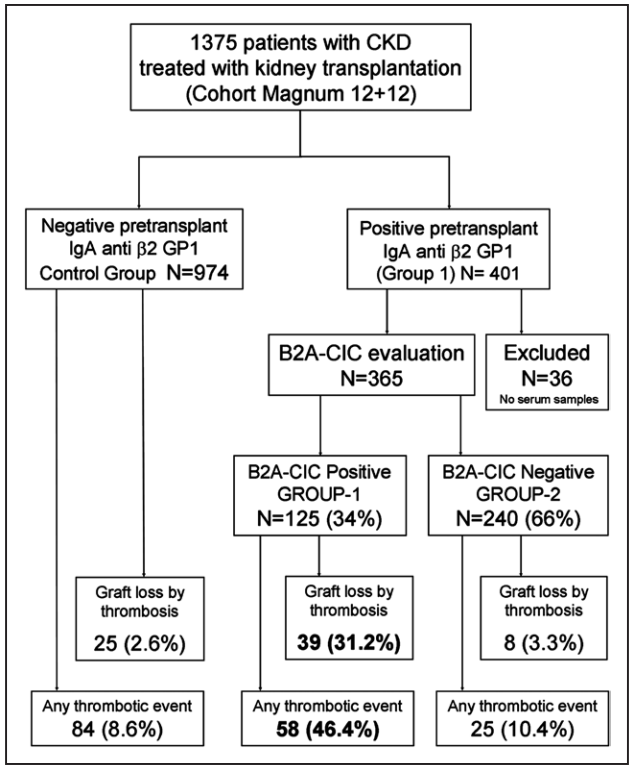

Figure 1. Description of the study.
Distribution of patients and main outcomes. B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I; CKD, chronic kidney disease; and GP1, glycoprotein I.

In a Kaplan-Meier analysis (Figure 2C), graft loss (for any cause) was significantly higher in group 1 versus the control group (HR, 6.46; 95% CI, 3.47–12.03; *P*<0.001) and versus group 2 (HR, 5.08; 95% CI, 2.51–10.31; *P*<0.001). Differences between group 2 and the control group were not significant (Table 2).

It is notable that the proportion of patients with a nonfunctioning graft (kidney that never functioned without experiencing rejection or thrombosis) was significantly higher (*P*=0.006) in group 1 than in the control group (4% versus 0.6%; *P*=0.006; relative risk, 6.36; 95% CI, 1.97–20.54). The differences between the groups were not significant in the remaining causes of graft loss (Table 2).

Other Thrombotic Events

In addition to the patients with GL-GT, 95 patients without graft loss (7.1%) presented thrombotic events. This thrombosis in patients with non–graft thrombosis was more frequent in patients in group 1 (15.2%) than in the control group (6.1%; *P*<0.001; Table 2). When patients who had experienced any type of thrombotic event (including GL-GT and other thrombosis) were considered, it was found that posttransplant thrombosis was significantly more frequent in the group 1 patients (46.4%), this frequency being significantly higher than that observed in group 2 (10.4%; relative risk, 4.45; *P*<0.001) and in the control group (8.6%; relative risk, 5.38; *P*<0.001).

There were no differences concerning posttransplant thrombosis among patients who received preventive treatment and those who did not (data not shown), with the exception of patients treated with low-dose aspirin who had a higher incidence of graft thrombosis (8.4% versus 4.8%; *P*=0.038).

Multivariate Analysis

Factors that showed an association with GL-GT with a *P* value of <0.150 (online-only Data Supplement Table III) were subjected to a Cox proportional hazards multivariate analysis (Table 3). The presence of B2A-CIC (HR, 14.75; 95% CI, 9.11–23.89; *P*<0.001), type 2 diabetes mellitus (HR, 1.97; 95% CI, 1.15–3.37; *P*=0.014), and transplant from a non–heart-beating donor (HR, 2.55; 95% CI, 1.38–4.71; *P*=0.001) were identified as independent variables for graft loss by thrombosis. Pretransplant presence of arterial hypertension was identified as a significant protection factor (HR, 0.40; 95% CI, 0.23–0.68; *P*<0.001).

A univariate analysis of the factors associated with any type of thrombosis was also performed (online-only Data Supplement Table IV).

The variables that were identified with a *P* value of <0.150 were subjected to a Cox multivariate analysis. Presence of B2A-CIC (HR, 6.72; 95% CI, 4.81–9.37; *P*<0.001) was identified as the independent variable for thrombosis having a stronger association. Sex, age, type 2 diabetes mellitus, and non–heart-beating donor were also identified as independent variables. Again, it was found that the presence of hypertension before transplantation acts as a significant protective factor against the incidence of thrombotic events (Table 3).

Transplant-Related Mortality

Patients in group 1 had a significantly higher mortality (*P*<0.001) in the first 6 months after transplant than that observed in group 2 (8% versus 1.8%) and the control group (8% versus 2.9%; Table 2 and Figure 3A).

Validation of the B2A-CIC Levels Cutoff

The values of IgA-based CIC units (AU) were analyzed by using the receiver operating characteristic curve (ROC curve) to validate whether B2A-CIC cutoff previously described for patients with non–transplant-related APS symptoms was also suitable for patients with posttransplant thrombosis. For GL-GT, an area under the ROC curve of 0.752 (95% CI, 0.704–0.795; *P*<0.001; Figure 4A) was obtained. The optimal cutoff (defined with the maximum value of the Youden J index) was 20.83 AU (sensitivity, 85.1%; specificity, 73.0%).

Table 2. Outcomes in the First Semester After Transplant in Patients in the 3 Groups

Condition	B2A-CIC (+) n=125		B2A-CIC (–) n=240		B2A-CIC (–) n=240		*P* Value*		
	n	%	n	%	n	%	B2A-CIC (+) vs B2A-CIC (–)	B2A-CIC (+) vs Control Group	B2A-CIC (–) vs Control Group
Patients with thrombotic events in the first semester									
Including graft loss by thrombosis†	58	46.4	25	10.4	84	8.6	<0.001	<0.001	1.000
Excluding graft loss by thrombosis†	19	15.2	17	7.1	59	6.1	0.066	<0.001	1.000
Deep venous thrombosis	12	9.6	15	6.3	44	4.5	1.000	0.081	1.000
Pulmonary thromboembolism	2	1.6	4	1.7	8	0.8	1.000	1.000	1.000
Arterial thrombosis	6	4.8	2	0.8	11	1.1	0.114	0.018	1.000
Myocardial infarction	1	0.8	2	0.8	5	0.5	1.000	1.000	1.000
Stroke	1	0.8	3	1.3	4	0.4	1.000	1.000	0.864
Any antiphospholipid syndrome event ‡	59	47.2	33	13.8	112	11.5	<0.001	<0.001	1.000
Graft loss in the first 6 mo									
Total graft losses	48	38.4	22	9.2	70	7.2	<0.001	<0.001	1.000
Acute rejection	1	0.8	4	1.7	8	0.8	1.000	1.000	1.000
Nonfunctioning graft	5	4	3	1.3	6	0.6	0.555	0.006	1.000
Death	0	0	2	0.8	11	1.1	1.000	1.000	1.000
Graft thrombosis	39	31.2	8	3.3	25	2.6	<0.001	<0.001	1.000
Surgery related	0	0	2	0.8	2	0.2	1.000	1.000	1.000
Others	3	2.4	3	1.3	18	1.8	1.000	1.000	1.000
Mortality in the first 6 mo	10	8	7	2.9	18	1.8	0.087	<0.001	0.888
Cardiovascular diseases	5	4	4	1.7	8	0.8	0.519	0.006	0.708
Infections	3	2.4	3	1.3	7	0.7	1.000	0.186	1.000
Cancer	1	0.8	0	0	0	0	0.495	0.342	—
Others	1	0.8	0	0	3	0.3	0.495	1.000	1.000
Patients with cytomegalovirus infection	9	7.2	21	8.8	75	7.7	1.000	1.000	1.000

B2A-CIC, circulating immune complexes of IgA bound to β_2-glycoprotein I; and —, not calculated (zero elements).
**P* values were adjusted by the Bonferroni method for multiple comparisons.
†A patient may have >1 type of thrombotic event.
‡It includes thrombosis, myocardial infarction, and stroke.

The cutoff was also reevaluated using the presence of any type of thrombosis as a classification variable, obtaining an area under the ROC curve of 0.709 (95% CI, 0.659–0.755; Figure 4B) and practically the same optimum cutoff: 20.86 AU (sensitivity, 70.7%; specificity, 76.1%). Adopting 20.83 AU as a cutoff for the independent variable, the univariate odds ratio for graft loss by graft thrombosis was 16.23 (95% CI, 9.71–27.10; *P*<0.001) and for any thrombosis in the 6 months after transplant was 8.78 (95% CI, 5.87–13.13; *P*<0.001).

An additional ROC curve analysis of levels of B2A-CIC (AU) in patients with graft loss in the first 6 months after transplant with the exclusion of patients with GL-GT can be seen in the online-only Data Supplement Figure I.

Long-Term Outputs

The incidence of thrombotic events, graft losses, and mortality in patients who surpassed the first semester after transplant was low; significant differences were not found in the 3 groups of patients (online-only Data Supplement Table V and Figure 3B). There were also no significant differences in the causes of graft loss or death, with the exception of a slightly more significant mortal-

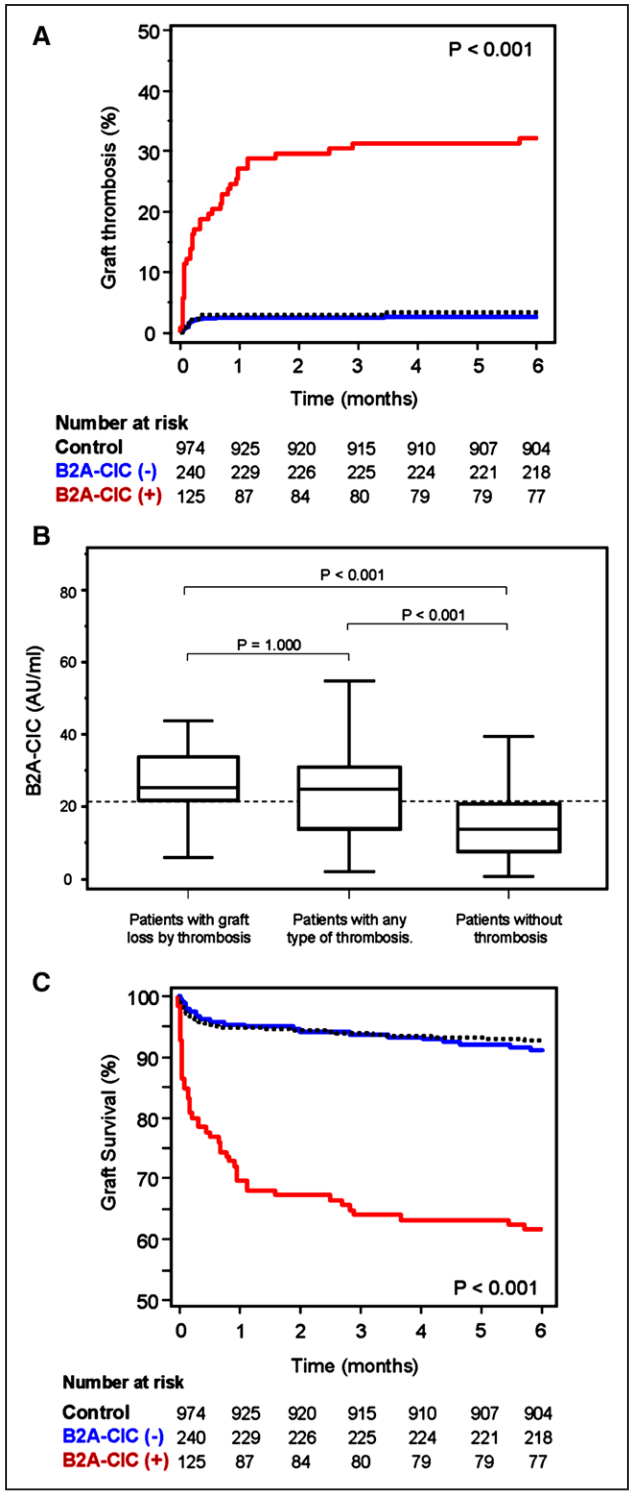

Figure 2. Graft survival and pretransplant serum levels of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC).
A, Incidence of graft thrombosis in the 3 groups during the 6 months of the follow-up. Patients in group 1 (B2A-CIC positive, red line) had significantly higher incidence of graft thrombosis survival (Kaplan-Meier survival analysis) than did the control group (black dotted line) (hazard ratio, 14.06; 95% confidence interval, 6.0–33.0) and group 2 (B2A-CIC negative, blue line) (hazard ratio, 10.9; 95% confidence interval, 4.1–28.8). (*Continued*)

ity from cardiovascular causes in B2A-CIC–negative patients than in controls (1.8% versus 0.2%; *P*=0.045; online-only Data Supplement Table V).

DISCUSSION

In this work, we are describing for the first time that the pretransplant presence of B2A-CIC identifies a subgroup of patients prone to develop posttransplant thrombosis, thus behaving as a predictive biomarker.

The condition of the patients studied in this work is special: end-stage renal disease treated with a kidney transplant. Patients with end-stage renal disease who are going to receive transplants make up an ideal model to study the occurrence of APS events, because they include a high percentage of asymptomatic patients with aPL (IgA-aB2GP1), and all of them are subjected to a well-known second hit, that is, transplant surgery.

Although this population may seem very complex for the study of APS events because they have an additional disease, the work focuses on exploring a new pathophysiological pathway of APS[24] that could help to better understand its pathogenesis and to establish new therapeutic strategies. It should be considered that most studies on APS also are based on groups of patients with concomitant conditions (autoimmune diseases). In this way, the cutoff for B2A-CIC obtained in this study with patients with end-stage renal disease is practically identical to that obtained with patients with non–transplant-related APS,[15] suggesting that both patients with end-stage renal disease APS and patients with conventional APS would be equivalent regarding B2A-CIC thrombotic risk.

IgA-aB2GPI antibodies behave as an independent risk factor for APS events, especially graft thrombosis. However, the presence of IgA-aB2GP1 is not sufficient to identify the population that is potentially at risk of throm-

Figure 2 Continued. The differences between B2A-CIC–negative patients and the control group were not significant. **B,** Pretransplant serum levels of B2A-CIC. The levels were significantly higher (*P*<0.001) in patients who lost the kidney by graft thrombosis (median, 25.1; interquartile range, 21.7–33.7) and in patients with any type of thrombosis in the first 6 months after transplantation (median, 24.9; interquartile range, 13.9–30.9) than in those patients who did not have any thrombosis in the first 6 months after transplantation (median, 13.6; interquartile range, 7.7–20.6). The *P* values were adjusted by the Bonferroni method for multiple comparisons. **C,** Graft survival at 6 months of follow-up (including all causes of graft loss). Patients who were B2A-CIC positive (red line) had a significantly lower graft survival (Kaplan-Meier survival analysis) than did patients in the control group (black dotted line; hazard ratio, 6.46; 95% confidence interval, 3.47–12.03) and B2A-CIC negative (blue line) (hazard ratio, 5.08; 95% confidence interval, 2.51–10.31). Differences between B2A-CIC negative and the control group were not significant.

Table 3. Multivariate Analysis (Cox Regression) of Outcomes in the First 6 Months After Transplantation

Variable	Univariate			Multivariate		
	Hazard Ratio	95% Confidence Interval	*P* Value	Hazard Ratio	95% Confidence Interval	*P* Value
Graft loss by graft thrombosis in the first 6 mo*						
B2A-CIC positive	13.35	8.39–21.24	<0.001	14.75	9.11–23.89	<0.001
Age, y	1.02	1.00–1.04	0.011	1.01	0.99–1.03	0.227
Type 2 diabetes mellitus	2.15	1.30–3.55	0.003	1.97	1.15–3.37	0.014
Non–heart-beating donor	1.69	0.94–3.03	0.078	2.55	1.38–4.71	0.003
Hypertension	0.62	0.37–1.03	0.066	0.40	0.23–0.68	<0.001
Any thrombosis in the first 6 mo†						
B2A-CIC positive	6.56	4.76–9.05	<0.001	6.72	4.81–9.37	<0.001
Male sex	0.64	0.47–0.87	0.004	0.63	0.46–0.85	0.003
Age, y	1.03	1.02–1.04	<0.001	1.03	1.02–1.04	<0.001
Type 2 diabetes mellitus	1.78	1.26– 2.51	0.001	1.42	0.99–2.04	0.057
Hypertension	0.67	0.47–0.95	0.023	0.46	0.32–0.65	<0.001
Non–heart-beating donor	1.37	0.91–2.07	0.133	2.09	1.35–3.24	0.001

B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

*Risk factors associated with graft loss by graft thrombosis in the first 6 months.

†Risk factors associated with any type of thrombosis, except for age (year) and cold ischemia time (hour). The remaining variables are dichotomous.

bosis, because only a small proportion of patients who were positive for these antibodies develop thrombotic events.[9,20] This situation is similar to the situation observed with other aPL of IgG and IgM isotypes.[6]

In this work, we have shown that patients who are positive for IgA-aB2GP1 and also present B2A-CIC are those having the highest risk of thrombotic events when they are subjected to a situation capable of triggering the occurrence of thrombotic events such as transplant surgery. Those who are negative for B2A-CIC have a risk similar to that found in the control population.

A statistically significant association exists between the presence of B2A-CIC with graft losses attributable to nonfunctioning grafts. A significant majority of these patients did not undergo nephrectomy because they had no serious complications. For this reason, a complete histopathologic study is not available for them. On the basis of the presence of B2A-CIC and the symptoms, we have been able to speculate that some of these patients might have experienced some form of silent and progressive thrombotic microangiopathy that would harm the organ by annulling its function, but which had not had serious systemic implications that required medical attention and nephrectomy.

In addition to GL-GT, the presence of B2A-CIC is also associated with other thrombotic events. Renal transplant recipients are at high risk of thromboembolic events in the first months posttransplant.[25] Although some of these events can be related to the transplant surgery, treatments, or time of hospitalization,[24] the presence of B2A-CIC can be considered the most important risk factor.

The biological significance of the presence of B2A-CIC is uncertain. APS is an autoimmune disease having a special situation because the antigen and antibody are present in the blood at the same time. Given the abundance of the antigen (B2GP1), we would have expected that all the antibodies would be permanently bound to their antigen and could not be detected in the laboratory, because the laboratory assays are only designed to evaluate unbound antibodies (the free form).

However, in reality, the antibodies exist in free form and are detected in the diagnostic test. One possible explanation is that antibodies found in blood in free form could have low affinity or be directed against B2GP1 epitopes that are not accessible in physiological conditions.

The biological behavior of the antibodies incorporated into B2A-CIC would be different from that of the free-form antibodies, not only because they have greater affinity, but also because they would be directed against epitopes that are only present in some conformations of the protein that would be more related to their anticoagulant function. The search for epitopes recognized by the antibodies integrated in the B2A-CIC and their possible association with the different physiological functions of B2GP1 should be studied in future research.

It is striking that patients with hypertension at pretransplant have a significantly lower risk of thrombosis. Some antihypertensive treatments might provide vascular protection by reversing endothelial dysfunction and pro-

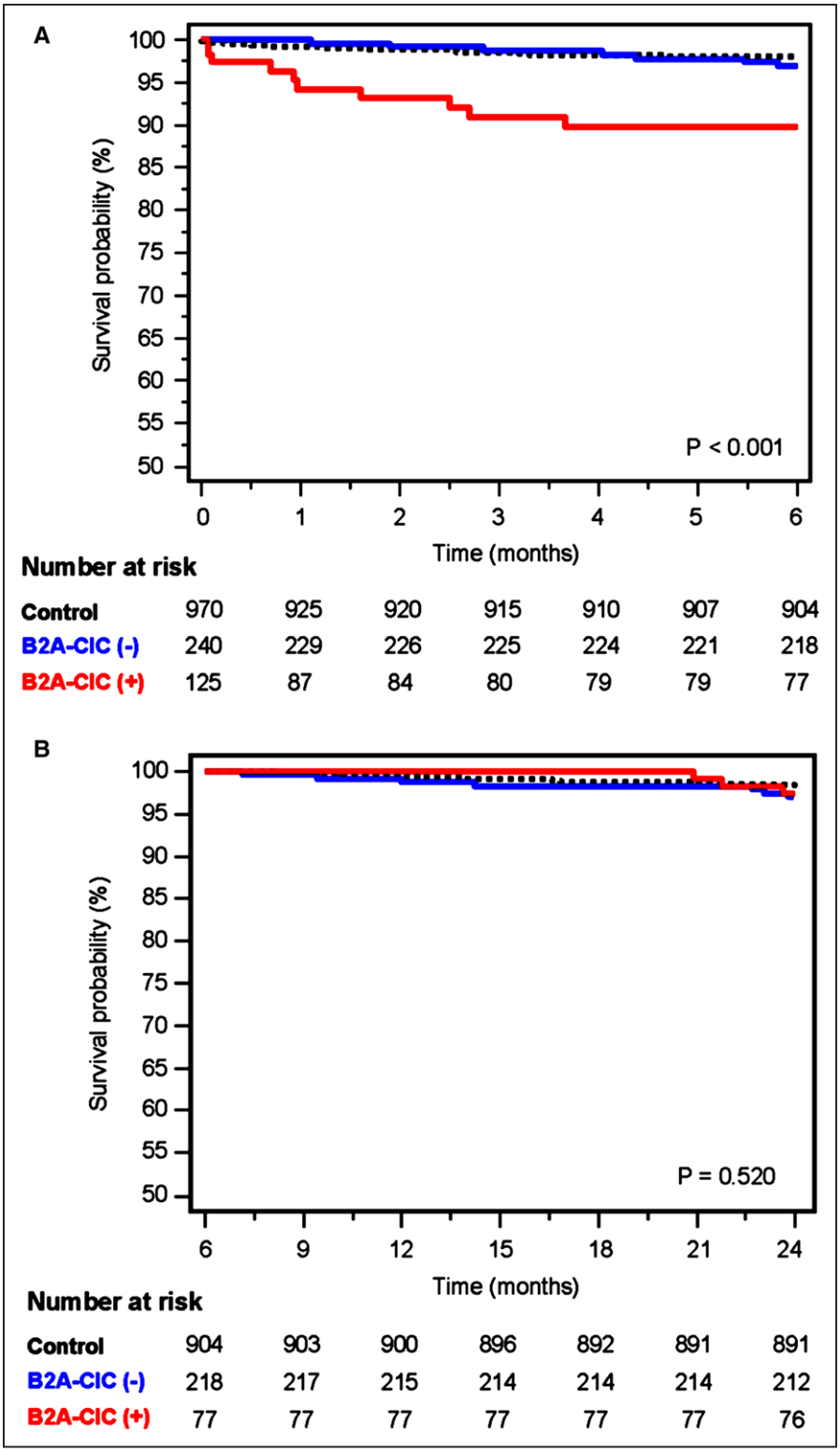

Figure 3. Patient survival analysis.
A, Survival in the first 6 months after transplantation (Kaplan-Meier survival analysis). Patients who were B2A-CIC positive had a higher mortality than did patients in the control group (hazard ratio, 4.48; 95% confidence interval, 1.39–14.42). In comparison with the B2A-CIC negative, the differences are not enough to be significant (hazard ratio, 2.85; 95% confidence interval, 0.74–11.00). **B,** Survival analysis up to 24 months after transplantation of patients who remained in the study at the end of the first semester. There were no significant differences among the 3 groups of patients. B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

thrombotic abnormalities, contributing to a reduction in thrombosis-related complications.[26] In this way, hemodialyzed patients treated with antihypertensive drugs have a significantly longer period of vascular access permeability (without vascular thrombosis) than do those who were not treated with them,[27] and renal transplant recipients treated with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers associated with vitamin D have a 60% lower rate risk of venous thromboembolism.[28] Therefore, it can be speculated that antihypertensive treatment before transplantation could have an endothelium-protecting role and could explain, at least in part, a lower risk of thrombosis. This possible protective effect should be investigated in further studies.

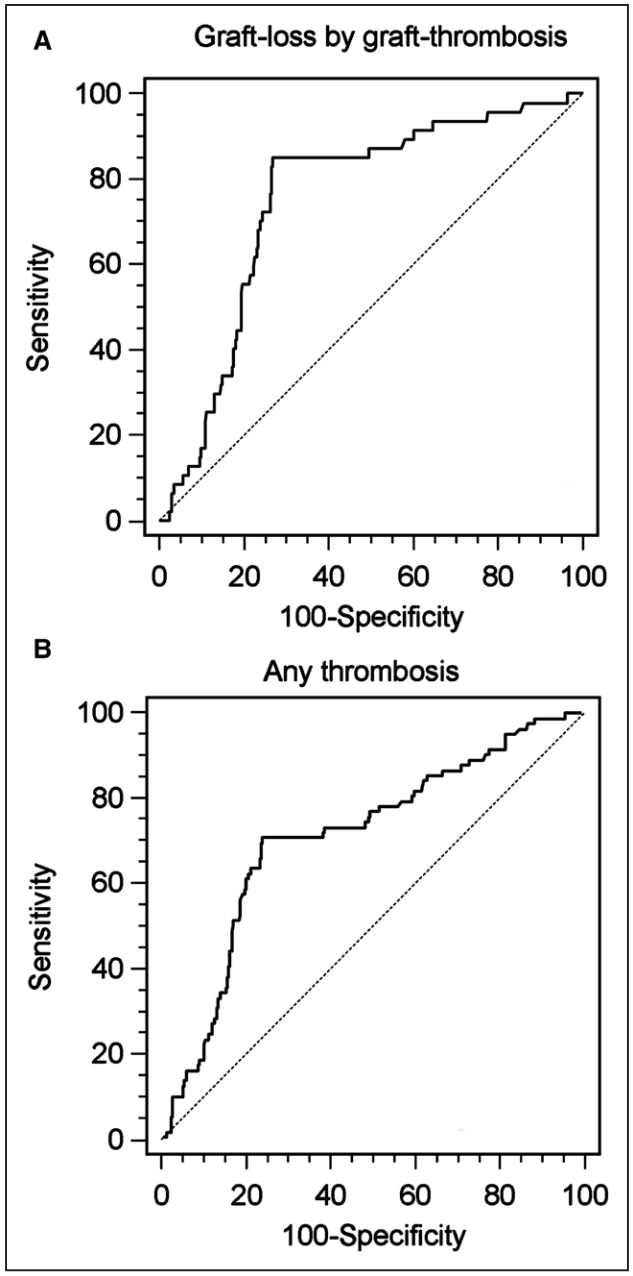

Figure 4. Receiver operating characteristic curves analysis for B2A-CIC evaluation.
A, Receiver operating characteristic curve of levels of B2A-CIC (AU/mL) for risk of graft loss by graft thrombosis in the first 6 months after transplantation (P<0.001). Area under the the receiver operating characteristic curve, 0.752 (95% confidence interval, 0.704–0.795). **B,** Receiver operating characteristic curve of levels of B2A-CIC (AU/mL) in patients with any type of thrombosis in the first 6 months after transplantation (P<0.001). Area under the receiver operating characteristic curve, 0.709 (95% confidence interval, 0.680–0.774). B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

This study has several limitations. Only CIC formed by B2GP1 bound to antibodies of IgA isotype were evaluated. The CIC integrated by antibodies of IgG and IgM isotypes were not evaluated because, in this cohort of patients, the prevalence of IgG and IgM aB2GP1 was very low and no association with thrombosis or graft loss was found.[20] Another weakness of the study is that, although it has collected the experience of 12 years, it is a single-center study with patients who have undergone transplantation. Thus, multicenter studies to confirm these findings, both with patients who have undergone transplantation and including other APS-associated situations, are mandatory.

B2A-CIC determination could help clinicians to distinguish which patients could receive preventive therapy. According to our results, positive CIC patients have a high risk for thrombosis and therefore could receive preventive therapy for posttransplant thrombosis. Low-dose aspirin was used most frequently as preventive treatment. Patients of group 1 exhibited a high frequency of this treatment in comparison with those of group 2 and the control group, because the group 1 patients presented more thrombotic antecedents before the transplantation. Patients preventively treated with low-dose aspirin had a significantly higher incidence of graft thrombosis than did other patients, which suggests that the low-dose aspirin therapy did not appear to be sufficiently preventive in our cohort. This coincides with others studies published in aPL carriers in which aPL-positive individuals did not benefit from low-dose aspirin for primary thrombosis prophylaxis.[29,30] Modern anticoagulation (with X factor inhibitors) and mainly hydroxychloroquine could be the therapy of choice for thrombosis prevention.[31,32] Patients who are positive for IgA-aB2GP1 and negative for CIC should receive therapy only if there are concurrent risk factors for thrombosis such as diabetes mellitus with severe cardiovascular disease. However, multicenter and randomized trials with this approach are mandatory before the use of this therapy can be established.

In summary, the presence of pretransplant B2A-CIC in patients who were positive for IgA-aB2GP1 is associated with thrombotic risk and can thus be considered as a biomarker of thrombotic complications. The presence of IgA-aB2GP1 without B2A-CIC implies a thrombosis risk similar to the general population. B2A-CIC determination could help clinicians to distinguish which patients could receive preventive therapy.

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DISCLOSURES

None.

AFFILIATIONS

From Department of Immunology (M.S., J.A.M.-F., D. Pérez, O.C., D. Pleguezuelo, E.P.-A., J.M.M., A.S.), and Department of Nephrology (F.G., E.G.), Instituto de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain (M.S., J.A.M.-F., D. Pérez, O.C., D. Pleguezuelo, E.P.-A., J.M.M., A.S.); Facultad de Medicina, Universidad Complutense de Madrid, Spain (M.S., E.P.-A.).

FOOTNOTES

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REFERENCES

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x.
2. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat Rev Rheumatol*. 2011;7:330–339. doi: 10.1038/nrrheum.2011.52.
3. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med*. 2013;368:1033–1044. doi: 10.1056/NEJMra1112830.
4. Ragusa MA, Costa S, Cefalù AB, Noto D, Fayer F, Travali S, Averna MR, Gianguzza F. RT-PCR and in situ hybridization analysis of apolipoprotein H expression in rat normal tissues. *Int J Mol Med*. 2006;18:449–455.
5. Willis R, Harris EN, Pierangeli SS. Pathogenesis of the antiphospholipid syndrome. *Semin Thromb Hemost*. 2012;38:305–321. doi: 10.1055/s-0032-1311827.
6. Gómez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun*. 2014;48-49:20–25. doi: 10.1016/j.jaut.2014.01.006.
7. Devreese K, Hoylaerts MF. Challenges in the diagnosis of the antiphospholipid syndrome. *Clin Chem*. 2010;56:930–940. doi: 10.1373/clinchem.2009.133678.
8. Ruiz-García R, Serrano M, Martínez-Flores JÁ, Mora S, Morillas L, Martín-Mola MÁ, Morales JM, Paz-Artal E, Serrano A. Isolated IgA anti-β2 glycoprotein I antibodies in patients with clinical criteria for antiphospholipid syndrome. *J Immunol Res*. 2014;2014:704395. doi: 10.1155/2014/704395.
9. Murthy V, Willis R, Romay-Penabad Z, Ruiz-Limón P, Martínez-Martínez LA, Jatwani S, Jajoria P, Seif A, Alarcón GS, Papalardo E, Liu J, Vilá LM, McGwin G Jr, McNearney TA, Maganti R, Sunkureddi P, Parekh T, Tarantino M, Akhter E, Fang H, Gonzalez EB, Binder WR, Norman GL, Shums Z, Teodorescu M, Reveille JD, Petri M, Pierangeli SS. Value of isolated IgA anti-β2 -glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. *Arthritis Rheum*. 2013;65:3186–3193. doi: 10.1002/art.38131.
10. Mehrani T, Petri M. Association of IgA Anti-beta2 glycoprotein I with clinical and laboratory manifestations of systemic lupus erythematosus. *J Rheumatol*. 2011;38:64–68. doi: 10.3899/jrheum.100568.
11. Pericleous C, Ferreira I, Borghi O, Pregnolato F, McDonnell T, Garza-Garcia A, Driscoll P, Pierangeli S, Isenberg D, Ioannou Y, Giles I, Meroni PL, Rahman A. Measuring IgA anti-β2-glycoprotein I and IgG/IgA anti-domain I antibodies adds value to current serological assays for the antiphospholipid syndrome. *PLoS One*. 2016;11:e0156407. doi: 10.1371/journal.pone.0156407.
12. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC, Pierangeli SS. International consensus guidelines on anticardiolipin and anti-β2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum*. 2012;64:1–10. doi: 10.1002/art.33349.
13. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramón E, Buonaiuto V, Jacobsen S, Zeher MM, Tarr T, Tincani A, Taglietti M, Theodossiades G, Nomikou E, Galeazzi M, Bellisai F, Meroni PL, Derksen RH, de Groot PG, Baleva M, Mosca M, Bombardieri S, Houssiau F, Gris JC, Quéré I, Hachulla E, Vasconcelos C, Fernández-Nebro A, Haro M, Amoura Z, Miyara M, Tektonidou M, Espinosa G, Bertolaccini ML, Khamashta MA; Euro-Phospholipid Project Group (European Forum on Antiphospholipid Antibodies). Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis*. 2015;74:1011–1018. doi: 10.1136/annrheumdis-2013-204838.
14. Martínez-Flores JA, Serrano M, Pérez D, Lora D, Paz-Artal E, Morales JM, Serrano A. Detection of circulating immune complexes of human IgA and beta 2 glycoprotein I in patients with antiphospholipid syndrome symptomatology. *J Immunol Methods*. 2015;422:51–58. doi: 10.1016/j.jim.2015.04.002.
15. Martínez-Flores JA, Serrano M, Pérez D, Cámara A G, Lora D, Morillas L, Ayala R, Paz-Artal E, Morales JM, Serrano A. Circulating immune complexes of IgA bound to beta 2 glycoprotein are strongly associated with the occurrence of acute thrombotic events. *J Atheroscler Thromb*. 2016;23:1242–1253. doi: 10.5551/jat.34488.
16. Hughes GR. Hughes syndrome/APS. 30 years on, what have we learnt? Opening talk at the 14th International Congress on antiphospholipid antibodies Rio de Janiero, October 2013. *Lupus*. 2014;23:400–406. doi: 10.1177/0961203314522341.
17. Ponticelli C, Moia M, Montagnino G. Renal allograft thrombosis. *Nephrol Dial Transplant*. 2009;24:1388–1393. doi: 10.1093/ndt/gfp003.
18. Morales JM, Marcen R, Andres A, Molina MG, Castillo DD, Cabello M, Capdevila L, Campistol JM, Oppenheimer F, Seron D, Vernet SG, Lampreave I, Valdes F, Anaya F, Escuin F, Arias M, Pallardo L, Bustamante J. Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. *Kidney Int Suppl*. 2008;74:S94–99.

19. Morales JM, Martinez-Flores JA, Serrano M, Castro MJ, Alfaro FJ, García F, Martínez MA, Andrés A, González E, Praga M, Paz-Artal E, Serrano A. Association of early kidney allograft failure with preformed IgA antibodies to β2-glycoprotein I. *J Am Soc Nephrol*. 2015;26:735–745. doi: 10.1681/ASN.2014030228.
20. Morales JM, Serrano M, Martínez-Flores JA, Pérez D, Castro MJ, Sánchez E, García F, Rodríguez-Antolín A, Alonso M, Gutierrez E, Morales E, Praga M, González E, Andrés A, Paz-Artal E, Martínez MA, Serrano A. The presence of pretransplant antiphospholipid antibodies IgA anti-β-2-glycoprotein I as a predictor of graft thrombosis after renal transplantation. *Transplantation*. 2017;101:597–607. doi: 10.1097/TP.0000000000001199.
21. Humar A, Johnson EM, Gillingham KJ, Sutherland DE, Payne WD, Dunn DL, Wrenshall LE, Najarian JS, Gruessner RW, Matas AJ. Venous thromboembolic complications after kidney and kidney-pancreas transplantation: a multivariate analysis. *Transplantation*. 1998;65:229–234.
22. Kazory A, Ducloux D. Acquired hypercoagulable state in renal transplant recipients. *Thromb Haemost*. 2004;91:646–654. doi: 10.1160/TH03-09-0568.
23. Brey RL, Chapman J, Levine SR, Ruiz-Irastorza G, Derksen RH, Khamashta M, Shoenfeld Y. Stroke and the antiphospholipid syndrome: consensus meeting Taormina 2002. *Lupus*. 2003;12:508–513. doi: 10.1191/0961203303lu390oa.
24. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. *Lancet*. 2010;376:1498–1509. doi: 10.1016/S0140-6736(10)60709-X.
25. Verhave JC, Tagalakis V, Suissa S, Madore F, Hébert MJ, Cardinal H. The risk of thromboembolic events in kidney transplant patients. *Kidney Int*. 2014;85:1454–1460. doi: 10.1038/ki.2013.536.
26. Remková A, Remko M. The role of renin-angiotensin system in prothrombotic state in essential hypertension. *Physiol Res*. 2010;59:13–23.
27. Chen FA, Chien CC, Chen YW, Wu YT, Lin CC. Angiotensin converting-enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers are associated with prolonged vascular access patency in uremic patients undergoing hemodialysis. *PLoS One*. 2016;11:e0166362. doi: 10.1371/journal.pone.0166362.
28. Moscarelli L, Zanazzi M, Bertoni E, Caroti L, Rosso G, Farsetti S, Annunziata F, Paudice N, Salvadori M. Renin angiotensin system blockade and activated vitamin D as a means of preventing deep vein thrombosis in renal transplant recipients. *Clin Nephrol*. 2011;75:440–450.
29. Erkan D, Harrison MJ, Levy R, Peterson M, Petri M, Sammaritano L, Unalp-Arida A, Vilela V, Yazici Y, Lockshin MD. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. *Arthritis Rheum*. 2007;56:2382–2391. doi: 10.1002/art.22663.
30. Barbhaiya M, Erkan D. Primary thrombosis prophylaxis in antiphospholipid antibody-positive patients: where do we stand? *Curr Rheumatol Rep*. 2011;13:59–69. doi: 10.1007/s11926-010-0149-3.
31. Al Marzooqi A, Leone A, Al Saleh J, Khamashta M. Current status and future prospects for the treatment of antiphospholipid syndrome. *Expert Rev Clin Immunol*. 2016;12:927–935. doi: 10.1080/1744666X.2016.1178573.
32. Belizna C. Hydroxychloroquine as an anti-thrombotic in antiphospholipid syndrome. *Autoimmun Rev*. 2015;14:358–362. doi: 10.1016/j.autrev.2014.12.006.

Capitulo 5

Autoanticuerpos y Trasplante cardiaco

La mortalidad temprana después del trasplante cardiaco se relaciona con la presencia prestrasplante de anticuerpos IgA anti-β2-glicoproteína I.

FONDO:
Los pacientes con insuficiencia renal terminal en espera de trasplante renal tienen una prevalencia de IgA anti-β2-glicoproteína I IgA anti B2GP1) cercana al 30%.La presencia de IgA anti B2GP1 se ha relacionado con la pérdida temprana del injerto después del trasplante renal.
Debido a que la β2-glicoproteína I es una proteína que fisiológicamente se elabora en riñón, corazón e hígado. Se ha hipotetizado que el proceso de elaboración de B2GP1 en un órgano enfermo conllevaría la formación de formas de la proteína con plegamiento aberrante que dejarían accesibles epítopos inmunogénicos que previamente estaban ocultos.
El objetivo de este trabajo es evaluar si los pacientes con insuficiencia cardiaca grave en espera de trasplante tienen una prevalencia elevada de estos anticuerpos y si la presencia de éstos pudiera estar relacionada con eventos vasculares y complicaciones graves en el periodo inmediato a un trasplante cardiaco.

MÉTODOS:
Se realizó un análisis de seguimiento durante dos años de 151 pacientes (consecutivos) que se sometieron a trasplante cardiaco en el Hospital 12 de Octubre entre 2004 y 2012 (ambos incluidos) para evaluar el papel de este tipo de anticuerpos preformados en la evolución del trasplante. La población se dividió en 2 grupos de acuerdo con la presencia o no de IgA anti B2GP1: el grupo 1 fue positivo para IgA anti B2GP1 (47 pacientes, 31,1%) y el grupo 2 fue negativo para IgA anti B2GP1 (104 pacientes, 68,9%).
RESULTADOS:
En los pacientes del Grupo 1 se observaron unas tasas de mortalidad temprana en los

primeros 3 meses tras el trasplante significativamente más altas (27.7%) que en el Grupo 2 (9.6%; p=0.009) . No se observaron diferencias significativas entre ambos grupos en las características del donante y del receptor o en las causas de muerte. Después de este período, no se encontró un aumento en la mortalidad o eventos trombóticos cuando se compararon los 2 grupos.

El análisis multivariable identificó la presencia de IgA anti B2GP1b, el sexo femenino y el grupo sanguineo A como factores de riesgo independientes para la mortalidad temprana después de la TH.

La incidencia de eventos trombóticos durante los primeros 3 meses post trasplante fue significativamente mayor en el Grupo 1 (23.4% vs 5.8%; p=0.002). Asimismo se observó una mayor presencia de factores de riesgo para eventos trombóticos, que podrían haberlos exacerbado.

CONCLUSIÓN:

La presencia pre-trasplante de IgA anti B2GP1 se asocia con mayores tasas de mortalidad temprana y de aparición de eventos trombóticos después del trasplante cardiaco.

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Early mortality after heart transplantation related to IgA anti–β2-glycoprotein I antibodies

Juan F. Delgado, MD, PhD,[a,b,c,d,1] Manuel Serrano, MD,[c,d,e,1] Laura Morán, MD,[a,c] Ana Belén Enguita, MD,[c,f] José Angel Martínez-Flores, PhD,[c,e] Carlos Ortiz-Bautista, MD,[a,c] Adriana Rodríguez-Chaverri, MD,[a,b,c] Inés Ponz de Antonio, MD,[a,b,c] Maria Dolores García Cosio, MD,[a,c] María José Castro Panete, PhD,[c,e] José María Cortina, MD,[c,g] and Antonio Serrano, MD, PhD[c,e]

From the [a]Heart Failure and Heart Transplantation Program, Cardiology Department, 12 de Octubre University Hospital, Madrid, Spain; [b]CIBER en enfermedades cardiovasculares; [c]I+12 Investigation Institute, 12 de Octubre University Hospital, Madrid, Spain; [d]Facultad de Medicina. Complutense University of Madrid, Madrid, Spain; [e]Immunology Department, 12 de Octubre University Hospital, Madrid, Spain; [f]Pathology Department, 12 de Octubre University Hospital, Madrid, Spain; and the [g]Cardiac Surgery Department, 12 de Octubre University Hospital, Madrid, Spain.

KEYWORDS:
anti-phospholipid syndrome;
early graft failure;
early mortality;
heart transplantation complications;
IgA anti–β_2-glycoprotein I antibodies;
primary graft failure;
thrombotic events

BACKGROUND: The presence of pre-formed IgA anti–β_2-glycoprotein I antibodies (IgA-aB2GP1ab) has been related to early graft loss after kidney transplant. Because β_2-glycoprotein I is produced in both the kidney and heart, we aimed to assess whether the presence of these antibodies may also be associated with poor outcomes after heart transplantation (HT).

METHODS: A 2-year follow-up retrospective analysis of 151 consecutive patients who underwent HT between 2004 and 2012 was performed to assess the role of this pre-formed antibody type in HT. The population was divided into 2 groups according to the presence of IgA: Group 1 was positive for IgA-aB2GP1ab (47 patients, 31.1%), and Group 2 was negative for IgA-Ab2GP1ab (104 patients, 68.9%).

RESULTS: Early mortality rates within the first 3 months were higher in Group 1 (27.7%) than in Group 2 (9.6%). No differences in donor and recipient characteristics or in causes of death were observed between groups. Multivariate analysis identified the presence of IgA-aB2GP1ab, female gender and blood type A as independent risks factors for early mortality after HT. A greater incidence of thrombotic events during the first 3 months post-HT in Group 1 (23.4% vs 5.8%) and a greater presence of risk factors for thrombotic events, which may have exacerbated them, were observed. After this period, no increase in mortality or in thrombotic events was found when the 2 groups were compared.

CONCLUSION: Pre-transplant presence of IgA-aB2GP1ab is associated with both increased early mortality rates and higher thrombotic events after HT.

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Heart transplantation (HT) is still the leading treatment for end-stage heart failure, with excellent post-HT survival. The most recent data of the Registry of the International Society for Heart and Lung Transplantation indicate a current 1-year survival rate of 84.5% and a 5-year survival

[1]J.F.D. and M.S. have contributed equally to this work.

Reprint requests: Juan F. Delgado, MD, PhD, Cardiology Department, University Hospital 12 de Octubre, Avenida de Córdoba sn, 28041 Madrid, Spain. Telephone: +346-3912-2454. Fax: +34 91 469 57 75.

E-mail address: juan.delgado@salud.madrid.org

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of 72.5%.[1] This significant improvement, compared with the 76.9% 1-year survival and 62.7% 5-year survival in the 1980s, can be attributed to the development of new surgical techniques, improved mechanical circulatory support and strategies for immunosuppression as well as in post-operative care.

Despite these advances, primary graft failure (PGF) continues to be a leading cause of death within the first 30 days post-HT, along with multiple-organ failure and infection, which account for 38.7%, 18% and 13.1% of deaths after HT, respectively.[1] The high morbidity associated with PGF and its treatment has likely been a major contributor to deaths that have otherwise been attributed to different causes, such as infection and rejection, over subsequent months.

The pathogenic mechanism of PGF is unknown. Acute ischemia–reperfusion injury with myocardial stunning has been postulated as a predominant factor in the development of PGF. However, preventive, diagnostic and therapeutic strategies focused on PGF still need to be further addressed.[2]

Due to a PGF sentinel case seen in our hospital that could remind us of a catastrophic anti-phospholipid syndrome and studies in renal transplantation showing a positive association between the presence of immunoglobulin A anti–β_2-glycoprotein I antibodies (IgA-aB2GP1ab) and early graft loss,[3] we decided to conduct a retrospective analysis to determine the prevalence of IgA-aB2GP1ab in patients with end-stage heart failure awaiting HT and its association with outcomes and cardiovascular events post-HT.

Methods

Study design

We performed a historical cohort follow-up study that included all consecutive HT patients who had undergone HT during a 9-year period (from January 1, 2004 to December 31, 2012) at our hospital. During 2014 to 2015, IgA-aB2GP1ab was retrospectively examined in pre-HT serum samples.

Primary aims

1. To determine the prevalence of IgA-aB2GP1ab in patients in end-stage heart failure who are awaiting HT and its association with cardiovascular events.
2. To determine the possible influence of preformed IgA-aB2GP1ab on early outcomes (first 3 months after transplant).

Secondary aims

1. To evaluate the prevalence of pre-transplant and early post-transplant thrombotic events in positive vs negative patients for IgA-aB2GP1ab.
2. To investigate delayed clinical outcomes (7 to 24 months) in positive vs negative patients for IgA-aB2GP1ab.

The institutional review board of our institution (CEIC15/008) approved this study.

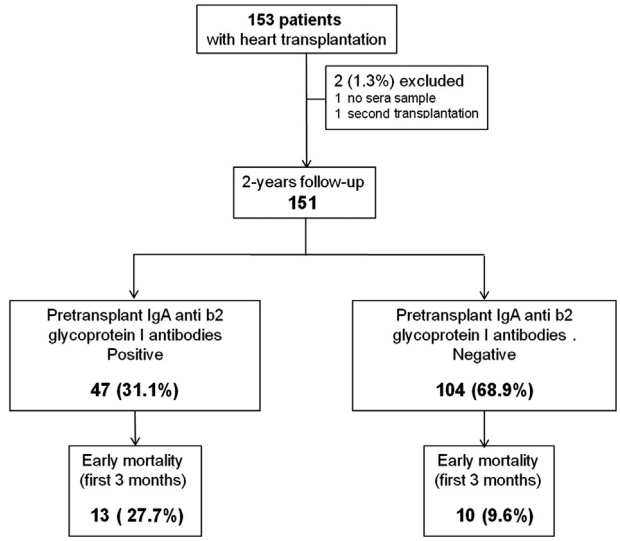

Figure 1 Algorithm of disposition and outcomes.

Patients

A total of 153 consecutive patients were included, 2 of whom were subsequently excluded: 1 was excluded due to unavailability of pre-HT serum sample and the other for being a retransplantation case in which only the second procedure was included, so a total of 151 patients were evaluated. The disposition algorithm and main outcomes are shown in Figure 1.

Database

Pre-transplant recipient characteristics, including age, original disease, blood type, body mass index, arterial hypertension, hyperlipidemia, diabetes, smoking, immunologic data and other associated diseases, were specifically recorded in our database. Data related to donor characteristics, immunosuppressive treatment, incidence of thrombotic and cardiovascular events, anti-coagulation therapy, risk factors for thrombotic events, patient survival rates and causes of death were added after surgery.

Immunosuppressive and anti-coagulant treatment

Post-operative immunosuppressive treatment consisted of 2 injections of a 20-mg intravenous (IV) bolus of basiliximab on Days 0 and 4 post-HT. Cyclosporine (CsA; 5 to 8 mg/kg/day to maintain trough levels of 250 to 350 ng/ml during the first year), mycophenolate mofetil (MMF 2 to 3 g/day) and steroids (methylprednisolone 500 mg IV before and during surgery and 125 mg intravenously every 8 hours for 3 doses after transplantation, followed by prednisone 1 mg/kg/day orally, tapered 0.1 mg/kg every other day to 0.2 mg/kg/day and reduced to 0.1 mg/kg/day within the first year post-transplant).

In accordance with their clinical situation, patients with an indication for anti-coagulation in the post-transplant period received enoxaparin 1 mg/kg subcutaneously twice daily or unfractionated heparin 80 U/kg IV bolus followed by infusion of 18 U/kg/h IV to maintain activated partial thromboplastin time (aPTT) within the therapeutic range, or acenocumarol to maintain international normalized ratio (INR) at 2 to 3.

Definitions

PGF was defined as a significant impairment of systolic graft function; severe hemodynamic compromise requiring at least 2 inotropes/vasopressors, including epinephrine or norepinephrine; or mechanical support in the first 24 hours post-HT, in the absence secondary causes.[4,5]

Thrombotic events were defined as venous or arterial thrombosis, intracavitary thrombosis, pulmonary thromboembolism, thrombotic microangiopathy, transient ischemic attack or acute stroke.

Risk factors for thrombotic events included atrial arrhythmias, mechanical support (intra-aortic balloon pump and ventricular assist device), infections or sepsis, new surgery (cardiac surgical reintervention, vascular surgery, abdominal surgery), central catheters and pacemaker leads.

Cardiovascular events included stroke, myocardial infarction, coronary revascularization, death or re-transplant.

Allocation was conducted by 2 independent researchers (M.S. and L.M.) who were blinded to the presence or absence of antibodies determinations. Discrepancies were re-evaluated by a third senior researcher (J.F.D.).

Laboratory determinations

Autoantibodies were measured in pre-transplant serum used for crossmatch. Anti-cardiolipin or anti-B2GP1 of IgG and IgM isotypes were evaluated using an immunoassay system (BioPLex 2200 Multiplex, Bio-Rad Laboratories, Hercules CA). Antibody titers were considered positive when >18 U/ml.

IgA aCL and aBGPI antibodies were quantified by enzyme-linked immunosorbent assay (ELISA; IgA-aCL and IgA-aB2GP1 QUANTA Lite, INOVA Diagnostics, Inc., San Diego, CA). Antibody titers were considered positive when >20 U/ml.[6] This cut-off was established in accordance with the assay manufacturer's guidelines and coincided with the 99th percentile of a healthy population at our hospital.[7]

Statistical methods

Results are expressed as absolute frequency, percentage or mean ± standard deviation. Association between qualitative variables was determined with Pearson's chi-square test or Fisher's exact test, as appropriate. Comparison of continuous variables was performed using the Student's *t*-test or Mann–Whitney *U*-test, as appropriate. Kaplan–Meier curves were used to calculate the patient survival probability and the differences between the distributions of survival were assessed with the log-rank test. Mortality risk was also estimated by multivariate analysis using the logistic regression model. $p < 0.05$ was considered significant. Data were processed and analyzed using MEDCALC for Windows, version 15.5 (MedCalc Software, Ostend, Belgium).

Results

Anti-cardiolipin antibodies

Mean levels of anti-phospholipid antibodies (aPL) antibodies were: IgG 1.9 ± 0.2 IU/ml; IgM 1.7 ± 0.3 IU/ml; and IgA 1.8 ± 0.2 IU/ml. Anti-B2GP1 antibodies mean levels were: IgG 2.0 ± 0.4 IU/ml; IgM 1.8 ± 0.4 IU/ml; and IgA 24.8 ± 3.1 U/ml (Figure 2).

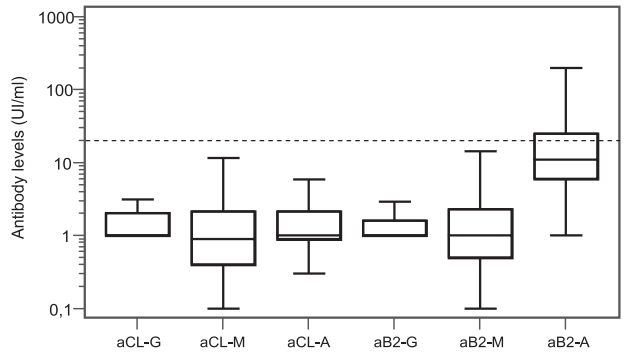

Figure 2 Anti-phospholipid antibody levels in the pre-transplant sera of patients who received a heart transplant in the 2004 to 2011 period. Dotted line indicates cut-off.

Forty-seven patients (31%) had values of IgA-aB2GP1ab above the cut-off level (Group 1), whereas 104 were negative (Group 2). Positivity of other aPL antibodies was in keeping with the normal population (<2%). Non-significant differences between the groups were observed for pre-transplant characteristics (Table 1), etiology of heart dysfunction before HT and donor cause of death (refer to Tables S1 and S2 in Supplementary Material, available online at www.jhltonline.org).

Post-transplant mortality

Post-HT mortality was concentrated in the early post-transplant period (first 3 months) (Figure 3). Early mortality was significantly higher in Group 1 (27.7% vs 9.6%, $p = 0.009$). Kaplan–Meier survival analysis (Figure 3) showed significantly lower survival rates among Group 1 at both 3 months (odds ratio [OR] = 3.1; $p = 0.004$) and 24 months (OR = 2.6; $p = 0.007$).

The only significant differences found when comparing deceased patients versus those who survived in the first 3 months (Table 2) were a higher proportion of females (34.8% vs 15.6%, $p = 0.030$), blood group A (65.2% vs 37.5%; $p = 0.024$) and presence of IgA-aB2GP1ab (56.5% vs 26.6%; $p = 0.009$). No significant differences in cause of death were observed between Group 1 and Group 2 patients (Table 3), nor were differences observed in donor characteristics of deceased patients and survivors (see Table S3 in Supplementary Material online).

Those factors significantly associated with early mortality in the univariate analysis were included in a logistic regression multivariate analysis (Table 4). After adjusting for cofounding variables, presence of IgA-aB2GP1ab (OR = 3.16; 95% confidence interval [CI] 1.22 to 8.21; $p = 0.018$), blood type A (OR = 3.83; 95% CI 1.38 to 10.62; $p = 0.01$) and female gender (OR = 3.52; 95% CI 1.17 to 10.58; $p = 0.025$) were found to be independently associated with early mortality post-HT (Table 4).

Post-transplant thrombotic events

Patients in Group 1 had more thrombotic events than those in Group 2 (23.4% vs 5.8%; OR = 4.99; $p = 0.002$;

Table 1 Pre-transplant Characteristics of Patients in Group 1 and Group 2 and Main Outcomes in the First 3 Months Post-transplant

Condition	Group 1 ($N = 47$) [n (% of sample)]	Group 2 ($N = 104$) [n (% of sample)]	p
Gender (female)	12 (25.5%)	16 (15.4%)	NS
Age (years)[a]	51.2 ± 1.7	46.8 ± 1.3	NS
Death in the first 3 months	13 (27.7%)	10 (9.6%)	0.009
Death in 2 years	15 (31.9%)	14 (13.5%)	0.015
Blood type			
Group 0	20 (42.6%)	48 (46.2%)	NS
Group A	22 (46.8%)	41 (39.4%)	NS
Group B	5 (10.6%)	10 (9.6%)	NS
Group AB	0 (0%)	5 (4.8%)	NS
Rh-positive	43 (91.5%)	85 (81.7%)	NS
Causes of heart dysfunction			
Ischemic	17 (36.2%)	27 (26%)	NS
Idiopathic	13 (27.7%)	45 (43.3%)	NS
Restrictive	4 (8.5%)	5 (4.8%)	NS
Valvular	5 (10.6%)	6 (5.8%)	NS
Others	8 (17%)	21 (16.4%)	NS
Diabetes	9 (19.1%)	27 (21.1%)	NS
Renal dysfunction	7 (14.9%)	21 (20.2%)	NS
HBP antecedents	10 (21.3%)	33 (31.7%)	NS
Dyslipidemia	16 (34%)	33 (25.8%)	NS
Hyperuricemia	4 (8.5%)	14 (13.5%)	NS
Hyperbilirubinemia	9 (19.1%)	25 (24%)	NS
High levels of ALT/AST	7 (14.9%)	30 (28.8%)	NS
Previous infection	8 (17%)	15 (14.4%)	NS
Patients with thrombotic antecedents[b]	1 (2.1%)	6 (5.8%)	NS
Deep venous thrombosis	1 (2.1%)	3 (2.9%)	NS
Pulmonary embolism	1 (2.1%)	4 (3.8%)	NS
Previously anti-coagulated	28 (59.6%)	56 (53.8%)	NS
Other vascular diseases			
Peripheral vascular disease	5 (10.6%)	4 (3.8%)	NS
Paroxysmal atrial flutter	8 (17%)	26 (25%)	NS
Permanent atrial flutter	18 (38.3%)	23 (22.1%)	NS
Thrombosis AV	1 (2.1%)	8 (7.7%)	NS
Thrombophlebitis	0 (0%)	2 (1.9%)	NS
Ethnicity Caucasian	45 (95.7%)	100 (96.2%)	NS
Ethnicity other	2 (4.3%)	4 (3.8%)	NS

ALT, alanine aminotransferase; AST, aspartate transaminase; HBP, high blood pressure; NS, not significant.

[a]Data expressed as mean ± standard error of the mean.

[b]Some patients had more than one event.

Table 3). The OR of the probability that the accumulation of events in terms of time would occur was 4.47 (95% CI 1.57 to 12.72; Figure 4A).The presence of an intracavitary thrombus in 6 patients in Group 1 (12.8%) vs 0 in Group 2 was the most prevalent event ($p = 0.001$; Table 3). These thrombi were detected by transesophageal echocardiography performed according to the protocol in the second week after transplantation, before the first endomyocardial biopsy (left atrium in 3 patients, superior vena cava in 2 patients and right atrium in 1 patient).

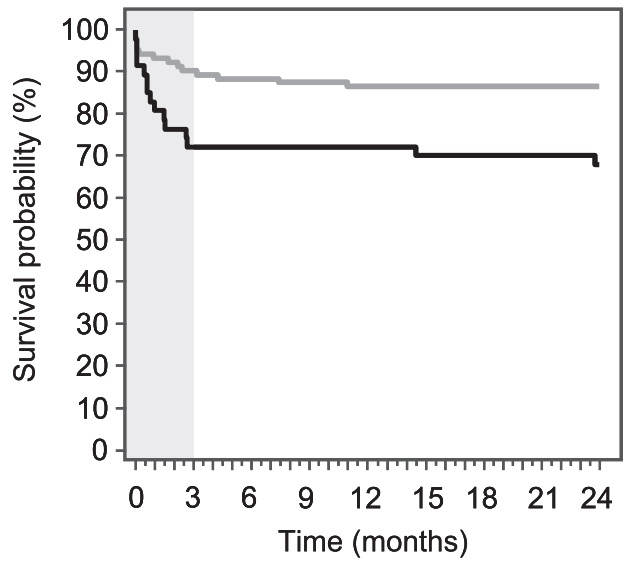

Figure 3 Survival at 2 years in patients in Group 1 (black) and in Group 2 (gray). Group 1 HR at 3 months: 3.10 (95% CI 1.27 to 7.61; $p = 0.004$); Group 1 HR at 2 years: 2.6 (95% CI 1.18 to 5.85; $p = 0.007$).

Presence of precipitating factors for the development of thrombosis (risk factors for thrombotic events) was higher in patients in Group 1 (51.1% vs 26.9%; $p = 0.007$). Post-transplant thrombosis in patients with risk factors was also higher among patients in Group 1 (40.9% vs 9.1%; $p = 0.033$). The OR for the accumulation of events was 5.38 (95% CI 1.64 to 17.63; $p = 0.015$; Figure 4B).

Post-transplant anti-coagulation

A total of 21 patients were anti-coagulated post-HT (15 preventively [6 in Group 1 and 9 in Group 2] and 6 after having a thrombotic event [5 in Group 1 and 1 in Group 2]). Mortality rate in the first trimester in patients with preventive anti-coagulation did not differ significantly from that observed in the non–anti-coagulated (total: 14.7% vs 20.0%; $p = 0.704$). Although the incidence of thrombotic events was higher in patients with preventive anti-coagulation compared with those not anti-coagulated (4 of 15 patients [26.6%] vs 13 of 136 patients [9.6%]), these differences did not reach significance (Fisher's test, $p = 0.069$).

Discussion

The main finding of our study was that IgA-aB2GPab was present in 31% of HT candidates and that it was independently associated with early mortality and thrombotic events after transplantation. To the best of our knowledge, our study is the first to describe this relationship in HT recipients.

Antiphospholipid antibodies (aPL) are a group of autoantibodies directed against epitopes on phospholipid-binding plasma proteins, phospholipids or both.[7] Antiphospholipid syndrome (APS) is a multisystemic auto-

Table 2 Characteristics of Patients Dead in the First 3 Months vs Alive Patients

Condition	Dead ($N = 23$) [n (% of sample])	Alive ($N = 128$) [n (% of sample])	p
Gender (female)	8 (34.8%)	20 (15.6%)	0.030
Age (years)[a]	50.4 2.6	47.8 1.6	NS
IgA-aB2GP1ab–positive	13 (56.5%)	34 (26.6%)	0.009
Smoking			
No smoking	11 (47.8%)	64 (50%)	NS
Ex-smoker	5 (21.7%)	36 (28.1%)	NS
Active smoker	7 (30.4%)	28 (21.9%)	NS
Diabetes	5 (21%)	31 (24.2%)	NS
Renal dysfunction	5 (21.7%)	23 (18%)	NS
Dyslipidemia	8 (34.8%)	41 (32.3%)	NS
Hyperuricemia	1 (4.3%)	17 (13.3%)	NS
Hyperbilirubinemia	9 (39.1%)	25 (19.5%)	NS
ALT/AST high levels	3 (13%)	34 (26.6%)	NS
HBP antecedents	5 (21.7%)	38 (29.7%)	NS
Mechanical ventilation	6 (26.1%)	16 (12.5%)	NS
Previous infection	5 (22.7%)	18 (14.1%)	NS
Thrombotic antecedents[b]	1 (4.3%)	6 (4.7%)	NS
Pulmonary embolism	1 (4.3%)	4 (3.1%)	NS
Deep venous thrombosis	1 (4.3%)	3 (2.3%)	NS
Previously anti-coagulated	15 (65.2%)	69 (53.9%)	NS
Other vascular diseases			
Peripheral vascular disease	3 (13%)	6 (4.7%)	NS
Paroxysmal atrial flutter	7 (30.4%)	27 (21.1%)	NS
Permanent atrial flutter	7 (30.4%)	34 (26.6%)	NS
Thrombosis a/V	1 (4.3%)	8 (6.3%)	NS
Thrombophlebitis	0 (0%)	2 (1.6%)	NS
Ethnicity Caucasian	22 (95.7%)	123 (96.1%)	NS
Ethnicity: other	1 (4.3%)	5 (3.9%)	NS
Blood type			
Group 0	7 (30.4%)	61 (47.7%)	NS
Group A	15 (65.2%)	48 (37.5%)	0.024
Group B	1 (4.3%)	14 (10.9%)	NS
Group AB	0 (0%)	5 (3.9%)	NS
Rh-positive	21 (91.3%)	107 (85.6%)	NS

ALT, alanine aminotransferase; AST, aspartate transaminase; a/V, arterial/venous; HBP, high blood pressure; NS, not significant.
[a]Data expressed as mean ± standard error of the mean.
[b]Some patients had more than 1 event.

immune disorder characterized by recurrent thrombosis and/or gestational morbidity and the presence of aPL.[8] The aPL having the highest association with vascular pathology is the one directed against β_2-glycoprotein I (B2GP1),[9] a protein synthesized mainly in the liver, but also in the heart and kidneys, and found in the serum and membranes of endothelial cells and platelets.[10,11]

The 2004 International Consensus Statement on the diagnosis of APS (Sidney/Sapporo Consensus) only recognized antibodies of IgG and IgM isotypes as diagnostic. However, since 2010 (Galveston criteria), IgA testing has been recommended when IgG and IgM isotypes are negative and APS is suspected.[12]

Prevalence of IgA-aB2GP1ab is found in one third of advanced heart failure cases, similar to that previously reported in chronic renal failure, hemodialysis and renal transplantation.[3] Although the immune mechanism involved in its production is unknown, we can hypothesize that development of IgA-aB2GP1ab may occur either after an antigen presentation of abnormally folded protein in the setting of mucosal infection or by cross-reaction with antibodies against epitopes detected on mucosal infections by pathogens with molecular mimicry with B2GP1.[13] Therefore, further study of this proposal is recommended.

The presence of antibodies is not sufficient to induce thrombotic events. In fact, positive IgA-aB2GP1ab patients in our study did not have more thrombotic events pre-HT than the negative patients. Patients with aPL remain asymptomatic for a long period and the development of thrombotic events only occurs if a "second hit" or a risk factor involved in innate immunity activation (such as infection, inflammation or surgery) triggers the process.[14] Post-transplant thrombosis could be considered as a latent immune process reactivated by surgery, rather than just a surgical complication.[15]

The exact mechanisms of IgA-aB2GP1ab–mediated thrombosis are unknown. Inhibition of B2GP1 anti-coagulant activity has been suggested as a pathogenic

Table 3 Mortality and Post-transplant Thrombotic Events in the First Trimester in Patients in Group 1 vs Group 2

Condition	Total ($N = 151$) [n (% of sample)]	Group 1 (N = 47) [n (% of sample)]	Group 2 ($N = 104$) [n (% of sample)]	p	OR	95% CI OR
Early outcomes (first 3 months)						
Patients dead	23 (15.2%)	13 (27.7%)	10 (9.6%)	0.004	3.59	1.44 to 8.96
Graft failure	9 (39%)[a]	4 (8.5%)	5 (4.8%)	NS	—	—
Hyperacute rejection.	1 (4%)[a]	0	1 (1%)	NS	—	—
Sneddon's syndrome–like	1 (4%)[a]	1 (2.1%)	0	NS	—	—
Infection	5 (22%)[a]	3 (6.4%)	2 (1.9%)	NS	—	—
Cardiacn arrest	4 (17%)[a]	3 (6.4%)	1 (1%)	NS	—	—
Cerebrovascular hemorrhage	1 (4%)[a]	1 (2.1%)	0	NS	—	—
Multiple-organ failure	2 (9%)[a]	1 (2.1%)	1 (1%)	NS	—	—
Patients with thrombotic events	17 (11.3%)	11 (23.4%)	6 (5.8%)	0.002	4.99	1.72 to 14.48
Total thrombotic events[b]	20	13	7	<0.001	5.30	1.95 to 14.38
Deep venous thrombosis	2 (1.3%)	0 (0%)	2 (1.9%)	NS	—	—
Pulmonary embolism	2 (1.3%)	1 (2.1%)	1 (1%)	NS	—	—
Intracavitary thrombus	6 (4%)	6 (12.8%)	0 (0%)	<0.001	32.7	1.8 to 594
Arterial thrombus	3 (2%)	2 (4.3%)	1 (1%)	NS	—	—
Stroke	7 (4.6%)	4 (8.5%)	3 (2.9%)	NS	—	—
Mortality in 24 months	29 (19.2%)	15 (31.9%)	14 (13.5%)	0.015	3.01	1.31 to 6.93
Mortality from Months 4 to 24	6 (4%)	2 (4.3%)	4 (3.8%)	NS	—	—

CI, confidence interval; NS, non-significant; OR, odds ratio.
[a]Cause of death percentage refers to total deaths.
[b]Several patients had more than 1 event.

mechanism.[11] The presence of IgA-aB2GP1ab is an independent risk factor for early graft thrombosis after renal transplantation. Although not all deaths could be directly attributed to thrombotic processes, the presence of this antibody may play a role in surgical complication–related deaths.[15]

In the HT patients in our study, the presence of IgA-aB2GP1ab was clearly related to early mortality and thrombotic events. Although no significant differences were found between the causes of death post-HT between patients with or without antibodies, $>50\%$ of patients positive for IgA-aB2GP1ab who died had early graft dysfunction.

As stated earlier, PGF remains a leading cause of death in the first 30 days post-HT. In addition, the high mortality level directly associated with PGF and its treatment, likely attributable to other causes over subsequent months, must also be taken into account. However, despite its clinical relevance, this complication has received little attention in the literature compared with other common causes of death. This is probably due to the complexity of its study, which involves multifactorial conditions at the time of HT, the difficulty of accurately assessing graft function in the operative setting, and the lack of a clear-cut graft failure definition.[4] The pathophysiology of PGF in this setting is poorly understood but probably involves the concerted action of multiple-donor-, procedure- and recipient-dependent mechanisms. Although acute ischemia–reperfusion injury with myocardial stunning has been postulated to be a major factor in the development of PGF, the exact pathogenic mechanism is still unknown.

The higher incidence of thrombotic events in the presence of IgA-aB2GP1ab in our study patients suggests the tissue damage that initiates the events leading to death of the patient could be microvascular thrombosis. In our sentinel case, microvascular thrombosis affected the heart and other organs. However, in less severe cases, we can hypothesize a less severe form of vascular involvement, mild thrombotic microangiopathy, which may be the substrate and the origin of other multiple cardiac and non-cardiac complications. In this regard, future research needs to be carried undertaken.

The presence of high titers of IgA-aB2GP1ab related to an increased risk of thrombotic events only appears in the first weeks after transplantation. In fact, after renal

Table 4 Multivariate Analysis ($p < 0.001$) of Factors Associated With Mortality in the First Trimester After Heart Transplant

	Univariate			Multivariate		
Variable	OR	95% CI	p	OR	95% CI	p
IgA-aB2GP1 antibodies	3.59	1.44 to 8.96	0.006	3.16	1.22 to 8.21	0.018
Blood group A	3.13	1.23 to 7.92	0.016	3.83	1.38 to 10.62	0.010
Gender (female)	2.88	1.08 to 7.69	0.035	3.52	1.17 to 10.58	0.025

Area under the receiver operating characteristic curve = 0.760; 95% CI 0.684 to 0.826. CI, confidence interval; IgA-aB2GP1, immunoglobulin A anti-β_2-glycoprotein I antibodies OR, odds ratio.

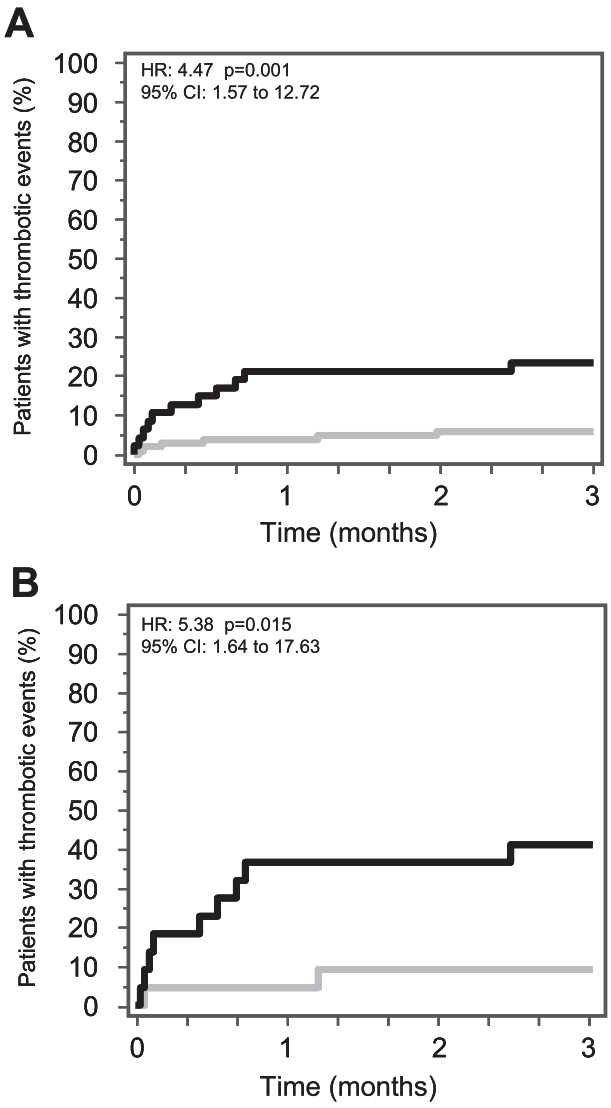

Figure 4 (A) Incidence of thrombotic events in the first 3 months in patients in Group 1 (dark line) and Group 2 (gray line). (B) Incidence of thrombotic events in patients with enablers for thrombotic activity. Group 1 (dark line) vs Group 2 (gray line).

transplantation, IgA-aB2GP1ab levels decrease, probably secondary to immunosuppressive therapy, and may even become negative, as has been observed in patients studied before a second renal transplant.[13] Thus, factors such as prophylactic anti-coagulation and progressive reduction of post-transplant antibodies in our study patients may help to explain the most favorable survival outcomes in patients positive for IgA-aB2GP1ab who survived the immediate post-HT period.

In addition to high pre-HT levels of IgA-aB2GP1ab, we found that ABO blood group A and female gender are independently associated with early mortality. The association between early mortality and presence of group A has been described previously in HT groups with a very similar population.[16] The ABO blood group system has been recognized as a thrombosis risk factor since the 1960s in relation to blood type–dependent variations of pro-coagulant factor VIII and von Willebrand factor blood levels (blood type O individuals have lower levels).[17,18] In this sense, it has been described that A, B and AB blood types are related to a higher incidence of thromboembolic events compared with type O patients.[19]

On the other hand, pathophysiologic mechanisms underlying gender-based differences throughout the spectrum of cardiovascular disease are still not completely understood.[20] The greater susceptibility to microvascular dysfunction among women could justify our result.[21]

Our study has some limitations, including those inherent in a retrospective study. Nonetheless, in our case, the key variables were collected prospectively and after a strict protocol. However, in certain cases, the allocation of clinical events in evolution was difficult. Likewise, causality between IgA-aB2GP1ab and outcome cannot be inferred, as it is only possible to obtain relationships or associations. Other limitations include enrollment limited to a single center and a largely Caucasian and male study group.

Despite these limitations, for the first time we have been able to demonstrate the presence of IgA-aB2GPab in 31% of HT candidates and their independent association with early mortality (mainly PGF) and thrombotic events.

Considering these findings, we suggest that the underlying pathogenic mechanism could be a form of APS and therefore microvascular thrombosis in a subgroup of advanced heart failure, IgA-aB2GPab–positive patients who develop PGF post-HT. If our findings are corroborated, it may be useful to recommend anti-coagulation early after HT in patients positive for IgA-aB2GPab.

Multicenter and prospective studies with a larger sample size are needed to confirm the usefulness of IgA-aB2GPab as an early transplant failure biomarker, to establish the relationship with PGF and to assess the effect of anti-coagulation in this setting.

Finally, our findings support the idea that autoimmunity is relevant post-HT, suggesting that APS could be involved in many other previously unexplored processes, including HT.

Disclosure statement

The authors have no conflicts of interest to disclose. We thank Margarita Sevilla for excellent technical assistance. This study was partially supported by the CIBER de enfermedades cardiovasculares and by the Fondo de Investigaciones Sanitarias (Grants PIE13/0045 and PI14-0360). All the grants were financed by Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, and cofinanced by the European Regional Development Fund.

Supplementary Materials

Supplementary materials can be found in the online version of this article at www.jhltonline.org/.

References

1. Yusen RD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-second official adult lung and heart–lung transplantation report—2015; Focus theme: Early graft failure. J Heart Lung Transplant 2015;34:1264-77.

2. Stehlik J, Feldman DS, Brown RN, et al. Interactions among donor characteristics influence post-transplant survival: a multi-institutional analysis. J Heart Lung Transplant 2010;29:291-8.
3. Morales JM, Martinez-Flores JA, Serrano M, et al. Association of early kidney allograft failure with preformed IgA antibodies to beta2-glycoprotein I. J Am Soc Nephrol 2015;26:735-45.
4. Cosio Carmena MD, Gomez Bueno M, Almenar L, et al. Primary graft failure after heart transplantation: characteristics in a contemporary cohort and performance of the RADIAL risk score. J Heart Lung Transplant 2013;32:1187-95.
5. Kobashigawa J, Zuckermann A, Macdonald P, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant 2014;33:327-40.
6. Mattia E, Ruffatti A, Tonello M, et al. IgA anticardiolipin and IgA anti-beta2 glycoprotein I antibody positivity determined by fluorescence enzyme immunoassay in primary antiphospholipid syndrome. Clin Chem Lab Med 2014;52:1329-33.
7. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295-306.
8. Gomez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. J Autoimmun 2014;48-49:20-5.
9. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. N Engl J Med 2013;368:1033-44.
10. Ragusa MA, Costa S, Cefalu AB, et al. RT-PCR and in situ hybridization analysis of apolipoprotein H expression in rat normal tissues. Int J Mol Med 2006;18:449-55.
11. Meroni PL, Borghi MO, Raschi E, et al. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol 2011;7:330-9.
12. Lakos G, Favaloro EJ, Harris EN, et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum 2012;64:1-10.
13. Serrano M, Martínez JA, Castro MJ, et al. Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with endstage renal disease. J Immunol Res 2014;2014:641962.
14. Harris EN, Pierangeli SS. Primary, secondary, and catastrophic antiphospholipid syndrome: what's in a name? Semin Thromb Hemost 2008;34:219-26.
15. Morales JM, Serrano M, Martinez-Flores JA, et al. The presence of pretransplant antiphospholipid antibodies IgA anti-beta-2-glycoprotein I as a predictor of graft thrombosis after renal transplantation. Transplantation 2017;101:597-607.
16. Almenar L, Vicente JL, Torregrosa S, et al. [Predictive variables of early mortality after orthotopic heart transplant in adults]. Rev Esp Cardiol 1997;50:628-34.
17. Muellner SK, Haut ER, Streiff MB, et al. ABO blood group as a potential risk factor for venous thromboembolism in acutely injured patients. Thromb Haemost 2011;105:5-13.
18. Dentali F, Sironi AP, Ageno W, et al. Non-O blood type is the commonest genetic risk factor for VTE: results from a meta-analysis of the literature. Semin Thromb Hemost 2012;38:535-48.
19. Vasan SK, Rostgaard K, Majeed A, et al. ABO blood group and risk of thromboembolic and arterial disease: a study of 1.5 million blood donors. Circulation 2016;133:1449-57.
20. Lew J, Sanghavi M, Ayers CR, et al. Sex-based differences in cardiometabolic biomarkers. Circulation 2017;135:544-55.
21. Bairey Merz CN, Shaw LJ, Reis SE, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part II: Gender differences in presentation, diagnosis, and outcome with regard to gender-based pathophysiology of atherosclerosis and macrovascular and microvascular coronary disease. J Am Coll Cardiol 2006;47(suppl):S21-9.

Capitulo 6

Validación de la importancia del riesgo de trombosis post trasplante en los portadores de IgA anti B2GP1 en un estudio multicéntrico prospectivo.

La presencia pretransplante de anticuerpos IgA anti-Beta 2 glicoproteína I es un predictor para la trombosis precoz del injerto después del trasplante renal en la práctica clínica. Un estudio multicéntrico y prospectivo.

FONDO:
La asociación de la presencia antes del trasplante de los autoanticuerpos IgA anti B2GP1 con la pérdida temprana del injerto, y especialmente con la pérdida del injerto debida a trombosis, descrita en los pacientes trasplantados en el Hospital 12 de octubre necesitaba una confirmación mediante un estudio multicéntrico.

METODOS:
En este trabajo se realizó un estudio observacional, multicéntrico y prospectivo a 10 años sobre 740 pacientes que recibieron un trasplante renal en el periodo 2000-2002 (tres años incluidos) en cinco hospitales del Grupo Forum Renal. Se trata de un estudio donde se comparan una cohorte de pacientes positivos para IgA anti B2GP1 (N=288) y otra de negativos para este autoanticuerpo (N=452).

RESULTADOS:
La pérdida de injerto en los 6 meses posteriores al trasplante fue mayor en el Grupo-1 (12.5 vs. 4.2% p <0.001), y la trombosis de los vasos del injerto, que se detectó solo en los tres primeros meses, continuó siendo la causa más frecuente de pérdida precoz del órgano trasplantado, especialmente en el Grupo-1 (6.9 vs. 0.4% p <0.001).

La presencia de IgA anti B2GP1 fue el más importante factor de riesgo independiente para la trombosis del injerto (Hazards ratio: 13.83; CI del 95%: 3.17-60.27, p <0.001). Además, la presencia de IgA anti B2GP1 también se comportó como el principal factor de riesgo independiente para la sufrir retraso en la funcionalidad del injerto (necrosis tubular aguda). La supervivencia del injerto a los 10 años fue menor en el grupo 1 respecto del grupo 2 (60,4% frente a 76,8%, p <0,001). La mortalidad fue significativamente mayor en el Grupo-1 (19.8% vs. 12.2%, p = 0.005).

Este estudio tiene la limitación de que todos los trasplantes proceden de donantes con muerte cerebral y no incluye ningún paciente trasplantado de donante a corazón parado, una práctica bastante común en la actualidad pero que aun no estaba desarrollada en el periodo de estudio. En adición, los receptores del estudio presentan menos complejidad que los pacientes que se trasplantan en la actualidad puesto que son más jóvenes y la proporción de hiperinmunizados y retrasplantes es baja.

CONCLUSIONES:

El estudio confirma que la presencia de IgA anti B2GP1 antes del trasplante fue el principal factor de riesgo para la trombosis del injerto y la pérdida temprana del injerto. Estas conclusiones justifican que se plantee la necesidad de un estudio prospectivo para evaluar la eficacia y la seguridad de la anticoagulación profiláctica para evitar estas graves complicaciones.

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Pretransplant IgA-Anti-Beta 2 Glycoprotein I Antibodies As a Predictor of Early Graft Thrombosis after Renal Transplantation in the Clinical Practice: A Multicenter and Prospective Study

Jose M. Morales[1,2†], Manuel Serrano[1,2†], Jose Angel Martinez-Flores[1,2], Fracisco Javier Gainza[3], Roberto Marcen[4,5], Manuel Arias[6,7], Fernando Escuin[8], Dolores Pérez[1,2], Amado Andres[1,2], Miguel Angel Martínez[1,2], Naroa Maruri[3], Eva Alvarez[3], José Luis Castañer[4,5], Marcos López-Hoyos[6,7] and Antonio Serrano[1,2]*

[1] Hospital 12 de Octubre, Nephrology Department, Healthcare Research Institute (Imas12), Madrid, Spain, [2] Hospital 12 de Octubre, Immunology Department, Healthcare Research Institute (Imas12), Madrid, Spain, [3] Nephrology Department, Hospital Universitario de Cruces, Biocruces Health Research Institute, Baracaldo, Spain, [4] Hospital Ramon y Cajal, Nephrology Department, Madrid, Spain, [5] Hospital Ramon y Cajal, Immunology Department, Madrid, Spain, [6] Hospital Marques de Valdecilla, Nephrology Department, Santander, Spain, [7] Hospital Marques de Valdecilla, Immunology Department, Santander, Spain, [8] Hospital la Paz, Nephrology Department, Madrid, Spain

Background: Graft thrombosis is a devastating complication after renal transplantation. We recently described the association of anti-beta-2-glycoprotein-I (IgA-ab2GP1) antibodies with early graft loss mainly caused by thrombosis in a monocenter study.

Methods: Multicenter prospective observational cohort study.

Setting and participants: Seven hundred forty patients from five hospitals of the Spanish Forum Renal Group transplanted from 2000 to 2002 were prospectively followed-up for 10 years.

Outcomes: Early graft loss and graft loss by thrombosis.

Measurements: The presence of IgA anti-B2GP1 antibodies in pretransplant serum was examined using the same methodology in all the patients.

Results: At transplantation, 288 patients were positive for IgA-B2GP1 (39%, Group-1) and the remaining were negative (Group-2). Graft loss at 6 months was higher in Group-1 (12.5 vs. 4.2% $p < 0.001$), vessel thrombosis being the most frequent cause of early graft loss, especially in Group-1 (6.9 vs. 0.4% $p < 0.001$). IgA-aB2GP1 was the most important independent risk factor for graft thrombosis (hazard ratio: 13.83; 95% CI: 3.17–60.27, $p < 0.001$). Furthermore, the, presence of IgA-aB2GP1 was associated with early graft loss and delayed graft function. At 10 years, survival figures were also lower in Group-1: graft survival was lower compared with Group-2 (60.4 vs. 76.8%, $p < 0.001$). Mortality was significantly higher in Group-1 (19.8 vs. 12.2%, $p = 0.005$).

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Edited by:
Olaf Penack,
Charité Universitätsmedizin
Berlin, Germany

Reviewed by:
Adriane Feijo Evangelista,
Barretos Cancer Hospital, Brazil
Katja Kotsch,
Charité Universitätsmedizin
Berlin, Germany

***Correspondence:**
Manuel Serrano
aserrano@h12o.es

[†]JM Morales and Manuel Serrano contributed equally to this work.

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Limitations: Patients were obtained during a 3-year period (1 January 2000–31 December 2002) and kidneys were only transplanted from brain-dead donors. Nowadays, the patients are older and the percentage of sensitized and retransplants is high.

Conclusion: In a prospective observational multicenter study, we were able to corroborate that pretransplant presence of IgA-aB2GP1 was the main risk factor for graft thrombosis and early graft loss. Therefore, a prospective study is needed to evaluate the efficacy and safety of prophylactic anticoagulation to avoid this severe complication.

Keywords: graft thrombosis, kidney transplant, autoimmunity, autoantibodies, antiphospholipid syndrome, antiphospholipid antibodies, B2GP1, IgA

INTRODUCTION

The introduction of modern immunosuppression, advances in the control of infections, better methodologies, and improvement in histocompatibility tests in recent decades have substantially increased short- and long-term results after renal transplantation. However, the percentage of patients who suffer graft loss in the first months posttransplant (mainly by thrombosis) has remained unchanged (5–8%) (1).

Humoral immune response to the allograft after kidney transplantation is one of the main factors responsible for the deterioration of graft function, or even graft loss. The main target of this immune attack is the donor major histocompatibility complex (alloreactivity) (2). It has been recently described that antibody-based autoimmune responses may also affect the outcome of renal transplantation (3). The main antigens associated with an autoimmune-mediated allograft response are angiotensin 1 receptor (4), endotelin 1 receptor (5), LG3 fragment of perlecan (6), and β_2 glycoprotein I (B2GP1) (7).

Antiphospholipid antibodies (aPL) are a group of autoantibodies directed against phospholipid-binding plasma proteins, including both those circulating in the blood and/or located in the plasma membrane of blood vessel cells. Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of circulating aPL associated with vessels thrombosis or adverse pregnancy outcome (8).

B2GP1 is a protein that is mainly synthesized not only in the liver but also in kidney and heart. It is localized in plasma and in the membrane of endothelial cells, the platelets being the most frequent antigen recognized by pathogenic aPL (9, 10). The prevalence of anti-B2GP1 antibodies of IgA isotype (IgA-aB2GP1) is higher in patients with chronic kidney disease than in the general population (30 vs. 1.5%) (11), and an association between these antibodies in patients undergoing hemodialysis and thrombotic events and mortality has been found (12, 13).

We recently described the association of a variety of IgA anti-beta-2 glycoprotein-I (aB2GP1) antibodies with early loss of kidney grafts, mainly from thrombosis (7). This observation was confirmed in a large historical cohort of patients transplanted over a 12-year period in a single hospital (14).

Abbreviations: OR, odds ratio; HR, hazard ratio; BMI, body mass index; PRA, panel reactive antibody score; aB2GP1, anti-beta-2 glycoprotein-I; aCL, anti-cardiolipin.

Our work has aimed to corroborate the association of IgA aB2GP1 with early graft thrombosis after renal transplantation in a prospective multicenter study.

MATERIALS AND METHODS

Study Design

We performed an observational, "non-interventional" follow-up study that included patients transplanted in five hospitals of the Spanish Forum Renal Group cohort. The original series ("Forum Renal") included all transplanted patients, without exclusions, during 2000–2002 in 14 renal transplant units in Spain ($N = 2,600$).

In our study, only patients from the five units with stored pretransplant serum samples were included, it not being possible to include the patients from the remaining centers because pretransplant serum samples were not available. Patients with hemolytic uremic syndrome, factor V Leiden, or primary APS were excluded.

A total of 740 patients who had received a kidney transplant in a 3-year period (from 01/01/2000 to 12/31/2002) were evaluated in a 10-year prospective follow-up study. Presence of aPL was examined in pretransplant serum samples used for donor–recipient crossmatch (disposition algorithm and flow diagram, **Figure 1**).

The centers and number of patients in each center included in the study were as follows: Unit-1 ($N = 269$): "Hospital 12 de Octubre" (Madrid); Unit-2 ($N = 86$): "Hospital Ramon y Cajal" (Madrid); Unit-3 ($N = 63$): "Hospital Marques de Valdecilla" (Santander); Unit-4 ($N = 282$): "Hospital de Cruces" (Bilbao); and Unit-5 ($N = 20$): "Hospital la Paz" (Madrid).

Primary Aim

The primary aim is to confirm the prevalence of pretransplant IgA-aB2GP1 antibodies and its influence on outcomes after kidney transplantation in a multicenter study. Main endpoints: (A) early graft loss and (B) graft loss by thrombosis. Secondary endpoint: delayed graft function (DGF).

Secondary Aims

The secondary aims are to perform a Replication Study comparing the results from the hospitals 2 to 5 with the results from hospital 1 that had previously described such a prevalence. Secondary endpoints: prevalence of IgA-aB2GP1 antibodies, graft and patient survival, and causes of graft loss and death.

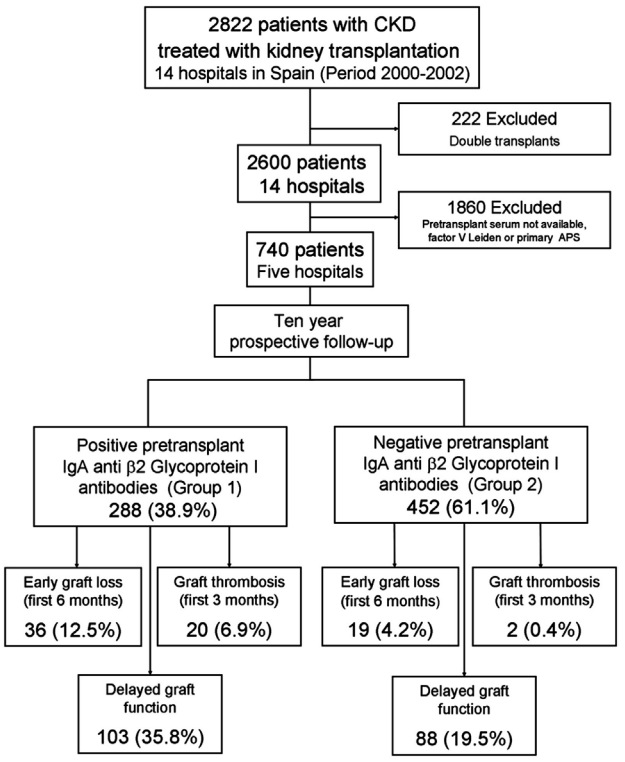

FIGURE 1 | Disposition of study and early outcomes. This cohort included 740 patients from five renal transplant units that were followed-up for 10 years.

Ethical Issues

The "Forum renal" study (prospective observational and non-interventional) was approved by all the Ethics Committees and Nephrology Departments of the 14 hospitals, assuring data confidentiality (15, 16). The patients were not asked to sign an informed consent because the Spanish legislation does not require it for observational studies without intervention. This study was approved by the ethics committee of the five hospitals, assuring data confidentiality. To control the center effect, each participating hospital was assigned a blinded code.

In addition, prior to centralizing the laboratory and database work, the study was submitted for approval to the Hospital 12 de Octubre Ethics Committee for Clinical Research and it received a favorable report (Reference Number CEIC-14/021).

Patients

Seven hundred forty patients (99.1% Caucasian) received a kidney transplant from heart-beating (brain dead) donors. Although an exhaustive coagulation study was not performed, all patients were negative for Factor V Leiden (disposition algorithm and outcomes, **Figure 1**). No patient was diagnosed of hemolytic uremic syndrome as an original disease. All of them were complement-dependent cytotoxicity crossmatch negative. All 740 patients were followed-up for a period of up to 10 years or until graft failure or death.

Database

The donor and recipient characteristics were stored in an anonymized database. The pretransplant variables included were gender, age, original disease, serology, immunological data, time on dialysis, and pretransplant conditions. In this way, hypertension, hyperlipidemia, diabetes, body mass index (BMI), smoking, and pretransplant cardiovascular disease were recorded. Immunosuppressive drug treatment was also included.

After surgery, incidence of thrombotic events, cardiovascular events, neoplasia, DGF, and acute rejection (AR) episodes were recorded. Patient and graft survival (GS), and causes of mortality and graft loss were also recorded.

Immunosuppressive Treatment

The most frequently used immunosuppressive protocol (88.5% of patients) was based on triple therapy with calcineurin inhibitors, mainly tacrolimus, associated with steroids and MMF with or without induction. In patients older than 60 years who received kidneys from donors older than 60 years, immunosuppression regimen was based on Cyclosporine (CsA), steroids, and MMF with or without induction. Immunosuppressive treatments in all the patients are described in Table S1 in Supplementary Material.

Definitions

Thrombotic Events

Thrombotic events were defined as venous thrombosis, arterial thrombosis, graft thrombosis, pulmonary thromboembolism acute stroke, or transient ischemic attack. Thrombotic episodes were diagnosed by imaging techniques or by histological study (9).

Graft Thrombosis

Graft thrombosis was diagnosed with imaging techniques. Furthermore, most of the patients who suffered graft thrombosis underwent a transplactectomy. Immediately after the surgery, the kidneys were studied in the Pathology Department, the results of macroscopic and histopathology analysis confirmed the presence of graft thrombosis.

Graft Loss

Death of the patient or loss of kidney function that requires the beginning of permanent renal replacement therapy. It is considered early graft loss if it occurs in the first 6 months after the transplant. Censored graft loss is graft loss excluding death of the patient.

Delayed Graft Function

Delayed graft function is a form of posttransplantation acute renal failure defined as a temporary graft non-function that requires hemodialysis during the first week after surgery. DFG was diagnosed once discarded hyperacute rejection, vascular complications, and urinary tract obstruction.

Arterial Hypertension

Arterial hypertension was defined as blood pressure greater than 140/90 mm Hg.

Primary Non-Function

Primary non-function was considered to exist in grafts with good perfusion that never functioned in which a biopsy study had excluded other causes of graft dysfunction as AR.

Cardiovascular Event

Cardiovascular event was considered if at least one of the following was present: heart failure, angina, coronary revascularization, myocardial infarction, stroke, or peripheral bypass.

Acute Rejection

Acute rejection was applied to acute deterioration in allograft function associated with specific histopathologic changes in the graft.

Clinically Suspected AR

Clinically suspected AR is defined as patients with AR clinical criteria lacking histological data that confirmed the diagnostic.

Panel Reactive Antibody Score (PRA)

Panel Reactive Antibody Score was defined as the percentage of the general population to which the patient reacts by preformed antibodies. PRA was studied by complement-mediated cytotoxicity using pooled lymphocyte panel with at least 35 unrelated genotypes. Patients were considered as "sensitized" with PRA values ≥50%.

Hyperlipidemia

Hyperlipidemia was defined as when hypertriglyceridemia (>150 mg/dL) or hypercholesterolemia (>200 mg/dL) were seen.

Diabetes Mellitus

Diabetes mellitus was diagnosed in patients with fasting plasma glucose greater than 126 mg/dL (7.0 mmol/L).

Normal Weight

Normal weight was defined by a BMI range from 18.5 to 25 kg/m^2. Overweight was defined when BMI is >30 kg/m^2.

Laboratory Determinations

Autoantibodies were measured in pretransplant serum used for crossmatch or in a serum sample obtained up to 15 days before transplantation. All the aPL determinations were performed in center 1.

IgA-aB2GP1 antibodies were quantified in all the centers by enzyme-linked immunosorbent assay (ELISA) using the QUANTA Lite B2 GPI IgA (INOVA Diagnostics Inc., San Diego, CA, USA). A unique assay lot was used for the analysis of the samples from centers 2, 3, 4, and 5.

The anti-cardiolipin (aCL) and aBGPI antibodies of IgG and IgM isotypes in patients from center 1 were measured with QUANTA Lite aCL IgG, QUANTA Lite B2 GPI IgG, QUANTA Lite aCL IgM, and QUANTA Lite B2 GPI IgM (INOVA Diagnostics Inc.). In patients from centers 2, 3, 4 and 5, these were measured using BioPLex 2200 multiplex immunoassay system APLS IgG and IgM (Bio-Rad, Hercules CA, USA).

Antibody levels higher than 18 U/mL were considered positive for aPL of IgG and IgM isotypes and higher than 20 U/mL were considered positive for IgA-aB2GP1. The cutoff values were those recommended by the manufacturer, which coincided with those determined in the healthy population in our country (17, 18).

Statistical Methods

Results were expressed as mean ± SE, or absolute frequency and percentage. Association between qualitative variables was determined with Pearson's Chi-square test or Fisher's exact test, when appropriate. Comparisons were performed using the Mann–Whitney *U* test in scaled variables with two categories. Probabilities less than 0.05 were considered significant.

Survival was calculated using the Kaplan–Meier Method and differences between the distributions of survival were assessed with the log-rank test.

Multivariate analyzes of graft loss and graft thrombosis-associated variables were performed using Cox regression (proportional hazards model). The relative measure of an effect was expressed as hazard ratio (HR).

Multivariate analysis of DGF (dichotomous outcome concentrated in a very short period of time) was performed using logistic regression model (19). Probabilities less than 0.05 were considered significant.

The policy regarding donor–recipient selection was based on trying to match recipients and donors with similar ages. Therefore, donor age is a recipient age-dependent variable. Donor age was not included as a statistical analysis variable except when studying DGF because it is more associated with donor age than recipient age in the literature (20).

Data were processed and analyzed using Medcalc for Windows version 16.1 (MedCalc Software, Ostend, Belgium).

RESULTS

Antiphospholipid Antibodies

The average pretransplant levels of aCL antibodies were IgM 5.4 U/mL ± 0.7 and IgG 4.0 U/mL ± 0.4. Mean levels of aB2GP1 antibodies were as follows: IgM 4.3 U/mL ± 0.8, IgG were 4.1 U/mL ± 0.5, and IgA were 32.4 U/mL ± 1.8 (Table S2 in Supplementary Material). Patients whose antibody levels were above the cutoff were considered positive. Prevalence of aCL positive patients was 1.1% for IgM and 1.2% for IgG. Prevalence of aB2GP1 antibodies patients was 1.6% for IgM and 1.2% for IgG.

Patients with IgA-aB2GP1 Antibodies

Two hundred eighty-eight (38.9%) patients were positive for IgA-aB2GP1 antibodies (Group-1) and 452 were negative (Group-2). Patients in Group-1, were immunologically less complex and there were fewer retransplanted patients (10.8 vs. 17.5%; $p = 0.017$) and less hyperimmunized patients (6.6 vs. 11.9%; $p = 0.024$). The prevalence of dyslipidemia and hypertension was slightly higher in Group-1. The remaining pretransplant characteristics did not differ between both groups (**Table 1**). The correlation between recipient age and IgA-aB2GP1 levels was very weak (Correlation coefficient $r = 0.184$, 95% CI: 0.114–0.253).

Clinical Events and Course in the Early Posttransplant Period (6 Months)

Thirty-six patients in Group-1 (12.5%) lost their graft during the first semester after transplantation vs. 19 in the Group-2 (4.2%, $p < 0.001$). At 3 months, the percentage of patients with graft

TABLE 1 | Pretransplant condition of patients in Group-1 (positive for IgA-aB2GP1 antibodies) and in Group-2 (negative patients).

Condition	Group-1 (*N* = 288)		Group-2 (*N* = 452)		*P*-value
	Patients/ mean	%/SE	Patents/ mean	%/SE	
Sex (women)	107	37.2%	198	43.8%	N.S.
Age (years)[a]	51.9	±0.8	47.4	±0.6	<0.001
Donor age (years)[a]	47.9	±1	44.2	±0.8	N.S.
Body mass index[a]	25.5	±0.3	24.9	±0.2	N.S.
Time on dialysis (months)[a]	36.5	±2.2	28.8	±2.0	N.S.
Type of dialysis					
Hemodialysis	217	75.3%	342	75.7%	N.S.
Peritoneal dialysis	58	20.1%	100	22.1%	N.S.
Both	12	4.2%	8	1.8%	N.S.
Undialyzed	1	0.3%	2	0.4%	N.S.
Panel reactive antibody score (PRA) at transplantation >50%	5	1.7%	19	4.2%	N.S.
Historical PRA >50%	19	6.6%	54	11.9%	0.024
Previous kidney transplant	31	10.8%	79	17.5%	0.017
Cold ischemia (h)[a]	19.5	±0.3	19.8	±0.3	N.S.
Associated conditions					
Diabetes mellitus	36	12.5%	41	9.1%	N.S.
Type 1 diabetes	14	4.9%	17	3.8%	N.S.
Type 2 diabetes	22	7.6%	24	5.3%	N.S.
Dyslipidemia	90	31.2%	98	21.7%	0.004
Hypertension	230	79.9%	311	68.8%	0.001
Causes CKD					
Chronic glomerulonephritis	73	25.3	137	30.3%	N.S.
Interstitial kidney disease	41	14.2%	59	13.1%	N.S.
Nephroangiosclerosis	20	6.9%	40	8.8%	N.S.
Polycystic kidney disease	47	16.3%	71	15.7%	N.S.
Diabetes mellitus	27	9.4%	29	6.4%	N.S.
Unknown	45	15.6%	67	14.8%	N.S.
Others	35	12.2%	49	10.8%	N.S.

N.S., non-significant.

[a]*Mann–Whitney test was used because variable is not normally distributed.*

TABLE 2 | Posttransplant events of patients in Group-1 (positive for IgA-aB2GP1 antibodies) and in Group-2 (negative patients).

Condition	Group-1 (*N* = 288)		Group-2 (*N* = 452)		*P*-value
	Patients/ mean	%/SE	Patents/ mean	%/SE	
Delayed graft function (DGF)	103	35.8%	88	19.5%	<0.001
Graft loss on the complete follow-up (global 29.6%)	114	39.6%	105	23.2%	<0.001
First-month (global 3.9%)	20	6.9%	9	2%	0.001
First-trimester (global 5.9%)	31	10.8%	13	2.9%	<0.001
First semester (global 7.4%)	36	12.5%	19	4.2%	<0.001
First year (global 8.5%)	38	13.2%	25	5.5%	<0.001
Causes graft loss first semester					
Acute rejection (AR)	7	2.4%	1	0.2%	0.014
Non-functioning graft	1	0.3%	6	1.3%	N.S.
Death (with a functioning kidney)	4	1.4%	4	0.9%	N.S.
Cardiovascular diseases (CVDs)	1		0		N.S.
Infections	3		0		N.S.
Sudden death	0		2		N.S.
Others	0		2		N.S.
Graft thrombosis	20	6.9%	2	0.4%	<0.001
Others	4	1.4%	6	1.3%	N.S.
Graft loss from month 7 to end of follow-up	78	27.1%	86	19%	<0.001
AR	5	1.7%	0	0%	0.019
Death (with a functioning kidney)	41	14.2%	47	10.4%	N.S.
CVDs	9		11		N.S.
Infections	8		7		N.S.
Cancer	6		10		N.S.
Sudden death	3		3		N.S.
Others	15		16		N.S.
Chronic allograft nephropathy	26	9%	32	7.1%	N.S.
Others	6	2.1%	7	1.5%	N.S.
Cardiovascular events					
Myocardial infarction	7	2.4%	18	4%	N.S.
Stroke	18	6.3%	9	2%	0.005
Angina pectoris	9	3.1%	7	1.5%	N.S.
Patients with AR episodes	28	9.7%	43	9.5%	N.S.
Death in follow-up	57	19.8%	55	12.2%	0.005
Death first semester	11	3.8%	8	1.8%	N.S.
Death from months 7 to 24	46	16%	47	10.4%	0.034

N.S., non-significant.

loss in the Group-1 was also significantly higher than in Group-2 (10.8 vs. 2.9%, $p < 0.001$) (**Table 2**). Differences between patients with early graft loss (<6 months) and remaining patients were age (55.7 ± 1.7 vs. 48.6 ± 0.5 years, $p < 0.001$), presence of DGF (50.9 vs. 23.8%, $p < 0.001$), positivity for IgA-aB2GP1 antibodies (65.5 vs. 36.8%, $p < 0.001$), and a higher proportion of patients with nephroangiosclerosis as cause of end-stage renal disease (ESRD) (20 vs. 7.2%, $p = 0.001$) (**Table 3**). As the risk of graft loss and graft thrombosis is partially dependent on the donor factors, we performed an analysis of same-donor paired kidneys (21) showing the same results (data not shown).

A Kaplan–Meier survival analysis showed significantly lower 6-month GS rates in Group-1 (HR: 3.10, 95% CI: 180–5.35, $p < 0.001$, **Figure 2A**). Graft thrombosis was the most common cause of graft loss (22 patients, 61% of losses), this occurring more frequently in Group-1 (6.9 vs. 0.4%, $p < 0.001$, **Figure 2B**).

Delayed graft function was also significantly higher in Group-1 (35.8 vs. 19.5%; $p < 0.001$). There were no differences regarding AR episodes in both groups (**Table 2**).

IgA anti-B2GP1 Antibodies Are an Independent Risk Factor for Early Graft Loss

Early graft loss-associated factors that were significant in the univariate analysis (**Table 3**) were included in a multivariate analysis [**Table 4** (A)].

Presence of IgA-aB2GP1 antibodies continued to be an independent and significant risk factor for graft loss after adjusting for other risk factors (HR: 2.49; 95% CI: 1.40–4.43, $p = 0.002$). Recipient age, presence of DGF, and nephroangiosclerosis as cause of ESRD were also independent risk factors for early graft loss [**Table 4** (A)].

TABLE 3 | Clinical characteristics of patients with early graft loss vs. patients with functioning graft at 6 months posttransplant.

Condition	Early graft loss (*N* = 55)		Functioning graft (*N* = 685)		
	N/ mean	%/SE	*N*/ mean	%/SE	*P*
Sex (women)	17	30.9%	288	42%	N.S.
Age (years)[a]	55.7	±1.7	48.6	±0.5	**<0.001**
Donor age (years)[a]	55.2	±2.2	44.9	±0.7	<0.001
Body mass index[a]	25.6	±0.6	25.1	±0.2	N.S.
Time on dialysis (months)[a]	44.9	±7.1	32.6	±1.6	N.S.
Pretransplant clinical characteristics					
Diabetes mellitus	5	9.1%	72	10.5%	N.S.
Type 1 diabetes	2	3.6%	29	4.2%	N.S.
Type 2 diabetes	3	5.5%	43	6.3%	N.S.
Dyslipidemia	18	32.7%	170	24.8%	N.S.
Hypertension	44	80%	497	72.6%	N.S.
Patients IgA-aB2GP1 positive	36	65.5%	252	36.8%	**<0.001**
Causes CKD					
Chronic glomerulonephritis	15	27.3%	195	28.5%	N.S.
Interstitial kidney disease	9	16.4%	91	13.3%	N.S.
Nephroangiosclerosis	**11**	**20%**	**49**	**7.2%**	**0.002**
Polycystic kidney disease	6	10.9%	112	16.4%	N.S.
Diabetes mellitus	3		53		N.S.
Unknown	4		108		N.S.
Others	7		77		N.S.
Transplant-associated factors					
Previous kidney transplant	13		97		N.S.
Panel reactive antibody score (PRA) at time of transplant >50%	4	7.3%	20	2.9%	N.S.
Historical PRA >50%	7		66		N.S.
Cold ischemia (h)[a]	20.7	±0.6	19.6	±0.2	N.S.
DGF	**28**	**50.9%**	**163**		**<0.001**

N.S., non-significant.
The variables that were selected for the multivariate analysis are marked in bold.
[a]*Mann–Whitney test was used because variable is not normally distributed.*

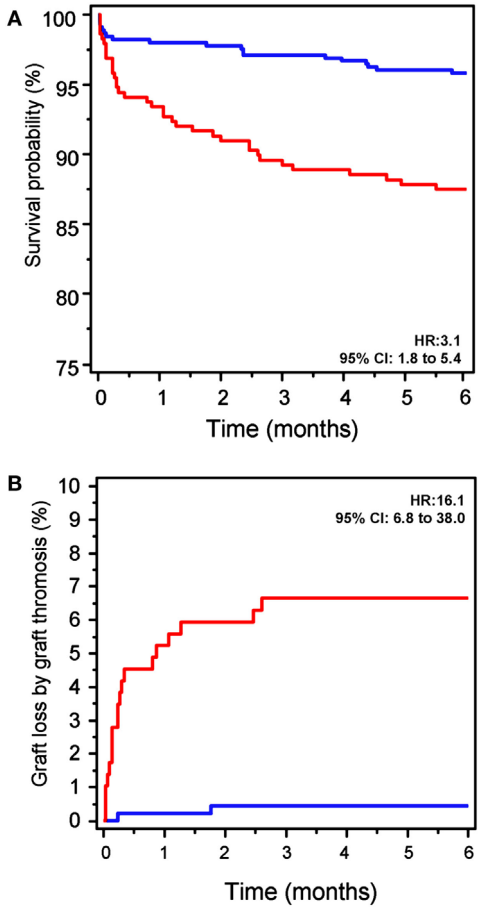

FIGURE 2 | Early graft loss. **(A)** Evolution of graft survival (GS) at the first 6 months of the follow-up. GS in patients in Group-1 (positive for IgA-aB2GP1 antibodies, red line) was significantly lower than that observed in Group-2 (blue line). HR, hazard ratio (Kaplan–Meier analysis). **(B)** Graft loss by thrombosis in the 6 months after transplantation was significantly higher in patients in Group-1 (red) than in patients in Group-2 (blue).

IgA-aB2GP1 Antibodies Are an Independent Risk Factor for Graft Thrombosis

IgA-aB2GP1, cold ischemia time, and age were identified in univariate analysis as significant and associated factors for graft thrombosis (Table S3 in Supplementary Material). In a Cox proportional regression multivariate analysis, cold ischemia time, DGF, and especially IgA-aB2GP1-ab (HR: 13.83; 95% CI: 3.17–60.27; $p < 0.001$) were identified as independent risk factors for graft thrombosis [**Table 4** (B)].

No significant differences were observed between the group I patients who suffered graft loss due to thrombosis and the remaining patients of this same group who had cardiovascular risk factors: dyslipidemia (5.6 vs. 7.6%; $p = 0.533$), hypertension (6.1% vs. 10.3%; $p = 0.255$), type 2 diabetes (9.1 vs. 6.8%; $p = 0.681$), and BMI (23.9 ± 0.8 vs. 25.6 0.3; $p = 0.128$).

IgA-aB2GP1 Antibodies Are an Independent Risk Factor for DGF

Variables previously significantly associated to DGF (Table S4 in Supplementary Material) were analyzed in a logistic regression univariate analysis. Those that continued to be significant were studied in a multivariate analysis: donor age, BMI, hypertension, and especially IgA-aB2GP1 antibodies [odds ratio (OR): 2.08, 95% CI: 1.47–2.95; $p < 0.001$] were identified as independent risk factors for DGF [**Table 4** (C)].

Late Posttransplant Period (from 7 Months to 10 Years)

Graft survival (**Figure 3**) was significantly lower (Kaplan–Meier analysis) in Group-1, both non-censored GS (HR: 1.63; 95% CI: 1.19–2.25; $p = 0.002$) and death-censored GS (HR: 1.80; 95% CI: 1.12–2.89; $p = 0.009$).

Causes of graft loss in this period were similar in both groups except for death with a functioning kidney that was more frequent in Group-1 patients (OR: 1.60; 95% CI: 1.02–2.51; $p = 0.042$).

Complete Follow-up (0–120 Months)

Non-censored GS was 70.4% at 10 years when the total group was considered. GS analysis (Kaplan–Meier) showed that graft loss

TABLE 4 | Multivariate analysis.

Factors	Univariate			Multivariate		
	Hazard ratio (HR)	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
A. Early graft loss						
Patients IgA-aB2GP1 positive	3.11	1.78–5.41	<0.001	**2.49**	**1.40–4.43**	**0.002**
Recipient age (years)	1.04	1.02–1.06	<0.001	**1.02**	**1.00–1.05**	**0.042**
Nephroangiosclerosis	3.01	1.55–5.82	0.001	**2.61**	**1.32–5.17**	**0.006**
Delayed graft function (DGF)	3.14	1.85–5.33	<0.001	**2.35**	**1.37–4.04**	**0.002**
B. Early graft loss by thrombosis						
IgA-aB2GP1 positive	16.09	3.76–68.85	<0.001	**13.83**	**3.17–60.27**	**<0.001**
DGF	4.29	1.83–10.03	<0.001	2.42	1.01–5.81	0.047
Cold ischemia time (h)	1.09	1.01–1.17	0.024	**1.09**	**1.056–3.233**	**0.041**
	Odds ratio (OR)	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
C. DGF						
IgA-aB2GP1 positive	2.30	1.65–3.22	<0.001	**2.08**	**1.47–2.95**	**<0.001**
Donor age (years)	1.02	1.01–1.03	<0.001	**1.02**	**1.01–1.03**	**0.003**
Cold ischemia (h)	1.04	1.01–1.07	0.011	1.03	1.00–1.07	0.050
Body index mass	1.07	1.04–1.11	<0.001	**1.06**	**1.02–1.10**	**0.002**
Hypertension	1.67	1.12–2.49	0.012	**1.56**	**1.03–2.37**	**0.039**
Time on dialysis (months)	1.00	1.00–1.01	0.307	–	–	–

(A) Cox proportional regression multivariate analysis (p < 0.001) of graft loss-associated variables significant in univariate analysis. (B) Cox proportional regression multivariate analysis (p < 0.001) of variables associated with graft loss by thrombosis. (C) Logistic regression multivariate analysis (p < 0.001) of DGF-associated variables significant in univariate analysis. The variables that were selected for the multivariate analysis are marked in bold.

was higher in Group-1 vs. Group-2: survival was 60.4 vs. 76.8%; HR: 1.91; 95% CI: 1.45–2.52; $p < 0.001$ (**Figure 3B**, dotted lines).

Death-censored GS (Kaplan–Meier analysis) was lower in Group-1 than Group-2: 76.1 vs. 86.5%; HR: 2.34; 95% CI: 1.61–3.39; $p < 0.001$ (**Figure 3B**, solid lines).

Global mortality in the follow-up was 15.1%, this being significantly higher in Group-1 (19.8 vs. 12.2%, $p = 0.005$; **Table 2**). Therefore, survival probability was lower in Group-1 (HR: 1.53; 95% CI: 1.07–2.18; $p = 0.015$, **Figure 4A**).

GS and Outcomes Excluding Center 1

When patients from centers 2, 3, 4, and 5 were considered alone with the exclusion of center 1, GS (**Figure 3C**) was also significantly lower in Group-1 vs. Group-2 patients, at 6 months (86.9 vs. 94.1%; HR: 2.30; 95% CI: 1.24–4.24; $p = 0.007$) and at 10 years (71.6 vs. 86.5%; HR 2.07 95% CI: 1.47–2.91; $p < 0.001$).

Notably, graft thrombosis (6 vs. 0.7%; OR: 8.66; 95% CI: 1.92–39.16; $p = 0.005$; **Figure 4B**) and DGF (39.7 vs. 22.4%; OR: 2.28; 95% CI: 1.52–3.40; $p < 0.001$) were significantly more frequent in patients in Group-1 than observed in Group-2 patients.

Remarkably, in a Cox proportional regression multivariate analysis (Table S5 in Supplementary Material), IgA-aB2GP1 antibodies continued to be an independent and significant risk factor associated with early graft-loss (HR: 1.90; 95% CI: 1.00–3.61; $p = 0.049$) and graft thrombosis (HR: 8.28; 95% CI: 1.75–39.07; $p = 0.008$). Likewise, IgA-aB2GP1 continued to be an independent risk factor for DGF in a multivariate logistic regression analysis (OR: 2.19; 95% CI: 1.43–3.37; $p < 0.001$).

Mortality was also higher on Group-1 (21.6 vs. 12.5%; $p = 0.008$; HR: 1.81; 95% CI 1.15–2.85; $p = 0.009$).

DISCUSSION

We previously described that the presence of pretransplant IgA-aB2GP1 is an independent risk factor for early graft loss in two unicenter studies (7, 14) and graft thrombosis (14) after renal transplantation. For the first time, herein, we have been able to demonstrate in an observational, multicenter, and prospective study including 740 renal transplant patients from five hospitals of Spain that preformed IgA-aB2GP1 is an independent risk factor for early graft thrombosis. Therefore, the presence of pretransplant IgA-aB2GP1 may be considered as a new tool to predict early graft loss by thrombosis after renal transplantation. In addition, these important results can be the rationale to investigate if prophylactic treatment of pretransplant IgA-aB2GP1 positive patients could improve this catastrophic early complication.

In this study we found that 55 (7.4%) of renal transplant patients suffered early loss in the first 6 posttransplant months and 22 of them (40%) due to graft thrombosis. Therefore, graft thrombosis was the main cause of early graft loss, representing 3% of the total group, a percentage almost similar to the other series (22). In addition, it is important to consider that these findings were obtained from a database representing the clinical practice in our country, including all transplanted patients from brain-death donors during 2000–2002, without exclusions. Notably, 90% of patients with graft thrombosis exhibited pretransplant IgA-aB2GP1 and consequently this parameter was the most important significant risk factor for graft thrombosis. This interesting finding was also corroborated when only centers 2, 3, 4, and 5 were analyzed. This partial analysis that excluded center 1 was done in order to demonstrate that the data from center 1 were not conditioning the final results. Therefore, the relationship between renal graft thrombosis and the presence of pretransplant IgA-aB2GP1 seems to be very clearly significant.

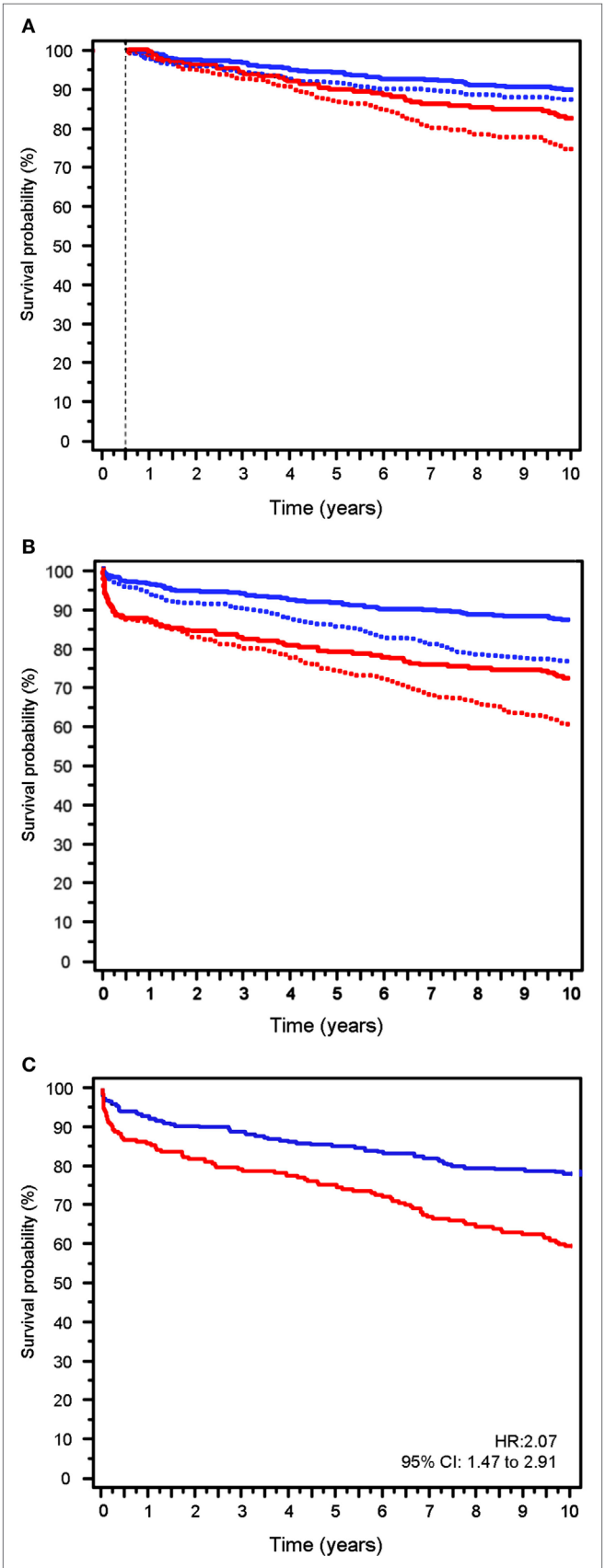

FIGURE 3 | Continued

FIGURE 3 | Graft survival (GS) on the complete follow-up. **(A)** GS from the end of the sixth month to the end of follow-up. Death-censored GS (solid line) vs. uncensored GS (dotted line). **(B)** GS during the 10 years of follow-up. Death-censored GS (solid line) vs. uncensored GS (dotted line). **(C)** Graft loss in the follow-up excluding Center 1 patients (uncensored data). Red lines patients in Group-1. Blue lines patients in Group-2.

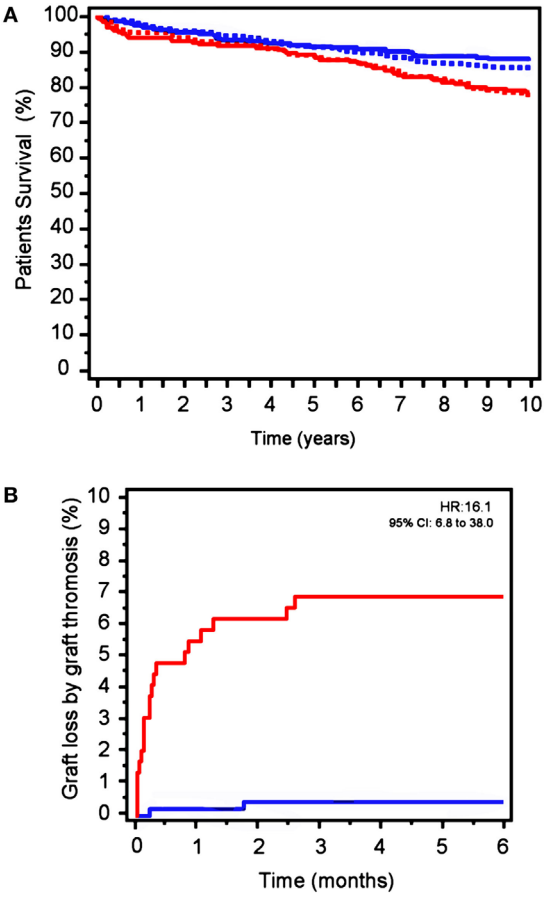

FIGURE 4 | **(A)** Mortality in patients in Group-1 (red) vs. Group-2 (blue) in the follow-up. Solid lines: excluding center 1. Dotted lines: complete study. **(B)** Graft-loss by graft thrombosis excluding center 1. Observation: the survival curves from the study that excludes center 1 and the survival curves from the complete study are so similar that they practically overlap and impede a correct visualization.

The prevalence of anti-B2GP1 IgA was significantly lower in retransplanted patients than in those receiving a first transplant. This finding confirms that observed in previous studies, that is, that the prevalence of these autoantibodies is lower in patients who have undergone immunosuppressant treatment prior to transplantation, either due to an autoimmune illness or a previous transplant (11, 14).

The prevalence of hyperlipidemia and hypertension in Group-1 patients is higher than in those in Group-2. We could hypothesize that this higher prevalence could be related to their older age; however, there is no known explanation for this finding. The presence of these factors could affect the development of vascular conditions such as arteriosclerosis. However, as the

prevalence of hyperlipidemia and hypertension does not show significant differences in the group I patients who have lost the graft due to thrombosis and the remaining group I patients who did not suffer graft loss, these factors do not seem to affect the vascular thrombosis of the graft.

Although it is known that the binding of antibodies with B2GP1 is critical to the development of events APS, the physiological functions of are unknown. Thus, the specific mechanism by which the antibodies act remains elusive (23).

Studies with electron microscopy suggest that the tridimensional structure of B2GP1 is not limited to a single conformation and it has been suggested that the geometry of the B2GP1 can alter their potential to interact with autoantibodies (24, 25).

Membrane-bound B2GP1 acquires a J-shaped structure and binding of anti-B2GP1 antibodies stabilizes the interaction of the protein with membrane phospholipids that is hypothesized to potentiate signaling through several receptors associated with prothrombotic cellular actions (26). Patients receiving a graft should undergo surgery, a known "second hit" to trigger the event. The risk of thrombotic events in carriers of IgA-aB2GP1 is higher for carriers of other thrombosis-associated risk factors such as smoking, infections, prolonged immobilization, use of estrogens, or surgical procedures (27). However, the contribution of these factors as second hits in association with IgA-aB2GP1 needs to be established in subsequent studies.

Since not all IgA-aB2GP1 positive patients develop thrombotic complications (18), the next step should be to find a marker that would identify the patients with a higher risk of thrombosis among those are IgA-aB2GP1 positive (28). We recently described that the presence of circulating immune complexes (CIC) of IgA bounded to B2GP1 was associated with occurrence of recent thrombotic events (29) and are a predictor of acute thrombotic events, including graft thrombosis after renal transplantation (30). However, we have not been able to detect the presence of CIC as, unfortunately, although the conditions of preservation of serum samples were adequate for the determination of IgA-aB2GP1, these conditions were not consistent with the maintenance of stable CIC and they had not been reliably determined in the present group of patients in some centers.

On the other hand, global results of this population at 10 years may be considered to be in agreement with other series of renal transplantation with deceased donors: patient survival 84.9%, DCGS and NCGS of 81 and 70.4%, respectively. It is important to note that our patients were closely followed-up in the renal transplant offices under the umbrella of a national health service with universal and lifelong support. These findings are in agreement with previous results in Spain (31). As we noted previously, IgAB2GP1 positive patients show lower survival figures than negative patients. Notably, the immunosuppressive protocol was based on calcineurin inhibitors and MMF. Only six patients did not receive calcineurin inhibitors as an initial immunosuppressive protocol, and therefore, we cannot discuss if m-TOR inhibitors can be useful in the prevention of vascular lesions associated with aPL (32). Furthermore, prior results have demonstrated a superior capacity of CNI over m-TOR inhibitors to inhibit alloantibodies production in renal transplantation (33, 34).

One of the main limitations is that these results were obtained during the first 3 years of the twenty-first century. Currently, donors and patients are older, show a high percentage of sensitized and retransplants as well as new forms of renal transplantation and with non-heart-beating donors. At present, and with these demographic changes, short- and long-term results could be different. At this point, such a limitation makes it necessary to design a long-term study.

Determination of IgA-aB2GPI antibodies in patients from all the centers was performed with the same diagnostic system (Quanta Lite ELISA). However, a different diagnostic system was used to quantify aPL of IgG and IgM isotype for center 1 (ELISA) than in rest of the centers (multiplex immunoassay). This change of methodology is irrelevant because the efficiency in determination of aPL of IgG and IgM isotypes using ELISA and multiplex diagnostic systems is very similar (35, 36). Furthermore, the average amount of antibody levels and the proportion of aPL of IgG and IgM isotype-positive patients was similar to those described in previous studies (7, 11).

The need to include the determination of aB2GP1 IgA in diagnostic protocols has been suggested recently. Currently, the IgA isotype is not included among the APS classification criteria of the APS, so that very few centers perform the IgA determination aB2GP1. For this reason, cases of IgA-mediated thrombosis are clearly underdiagnosed (37).

In summary, for the first time, we have been able to corroborate in a large cohort of patients from five hospitals that the presence of pretransplant IgA-aB2GP1 is an independent risk factor for graft thrombosis after renal transplantation, a devastating condition without available prevention and treatment. This finding can be the rationale for a prospective study to demonstrate if prophylactic anticoagulation can be useful to improve this early complication.

ETHICS STATEMENT

The study was submitted to the Ethics Committee for Clinical Research (ECCR) of Hospital "12 de Octubre" and received a favorable report (Reference Number CEIC-14/021).

AUTHOR CONTRIBUTIONS

AS and JM conceived the project, designed the research, discussed the results, and wrote the manuscript. AS, MS, and JM-F performed the antiphospholipid determinations and were responsible for the database and the statistical analysis. JM, JG, MA, RM, FE, AA, NM, and EA were responsible for the patients' care and clinical data collection. AS, MS, JM-F, JC, and ML-H were responsible for coordination of the Organ Transplant Waiting List Serum Bank. MM evaluated the histopathology. All authors contributed to the data interpretation, reviewed the manuscript, and agreed with the final version.

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REFERENCES

1. Hamed MO, Chen Y, Pasea L, Watson CJ, Torpey N, Bradley JA, et al. Early graft loss after kidney transplantation: risk factors and consequences. *Am J Transplant* (2015) 15(6):1632–43. doi:10.1111/ajt.13162
2. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* (1969) 280(14):735–9. doi:10.1056/NEJM196904032801401
3. Sigdel TK, Sarwal MM. Moving beyond HLA: a review of nHLA antibodies in organ transplantation. *Hum Immunol* (2013) 74(11):1486–90. doi:10.1016/j.humimm.2013.07.001
4. Giral M, Foucher Y, Dufay A, Van Huyen JP, Renaudin K, Moreau A, et al. Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. *Am J Transplant* (2013) 13(10):2567–76. doi:10.1111/ajt.12397
5. Dragun D, Catar R, Philippe A. Non-HLA antibodies in solid organ transplantation: recent concepts and clinical relevance. *Curr Opin Organ Transplant* (2013) 18(4):430–5. doi:10.1097/MOT.0b013e3283636e55
6. Soulez M, Pilon EA, Dieude M, Cardinal H, Brassard N, Qi S, et al. The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection. *Circ Res* (2012) 110(1):94–104. doi:10.1161/CIRCRESAHA.111.250431
7. Morales JM, Martinez-Flores JA, Serrano M, Castro MJ, Alfaro FJ, Garcia F, et al. Association of early kidney allograft failure with preformed IgA antibodies to beta2-glycoprotein I. *J Am Soc Nephrol* (2015) 26(3):735–45. doi:10.1681/ASN.2014030228
8. Gomez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun* (2014) 4(8–49):20–5. doi:10.1016/j.jaut.2014.01.006
9. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* (2006) 4(2):295–306. doi:10.1111/j.1538-7836.2006.01753.x
10. Klaerke DA, Rojkjaer R, Christensen L, Schousboe I. Identification of beta2-glycoprotein I as a membrane-associated protein in kidney: purification by calmodulin affinity chromatography. *Biochim Biophys Acta* (1997) 1339(2):203–16. doi:10.1016/S0167-4838(96)00233-6
11. Serrano M, Martinez-Flores JA, Castro MJ, Garcia F, Lora D, Perez D, et al. Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with endstage renal disease. *J Immunol Res* (2014) 2014:641962. doi:10.1155/2014/641962
12. Serrano A, Garcia F, Serrano M, Ramirez E, Alfaro FJ, Lora D, et al. IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. *Kidney Int* (2012) 81(12):1239–44. doi:10.1038/ki.2011.477
13. Hadhri S, Rejeb MB, Belarbia A, Achour A, Skouri H. Hemodialysis duration, human platelet antigen HPA-3 and IgA Isotype of anti-beta2glycoprotein I antibodies are associated with native arteriovenous fistula failure in Tunisian hemodialysis patients. *Thromb Res* (2013) 131(5):e202–9. doi:10.1016/j.thromres.2013.03.003
14. Morales JM, Serrano M, Martinez-Flores JA, Perez D, Castro MJ, Sanchez E, et al. The presence of pretransplant antiphospholipid antibodies IgA anti-beta-2-glycoprotein I as a predictor of graft thrombosis after renal transplantation. *Transplantation* (2017) 101(3):597–607. doi:10.1097/TP.0000000000001199
15. Morales JM, Marcen R, del Castillo D, Andres A, Gonzalez-Molina M, Oppenheimer F, et al. Risk factors for graft loss and mortality after renal transplantation according to recipient age: a prospective multicentre study. *Nephrol Dial Transplant* (2012) 27(Suppl 4):iv39–46. doi:10.1093/ndt/gfs544
16. Morales JM, Marcen R, Andres A, Molina MG, Castillo DD, Cabello M, et al. Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. *Kidney Int Suppl* (2008) 74(111):S94–9. doi:10.1038/ki.2008.547
17. Ruiz-Garcia R, Serrano M, Angel Martinez-Flores J, Mora S, Morillas L, Martin-Mola MA, et al. Isolated IgA anti-beta 2 glycoprotein I antibodies in patients with clinical criteria for antiphospholipid syndrome. *J Immunol Res* (2014) 2014:704395. doi:10.1155/2014/704395
18. Tortosa C, Cabrera-Marante O, Serrano M, Martinez-Flores JA, Perez D, Lora D, et al. Incidence of thromboembolic events in asymptomatic carriers of IgA anti ss2 glycoprotein-I antibodies. *PLoS One* (2017) 12(7):e0178889. doi:10.1371/journal.pone.0178889
19. Annesi I, Moreau T, Lellouch J. Efficiency of the logistic regression and Cox proportional hazards models in longitudinal studies. *Stat Med* (1989) 8(12):1515–21. doi:10.1002/sim.4780081211
20. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* (2004) 364(9447):1814–27. doi:10.1016/S0140-6736(04)17406-0
21. Louvar DW, Li N, Snyder J, Peng Y, Kasiske BL, Israni AK. "Nature versus nurture" study of deceased-donor pairs in kidney transplantation. *J Am Soc Nephrol* (2009) 20(6):1351–8. doi:10.1681/ASN.2008070715
22. Ponticelli C, Moia M, Montagnino G. Renal allograft thrombosis. *Nephrol Dial Transplant* (2009) 24(5):1388–93. doi:10.1093/ndt/gfp003
23. Brusch A. The significance of anti-beta-2-glycoprotein I antibodies in antiphospholipid syndrome. *Antibodies* (2016) 5(2):16. doi:10.3390/antib5020016
24. Ninivaggi M, Kelchtermans H, Lindhout T, de Laat B. Conformation of beta-2glycoprotein I and its effect on coagulation. *Thromb Res* (2012) 130(Suppl 1):S33–6. doi:10.1016/j.thromres.2012.08.269
25. Agar C, van Os GM, Morgelin M, Sprenger RR, Marquart JA, Urbanus RT, et al. Beta2-glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. *Blood* (2010) 116(8):1336–43. doi:10.1182/blood-2009-12-260976
26. de Groot PG, Meijers JC. beta(2)-Glycoprotein I: evolution, structure and function. *J Thromb Haemost* (2011) 9(7):1275–84. doi:10.1111/j.1538-7836.2011.04327.x
27. Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, et al. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. *Blood* (2005) 106(7):2340–6. doi:10.1182/blood-2005-03-1319
28. Martínez-Flores J, Serrano M, Morales J, Serrano A. Antiphospholipid syndrome and kidney involvement: new insights. *Antibodies* (2016) 5(3):17. doi:10.3390/antib5030017
29. Martinez-Flores JA, Serrano M, Perez D, Camara AG, Lora D, Morillas L, et al. Circulating immune complexes of IgA bound to beta 2 glycoprotein are strongly associated with the occurrence of acute thrombotic events. *J Atheroscler Thromb* (2016) 23(10):1242–53. doi:10.5551/jat.34488
30. Serrano M, Martinez-Flores JA, Perez D, Garcia F, Cabrera O, Pleguezuelo D, et al. Beta2-glycoprotein I/IgA immune complexes: a marker to predict thrombosis after renal transplantation in patients with antiphospholipid antibodies. *Circulation* (2017) 135(20):1922–34. doi:10.1161/CIRCULATIONAHA.116.025992
31. Ojo AO, Morales JM, Gonzalez-Molina M, Steffick DE, Luan FL, Merion RM, et al. Comparison of the long-term outcomes of kidney transplantation: USA versus Spain. *Nephrol Dial Transplant* (2013) 28(1):213–20. doi:10.1093/ndt/gfs287
32. Canaud G, Bienaime F, Tabarin F, Bataillon G, Seilhean D, Noel LH, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N Engl J Med* (2014) 371(4):303–12. doi:10.1056/NEJMoa1312890
33. Ruiz San Millan JC, Lopez-Hoyos M, Segundo DS, Quintela E, Rodrigo E, Gomez-Alamillo C, et al. Predictive factors of allosensitization in renal transplant patients switched from calcineurin to mTOR inhibitors. *Transpl Int* (2014) 27(8):847–56. doi:10.1111/tri.12334
34. Liefeldt L, Brakemeier S, Glander P, Waiser J, Lachmann N, Schonemann C, et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. *Am J Transplant* (2012) 12(5):1192–8. doi:10.1111/j.1600-6143.2011.03961.x

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00468/full#supplementary-material.

35. Perez D, Martinez-Flores JA, Serrano M, Lora D, Paz-Artal E, Morales JM, et al. Evaluation of three fully automated immunoassay systems for detection of IgA anti-beta 2-glycoprotein I antibodies. *Int J Lab Hematol* (2016) 38(5):560–8. doi:10.1111/ijlh.12543
36. Forastiero R, Papalardo E, Morin K, Quirbach C, Lakos G, Mattias K, et al. Comparative evaluation of different immunoassays for the detection of antiphospholipid antibodies: report of a wet workshop during the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum* (2010) 62(Suppl 10):2252. doi:10.1002/art.30166
37. Perez D, Tincani A, Serrano M, Shoenfeld Y, Serrano A. Antiphospholipid syndrome and IgA anti-beta2-glycoprotein I antibodies: when Cinderella becomes a princess. *Lupus* (2017) 27(2):177–8. doi:10.1177/0961203317738227

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DISCUSIÓN

Ya se conoce que los anticuerpos aB2GP1 de isotipo IgA son los más prevalentes en nuestra población con clínica de insuficiencia renal crónica (ERC) y en aquellos con APS no asociado con enfermedades autoinmunes concomitantes (PAPS)(108, 112). Sin embargo, no se conoce el origen de la presencia de los anticuerpos, sigue siendo desconocido.

Nuestro estudio de análisis de los polimorfismos relacionados con la aparición de anticuerpos antifosfolipídicos es el primer análisis de factores genéticos predisponentes para desarrollar anticuerpos IgA aB2GP1 en pacientes con enfermedad renal crónica (ERC). Los anticuerpos isotipo IgA son los anticuerpos antifosfolipídicos más comunes en pacientes con ERC tratados con hemodiálisis, además se asocian con mayor incidencia de eventos de origen cardiovascular, así como mayor mortalidad (108). No obstante, los pacientes tratados con diálisis peritoneal y los pacientes en prediálisis, muestran una prevalencia similar a la de los pacientes en hemodiálisis, excluyendo la posibilidad de que el origen de los anticuerpos sea un cambio de conformación de la proteína como consecuencia de la membrana de hemodiálisis (126).

En un estudio del origen de los aPL, se describió una predisposición genética para desarrollar anticuerpos aB2GP1 en aquellos sujetos que expresan el polimorfismo Val / Val 247 (127).

En 1999, un estudio de pacientes asiáticos mostró que la presencia del alelo Valina y el genotipo Val / Val 247 tuvo una asociación significativa con la presencia de anticuerpos aB2GP1 (128).

Los datos mostraron que el alelo valina está relacionado con la generación de anticuerpos aB2GP1 en pacientes asiáticos con SAD-APS y en pacientes caucásicos con PAPS. En la población mexicana mixta, la presencia de anticuerpos contra B2GP1 está asociada con el genotipo Leu / Leu. La expresión del polimorfismo 316 genera una protección para el desarrollo de estos anticuerpos (82, 91).

La unión del B2GP1 a los fosfolípidos aniónicos es esencial para la unión de los anticuerpos aCL puesto que se produce un cambio de conformación, que provoca la exposición de epítopos crípticos; por esta razón, la variación genética del B2GP1 que imposibilita la unión de los fosfolípidos con la proteína, afectando a la unión de anticuerpos

aCL. Este fenómeno fue examinado en 222 pacientes con lupus eritematoso sistémico, obteniéndose una distribución del polimorfismo Trp / Ser 316 con diferencias significativas entre los pacientes positivos y negativos para aCL. Esto probablemente sugiere una protección a la producción de aCL por el alelo mutado en la posición 316. Por el contrario, la presencia de aB2GP1 no se asoció con ningún polimorfismo en los pacientes con APS (91).

En nuestra población, no encontramos diferencias entre los pacientes positivos y negativos para IgA aB2GP1 y los polimorfismos estudiados. A la vista de los resultados en la búsqueda del origen de estos anticuerpos en la población con ERC, podemos poner en duda la influencia genética. Existe la hipótesis de que la membrana de hemodiálisis promueve un cambio conformacional en la proteína, exponiendo el epítopo crítico en la conformación fisiológica, sin embargo nuestro grupo en un trabajo previo ya demostró que no hay diferencias entre hemodiálisis, diálisis peritoneal y prediálisis (107).Por ello, podría hipotetizarse que la presencia de ciertos patógenos, por la teoría del mimetismo molecular la existencia de similitudes con B2GP1 podría inducir la aparición de autoanticuerpos anti fosfolipídicos (129).

Por lo tanto, podría ser interesante investigar si el origen de los anticuerpos IgA aB2GP1 es secundario a factores ambientales, como las infecciones.

En el estudio unicéntrico con 1375 pacientes trasplantados a lo largo de 12 años (capitulo 2) (2000-2011) demostramos por primera vez que la presencia de anticuerpos IgA aB2GP1 pretrasplante es un factor de riesgo independiente para la pérdida precoz del injerto por trombosis de éste. Además, si dividimos el período en 4 cuatrienios, se sigue manteniendo como factor de riesgo a pesar de los cambios acontecidos durante estos doce años, tanto en las características de los donantes y los receptores por criterios de expansión para el trasplante, como en los avances de las técnicas quirúrgicas. La supervivencia del injerto y del paciente a los seis meses fue significativamente menor en pacientes con IgA aB2GP1 pretrasplante en comparación con los que tenían anticuerpos negativos. Si bien es cierto que en estudios previos nuestro grupo ya describió que la presencia de IgA aB2GP1 preformada es un factor de riesgo independiente para la pérdida de injerto, especialmente por trombosis en el primer período después del trasplante (110).Sin embargo no pudimos demostrarlo estadísticamente debido a que la muestra era demasiado pequeña y el número de eventos no era suficiente para hacer un análisis multivariable. En este trabajo, hemos corroborado este hallazgo previo y demostramos claramente la presencia de anticuerpos IgA aB2GP1 pretrasplante es un factor de riesgo independiente para la trombosis temprana del injerto. Por lo tanto, podría

considerarse un fuerte predictor de trombosis del injerto y, en consecuencia, la pérdida precoz de éste y la mortalidad después del trasplante renal.

En la actualidad, a medida que la inmunosupresión se ha vuelto más efectiva, la trombosis del injerto vascular se ha ido convirtiendo en la causa más importante de pérdida precoz del injerto (130). Varios factores pueden estar involucrados en la patogénesis de la trombosis del injerto, como problemas derivados de la cirugía y errores técnicos, (131) compresión de la arteria renal o vena por colecciones de líquidos, y la hipercoagulabilidad postoperatoria (132). En nuestro estudio, la trombosis representó el 56.3% de la pérdida temprana del injerto después del trasplante, resultados similares a los reportados previamente (130, 133, 134).

La presencia de IgA aB2GP1 fue el principal factor de riesgo independiente para la pérdida de injerto inducida por trombosis, solo seguido de la diabetes mellitus tipo 2. Los pacientes mayores que recibieron riñones de donantes más viejos tenían una mayor prevalencia de pérdida de injerto y trombosis de vasos en comparación con el trasplante renal de cadáveres estándar (135).

Por otro lado, los pacientes con diabetes mellitus tipo 2 presentan un estado de hipercoagulabilidad (136) que aumenta el riesgo de trombosis del vaso (137).

Es bien sabido que la presencia de aPL es insuficiente para inducir la formación de trombosis, los pacientes con aPL durante largos períodos de tiempo necesitan un "segundo golpe" que involucre la activación de la inmunidad innata y un microambiente proinflamatorio para desencadenar episodios trombóticos (20, 138). La cirugía es un factor de riesgo importante para la trombosis(130), principalmente en pacientes urémicos con aterosclerosis grave. También puede ser un segundo desencadenante de la trombosis (117) en pacientes positivos para IgA aB2GP1 en el contexto del trasplante renal. Por lo tanto, la trombosis del injerto se puede considerar más como un proceso inmune latente reactivado por la cirugía que como una verdadera complicación quirúrgica. El papel potencial del "segundo golpe" de los factores del peritransplante, como los agentes infecciosos, el estado inflamatorio, los tratamientos inmunosupresores de acondicionamiento y otros factores no identificados (139) requieren más investigación.

Se desconoce por qué los pacientes con ERC muestran una prevalencia elevada de IgA aB2GP1 y cómo se genera su respuesta inmune. Se ha propuesto que el riñón en insuficiencia terminal produciría B2GP1 (140) plegada incorrectamente exponiendo epítopos detectados en infecciones de la mucosa por patógenos con mimetismo molecular, pudiendo reaccionar de forma cruzada con anticuerpos dirigidos frente a la B2GP1 (126).

Se deberían hacer nuevos estudios para confirmar esta hipótesis.
El pilar del tratamiento preventivo del APS trombótico es la profilaxis antitrombótica, generalmente con anticoagulación a largo plazo con antagonistas de la vitamina K (AVK) (141). El uso de AVK es especialmente difícil en pacientes trasplantados porque aumenta el riesgo de complicaciones hemorrágicas y, por lo tanto, debe utilizarse solo en los seleccionados (142).

Por este motivo, se deben investigar biomarcadores adicionales que puedan identificar entre los pacientes positivos para IgA aB2GP1 con mayor riesgo de trombosis.
Recientemente, el eculizumab se ha utilizado en casos aislados de APS catastrófico (143). Es posible que el eculizumab no sea útil en esta forma especial de APS porque el complemento no parece estar activado por la IgA (144). Sin embargo, para aclarar este punto, se requiere más investigación.

Recientemente se ha descrito que la vía mTOR está implicada en lesiones vasculares asociadas con el APS (145).De esta forma, la inmunosupresión inicial basada en inhibidores mTOR en pacientes con trasplante renal positivos para IgA aB2GP1 podría ser una alternativa al uso de AVK para prevenir eventos trombóticos. Se necesitan nuevos estudios prospectivos para confirmar la utilidad potencial de estos medicamentos. Un hallazgo importante de nuestro estudio es que la presencia de IgA aB2GP1 pretrasplante se asocia con una alta mortalidad temprana en el grupo global. En el análisis multivariable de Cox del período completo, solo encontramos que la edad es un factor de riesgo independiente. El valor de p de IgA aB2GP1 ($p = 0,060$) es insuficiente para considerarlo un factor de riesgo independiente. Sin embargo, cuando el análisis multivariable solo se realizó con el tercer cuatrienio (período con mayor mortalidad), la diabetes tipo 2 y el IgA aB2GP1 fueron factores de riesgo de muerte independientes. Este aspecto debe estudiarse más a fondo con un mayor número de pacientes.
Anteriormente describimos la relación entre función retardada del injerto (FRI) e IgA aB2GP1 (110). En los últimos años, se ha observado un aumento de los pacientes con FRI debido al aumento del trasplante renal de donante de cadáver en asistolia (146) y el trasplante de donante añoso para receptor añoso (“old for old”) (147).

En este estudio, el multivariable, el análisis ha identificado la existencia de un trasplante previo, la donación en asistolia, el tiempo de diálisis y la presencia de IgA aB2GP1 como factores de riesgo para FRI, de modo que podamos corroborar esta importante asociación. Podemos hipotetizar que algunos pacientes con FRI pueden tener compromiso vascular leve posterior al trasplante, equivalente a una microangiopatía leve reversible que podría estar relacionado con IgA aB2GP1.

Por lo tanto, la investigación futura en este área importante es obligatoria. Las limitaciones más importantes de nuestro estudio son que solo se incluyó nuestro centro, y que para el estudio pretrasplante de trombofilias, sólo se analizó el factor V Leiden debido a la naturaleza del presente estudio. No se realizaron pruebas adicionales como mutaciones de protrombina, resistencia a la proteína C activada, antitrombina, proteína C y actividad de la proteína S. Sin embargo, debe tenerse en cuenta que todos los pacientes trasplantados fueron reclutados sin limitaciones, lo que representa la práctica clínica real. Los factores estudiados, como la mortalidad y la pérdida de injerto, son objetivos y no dependen de ningún tipo de interpretación subjetiva.

Si se corrobora este hallazgo, el tratamiento profiláctico pretrasplante de los pacientes positivos para IgA B2GP1 podría mejorar esta complicación temprana. Además, puede ser un avance sobresaliente no solo evitar la pérdida del injerto sino también que el Servicio Nacional de Salud disminuya el presupuesto económico anual. Para confirmar el valor de IgA aB2GP1 como un biomarcador para pérdida precoz del injerto, es indispensable hacer nuevos estudios multicéntricos prospectivos.

Por lo tanto, podríamos estar describiendo una nueva forma de APS basada en el isotipo IgA, secundaria a ERC y diferente a la enfermedad renal secundaria al síndrome clásico de APS (IgG e IgM) (148, 149). Finalmente, este trabajo apoya la idea de que la autoinmunidad es relevante después del trasplante renal y también sugiere que APS, como propuso Grahams Hughes (quien describió por primera vez el APS), podría estar involucrado en muchos otros procesos inexplorados antes, como el trasplante renal (10).

En el trabajo detallado en el capitulo 3, hemos descrito por primera vez una alta prevalencia de inmuno complejos de IgA aB2GP1 unidos a B2GP1 (B2A-CIC) en pacientes con trombosis reciente y positivos aislados para anticuerpos IgA aB2GP1 en comparación con pacientes que presentaron trombosis antiguas y aquellos sin antecedentes trombóticos. Los anticuerpos IgA aB2GP1 son directamente trombogénicos pero se desconocen los mecanismos de la trombosis mediada por anticuerpos (113).

Aunque la presencia de anticuerpos a2GPI es una condición necesaria, solo un pequeño grupo de pacientes con anticuerpos positivos desarrollan complicaciones trombóticas. Se ha propuesto que la presencia de anticuerpos generaría un microambiente protrombótico, sin embargo, para que se formen trombos, se requieren factores protrombóticos adicionales ("segundo golpe"), que están relacionados con la respuesta inmune, aunque aún no se conocen bien (117). Por lo tanto, como se propone en estudios recientes, es necesario buscar nuevos marcadores que permitan la detección de pacientes realmente en riesgo de sufrir un evento trombótico (118).

La determinación de aCL detecta principalmente anticuerpos dependientes de B2GPI; sin embargo, nuestros pacientes presentan una positividad aislada de IgA para B2GPI (negativo para IgA aCL). Existen varios estudios que demuestran que la positividad de IgA aCL e IgA aB2GP1 son independientes (112, 113, 150).

Los epítopos reconocidos por IgA aB2GP1 se localizan principalmente en los dominios 4-5 de la proteína B2GPI, siendo esta región el área de unión de fosfolípidos . Cuando la cardiolipina se incorpora a B2GPI, los epítopos de unión a IgA no son accesibles y los pacientes solo presentan anticuerpos IgA aB2GP1 aislados (151, 152).

Hemos encontrado que los pacientes con trombosis aguda (grupo 1), tiene una mayor prevalencia de B2A-CIC positivos y niveles más altos de B2A-CIC que en pacientes con trombosis antiguas y para el grupo control. Tanto la prevalencia como los niveles disminuyen después del evento trombótico. Los pacientes del grupo 1 se reevaluaron entre los dos y seis meses después del evento, mostrando niveles de B2A-CIC y una prevalencia positiva similar a los del grupo 2 y del grupo control.

La presencia de B2A-CIC no es exclusiva de pacientes con antecedentes de eventos trombóticos. También aparece, pero con menor prevalencia, en pacientes asintomáticos. Esto sugeriría que la presencia de B2A-CIC no sería directamente trombogénica, sino que más bien se comportaría como un factor de riesgo adicional que favorecería el microambiente protrombótico, aumentando así la probabilidad de ocurrencia del evento trombótico.

El análisis multivariable para trombosis muestra que la positividad para B2A-CIC y la ausencia enfermedades autoinmunes como factores independientes para la trombosis reciente. Esto tiene sentido debido a la presencia de IgA aB2GP1 es más frecuente en pacientes con PAPS que en SAD-APS (112). Al realizar un estudio de seguimiento en pacientes con trombosis reciente, hemos sido capaces de detectar una caída en los niveles de B2A-CIC dos

meses después del evento trombótico. A pesar del pequeño número de pacientes, esta disminución es significativa y sugiere una formación de B2A-CIC durante el evento trombótico.

En el análisis de plaquetas, observamos que los niveles medios del Grupo-1 son más bajos que en el grupo control. Esto podría explicarse porque se habrían consumido durante el evento trombótico. Sin embargo, los pacientes con niveles elevados de B2A-CIC también tienen medios significativamente más bajos que aquellos en los niveles no elevados de B2A-CIC. Esto puede sugerir que B2A-CIC induciría un cierto grado de activación / agregación plaquetaria y podría activar las plaquetas para producir el consumo de plaquetas e incluso producir eventos trombóticos en algunos pacientes. La activación del complemento juega un papel importante en la patogénesis de la trombosis inducida por aPL. Por lo tanto, la hipocomplementemia es común en pacientes con APS (aPL de isotipos IgG o IgM) que refleja la activación y el consumo del complemento (153, 154), además el bloqueo del sistema del complemento ha sido propuesto como un tratamiento efectivo para formas complejas de APS (141).

En nuestro estudio, los niveles C3 y C4 son normales en pacientes con anticuerpos IgA aB2GPI y eventos APS, estos hallazgos son perfectamente consistentes porque la IgA no fija el complemento (144).

El mecanismo por el que los anticuerpos aB2GP1 producen la patología no está claro. Algunos estudios sugieren que B2GPI cambia su conformación después de unirse a la membrana plasmática de plaquetas y células endoteliales. Esto permitiría la unión del anticuerpo B2GPI, produciendo la activación endotelial (155), la activación de plaquetas (156) y un estado alterado de la coagulación(157, 158) que podría, a su vez, desencadenar un estado proinflamatorio y procoagulante. La presencia del B2A-CIC podría ayudar a que este proceso de activación sea más efectivo al aumentar la probabilidad de desencadenar eventos trombóticos en un mecanismo no relacionado con el complemento. Actualmente, no hay cura para APS y el tratamiento debe individualizarse y adaptarse a las características de cada paciente (159).

El riesgo de trombosis en individuos asintomáticos con aPL positivo es bajo, este riesgo aumenta con la concurrencia de otros factores de riesgo cardiovascular como el tabaquismo, uso de estrógenos, inmovilización prolongada, infecciones o procedimientos quirúrgicos res (94, 160).

A pesar de esto, al menos el 50% de los pacientes que desarrollan trombosis no tienen ningún otro factor de riesgo en el momento en que ocurre el evento (161). Las personas asintomáticas positivas para aPL sin otros factores de riesgo protrombóticos no requieren tratamiento (60).

Los pacientes con APS con antecedentes trombóticos generalmente se tratan para reducir el riesgo de tromboembolismo recurrente (159). El pilar principal del tratamiento es la tromboprofilaxis, que generalmente usa AVK. Sin embargo, no hay consenso con respecto a los criterios de detección del paciente y la duración del tratamiento porque los medicamentos anticoagulantes se encuentran entre los medicamentos más comunes que causan eventos adversos (162).

Por lo tanto, para seleccionar qué pacientes deben recibir tromboprofilaxis, se necesitan nuevos biomarcadores que lo hagan posible para identificar pacientes con un estado protrombótico y alto riesgo de eventos clínicos (163).

La identificación B2A-CIC podría ser un nuevo biomarcador para definir la población a ser tratada con riesgo de eventos trombóticos. Hemos seleccionado pacientes positivos solo para IgA aB2GP1 y deliberadamente excluyendo pacientes seronegativos y positivos para otros isotipos porque podrían haber sido un factor de confusión. Esta población debería incluirse en futuros estudios. Se debe realizar un estudio prospectivo de pacientes B2A-CIC positivos que no hayan tenido ningún evento con grupos grandes de pacientes y un control a largo plazo. En resumen, los anticuerpos IgA aB2GP1 son “per se” un factor de riesgo, pero no son suficientes para discriminar la población potencialmente en riesgo de trombosis. Hemos descrito por primera vez pacientes con niveles elevados de B2A-CIC con un mayor riesgo de desarrollar trombosis. Además, estos pacientes presentan más hipercoagulabilidad por activación plaquetaria. Sugerimos que B2A-CIC puede ser un marcador asociado con eventos trombóticos en pacientes positivos para IgA aB2GP1 y parece ser independiente del complemento. El estudio de B2A-CIC puede ayudarnos a comprender mejor el proceso de un estado protrombótico antes del desarrollo de un evento de APS. Desde un punto de vista clínico, puede ser útil para el diagnóstico de APS seronegativo y puede ayudar en la decisión de si el tratamiento con tromboprofilaxis sería útil o no.

En el trabajo detallado en el capítulo 4 describimos por primera vez que la presencia de B2A-CIC identifica un subgrupo de pacientes propensos a desarrollar trombosis postrasplante, comportándose así como un biomarcador predictivo. La condición de los pacientes estudiados en este trabajo es especial: pacientes con ERC tratada con un trasplante de riñón. Estos

pacientes que van a ser trasplantados conforman un modelo ideal para estudiar la ocurrencia de eventos de APS porque incluyen un alto porcentaje de pacientes asintomáticos con aPL (IgA aB2GP1) y todos ellos están sujetos a un segundo golpe bien conocido es decir, cirugía de trasplante.

Aunque esta población puede parecer muy compleja para el estudio de eventos de APS porque sufren una enfermedad adicional, el trabajo se centra en explorar una nueva ruta fisiopatológica de APS(164) que podría ayudar a comprender mejor su patogénesis y establecer nuevas estrategias terapéuticas. Se debe considerar que la mayoría de los estudios sobre APS también se basan en grupos de pacientes con enfermedades autoinmunes concomitantes. De esta forma, el punto de corte para B2A-CIC obtenido en este estudio con pacientes con ERC es prácticamente idéntico al obtenido con pacientes con APS no relacionados con trasplante (102) sugiriendo que tanto los pacientes con APS asociado a ERC como aquellos con APS convencional serían equivalentes con respecto al riesgo trombótico B2A-CIC.

Los anticuerpos IgA anti B2GPI se comportan como un factor de riesgo independiente para los eventos de APS, especialmente la trombosis del injerto. Sin embargo, la presencia de IgA aB2GP1 no es suficiente para identificar a la población que potencialmente está en riesgo de trombosis porque solo una pequeña proporción de pacientes con anticuerpos positivos desarrollan estos eventos trombóticos (113, 165). Esta situación es similar a la observada con otros aPL de isotipos IgG e IgM (32).

En este trabajo, hemos demostrado que los pacientes que son positivos para IgA aB2GP1 y B2A-CIC son los que tienen el mayor riesgo de eventos trombóticos cuando se les somete a una situación capaz de desencadenar eventos trombóticos como la cirugía de trasplante. Aquellos que son negativos para B2A-CIC tienen un riesgo similar al encontrado en la población de control.

Existe una asociación estadísticamente significativa entre la presencia de B2A-CIC con pérdidas de injerto. Una gran mayoría de estos pacientes no se sometieron a nefrectomía porque no presentaban complicaciones graves. Por esta razón, un estudio histopatológico completo no está disponible para ellos. En base a la presencia de B2A-CIC y los síntomas, hemos podido especular que algunos de estos pacientes podrían haber sufrido alguna forma de microangiopatía trombótica silenciosa y progresiva que dañaría el órgano anulando su función, pero que no había tenido graves consecuencias como implicaciones sistémicas que requirieron atención médica y nefrectomía.

Además de pérdida precoz del injerto por trombosis, la presencia de B2A-CIC también se asocia con otros eventos trombóticos. Los receptores de trasplante renal tienen un alto riesgo de eventos tromboembólicos en los primeros meses posteriores al trasplante (166).

Aunque algunos de estos eventos pueden estar relacionados con la cirugía de trasplante, los tratamientos o el momento de la hospitalización(1) la presencia de B2A-CIC puede considerarse el factor de riesgo más importante. El significado biológico de la presencia de B2A-CIC es incierto. La APS es una enfermedad autoinmune que tiene una situación especial ya que el antígeno y el anticuerpo están presentes en la sangre al mismo tiempo. Dada la abundancia del antígeno (B2GP1), hubiéramos esperado que todos los anticuerpos estuvieran permanentemente unidos a su antígeno y no pudieran detectarse en el laboratorio ya que los ensayos de laboratorio solo están diseñados para evaluar anticuerpos no unidos (la forma libre). Sin embargo, en realidad, los anticuerpos existen en forma libre y se detectan en la prueba de diagnóstico.

El comportamiento biológico de los anticuerpos incorporados en B2A-CIC sería diferente del de los anticuerpos de forma libre no solo porque tienen mayor afinidad sino también porque estarían dirigidos contra epítopos que solo están presentes en algunas conformaciones de la proteína que estar más relacionado con su función anticoagulante. La búsqueda de epítopos reconocidos por los anticuerpos integrados en el B2A-CIC y su posible asociación con las diferentes funciones fisiológicas B2GP1 deberían estudiarse en futuras investigaciones.

Es sorprendente que los pacientes con hipertensión en el pretrasplante tengan un riesgo significativamente menor de trombosis. Algunos tratamientos antihipertensivos pueden proporcionar protección vascular al revertir la disfunción endotelial y las anormalidades protrombóticas, contribuyendo a la reducción de las complicaciones relacionadas con la trombosis (167).

De esta forma, los pacientes hemodializados tratados con fármacos antihipertensivos tienen un período significativamente mayor de permeabilidad al acceso vascular (sin trombosis vascular) que aquellos que no fueron tratados (168), los receptores de trasplante renal que son tratados con inhibidores de la enzima convertidora de angiotensina y bloqueadores del receptor de angiotensina asociados con vitamina D tienen un 60% menor riesgo de tromboembolismo venoso (169).

Por lo tanto, se puede especular que el tratamiento antihipertensivo antes del trasplante podría tener un papel protector del endotelio y podría explicar, al menos parcialmente, un

menor riesgo de trombosis. Este posible efecto protector debe investigarse en estudios posteriores. Este estudio tiene varias limitaciones puesto que sólo se evaluaron los inmunocomplejos formados por B2GP1 unidos a anticuerpos del isotipo IgA. Los inmunocomplejos formados por anticuerpos de isotipos IgG e IgM no se evaluó porque en esta cohorte de pacientes la prevalencia de IgG e IgM aB2GP1 fue muy baja y no se encontró asociación con trombosis o pérdida de injerto (165). Otra debilidad del estudio es que aunque ha recogido la experiencia de 12 años, es un estudio unicéntrico. Por lo tanto, los estudios multicéntricos para confirmar estos hallazgos, tanto con pacientes trasplantados como con otras situaciones asociadas con APS, son obligatorios. La determinación de B2A-CIC podría ayudar a los médicos clínicos a distinguir qué pacientes podrían recibir terapia preventiva.

De acuerdo con nuestros resultados, los pacientes con B2A-CIC positivo tienen un alto riesgo de trombosis y, por lo tanto, podrían recibir terapia preventiva para la trombosis postrasplante. La aspirina en dosis baja fue la que se usó con más frecuencia como tratamiento preventivo. Los pacientes con B2A-CIC positivos presentaron una alta frecuencia de este tratamiento en comparación con los pacientes positivos para IgA aB2GP1 sin B2A-CIC y el grupo control, porque los pacientes B2A-CIC positivos antes del trasplante presentaron más antecedentes trombóticos. Los pacientes tratados preventivamente con dosis bajas de aspirina tuvieron una incidencia significativamente mayor de trombosis del injerto que otros pacientes, lo que sugiere que el tratamiento con dosis bajas de aspirina no pareció ser lo suficientemente preventivo en nuestra cohorte. Esto coincide con otros estudios publicados en portadores de aPL en los que individuos aPL positivos no se beneficiaron de una dosis baja de aspirina para la profilaxis de la trombosis primaria (161, 170).

La anticoagulación moderna (con inhibidores del factor Xa) y principalmente la hidroxicloroquina podrían ser la terapia de elección para la prevención de la trombosis (171, 172).

Los pacientes con anticuerpos IgA aB2GP1 negativos para inmunocomplejos sólo deberían recibir tratamiento si existen asociados otros factores de riesgo cardiovascular como diabetes con enfermedad cardiovascular severa. Sin embargo, los ensayos multicéntricos y aleatorizados con este enfoque son obligatorios antes de poder establecer el uso de esta terapia.

En resumen, la presencia de B2A-CIC pretrasplante en pacientes con anticuerpos IgA aB2GP1 positivos se asocia con riesgo trombótico y, por lo tanto, puede considerarse como un biomarcador de complicaciones trombóticas. La presencia de IgA aB2GP1 sin B2A-CIC

implica un riesgo de trombosis similar a la población general. La determinación de B2A-CIC podría ayudar a los médicos a distinguir qué pacientes podrían recibir terapia preventiva.

En el trabajo del capitulo 5 pusimos en prueba la hipótesis, previamente anticipada en las investigaciones que se hicieron en el contexto del estudio de trasplante renal, de que los pacientes con insuficiencia funcional de los órganos donde se elabora la B2GP1 tendrían un mayor riesgo de desarrollar autoanticuerpos aB2GP1

Desde el primer trasplante de corazón realizado en 1967 por Christiaan Neethling Barnard, el trasplante de corazón ha crecido en todo el mundo. Se informaron hasta 120.992 trasplantes de corazón en el 32º informe de trasplante de corazón, según los datos presentados por el registro de la Sociedad Internacional para el Corazón y los Trasplantes de Pulmón (ISHLT) que muestra una mejora significativa de los resultados de trasplante cardiaco a corto y largo plazo (124). Como resultado del desarrollo de nuevas técnicas quirúrgicas, el desarrollo y la mejora del soporte circulatorio mecánico, las estrategias actuales de inmunosupresión después del trasplante cardiaco y una mejor atención postoperatoria, las tasas de supervivencia progresivas más elevadas se informaron anualmente en la era reciente (excluyendo el trasplante corazón-pulmón) en comparación con las tasas de supervivencia del 82% entre 1982 y junio de 2013. A pesar de estos avances, el fallo primario del injerto (FPI) sigue siendo una de las principales causas de muerte dentro de los primeros 30 días postrasplante, junto con múltiples insuficiencia orgánica e infección, que representan respectivamente el 38,7%, 18% y 13,1% de las muertes después del trasplante cardiaco (124). El efecto sinérgico de varios factores de riesgo del donante y el receptor se ha asociado con la FPI. Sin embargo, las estrategias preventivas, diagnósticas y terapéuticas centradas en la FPI aún no se han abordado adecuadamente (125).

De acuerdo con estudios previos en trasplante renal realizados en nuestro centro que han determinado una asociación positiva entre la presencia de IgA aB2GP1 y la pérdida temprana del injerto (110) y dado que tanto el riñón como el corazón sintetizan B2GP1, decidimos investigar si este anticuerpo estaba presente en los pacientes en espera de trasplante cardiaco. El principal hallazgo de nuestro estudio fue que IgA aB2GP1 estaba presente en el 31% de los candidatos a trasplante cardiaco y que se asoció de forma independiente con la mortalidad temprana después del trasplante y los eventos trombóticos. Hasta donde tenemos conocimiento, nuestro estudio es el primer trabajo en describir esta relación en receptores de trasplante cardiaco.

La trombosis postrasplante podría considerarse un proceso inmune latente reactivado mediante cirugía, en lugar de solo una complicación quirúrgica (174). En el trasplante renal, se ha descrito la asociación entre la presencia de IgA aB2GP1 con la pérdida del injerto y el regreso a la terapia de diálisis. Aunque todas las muertes no se pueden atribuir directamente a los procesos trombóticos, la presencia de este anticuerpo podría tener un papel en las muertes relacionadas con complicaciones quirúrgicas. En nuestros pacientes con trasplante cardíaco, la presencia de IgA aB2GP1 está claramente relacionada con la mortalidad temprana, (a diferencia del trasplante renal, el fallo primario del injerto suele implicar la muerte del paciente) y los eventos trombóticos. Aunque no se encontraron diferencias significativas entre las causas de muerte después del trasplante cardiaco entre pacientes con o sin anticuerpos, más del 50% de los pacientes con anticuerpos IgA aB2GP1 que murieron estaban relacionados con fallo primario del injerto. La disfunción primaria del injerto sigue siendo una de las principales causas de muerte en los primeros 30 días después del trasplante cardiaco, lo que representa aproximadamente el 40% de la mortalidad total (124). Sin embargo, a pesar de su relevancia clínica, esta complicación ha recibido poca atención en la literatura en comparación con otras causas comunes de muerte.

Probablemente, esto se deba a la complejidad de su estudio, que involucra afecciones multifactoriales como donante, procedimiento y características del receptor en el momento de la HTA, la dificultad de evaluar con precisión la función del injerto en el entorno quirúrgico y la falta de un claro definición de FPI (175).En nuestros pacientes, la mayor incidencia de eventos trombóticos en presencia de IgA aB2GP1 sugiere que el daño tisular que inicia los eventos que conducen a la muerte del paciente podría ser una trombosis microvascular.. A veces, la causa principal de muerte en estos pacientes puede atribuirse a diferentes afecciones, como insuficiencia orgánica múltiple o sepsis, que no tienen nada que ver con el evento inicial que a menudo se desconoce. La presencia de altos títulos de IgA aB2GP1 relacionados con un mayor riesgo de eventos trombóticos, solo aparece en las primeras semanas después del trasplante. De hecho, después del trasplante renal, los niveles de IgA aB2GP1 disminuyen, probablemente de forma secundaria a la terapia inmunosupresora e incluso pueden volverse negativos, como se ha observado en pacientes estudiados antes de un segundo trasplante renal (107). Factores como la anticoagulación profiláctica y la reducción progresiva de anticuerpos postrasplante podrían ayudar a explicar los resultados de supervivencia más favorables en pacientes con anticuerpos IgA aB2GP1 positivos. Además de los altos niveles pretrasplante de anticuerpos IgA aB2GP1, nuestro estudio demostró que el grupo sanguíneo A y el sexo

femenino se asocian independientemente con la mortalidad temprana. La asociación entre la mortalidad temprana y la presencia del grupo A se ha descrito previamente en grupos de trasplante cardíaco con una población muy similar (176). El sistema de grupo sanguíneo ABO ha sido reconocido como un factor de riesgo de trombosis desde la década de 1960 en relación con las variaciones del factor VIII procoagulante y los niveles sanguíneos del factor de von Willebrand dependientes de la sangre (los individuos con sangre tipo O tienen niveles más bajos) (177, 178). En este sentido, se ha descrito que los tipos de sangre A, B y AB están relacionados con una mayor incidencia de eventos tromboembólicos en comparación con los pacientes de tipo O (179). Nuestro estudio tiene algunas limitaciones: en primer lugar, aquellas que son inherentes a un estudio retrospectivo en un solo centro. No obstante, en nuestro caso las variables clave se recolectaron prospectivamente y siguiendo un protocolo estricto. Sin embargo, la asignación de eventos en la evolución fue, en ciertos casos, difícil. Del mismo modo, la causalidad entre IgA aB2GP1 y el resultado no se puede inferir y solo se pueden establecer relaciones o asociaciones. En conclusión, los anticuerpos IgA aB2GP1 estuban presente en el 31% de los candidatos a trasplante cardiaco y se asociaron de manera independiente con mortalidad temprana y eventos trombóticos. Esta asociación nunca se ha descrito previamente en la literatura. El mecanismo patogénico subyacente parece ser la trombosis microvascular y, en consecuencia, la anticoagulación podría modular sus efectos nocivos. Se necesitan estudios multicéntricos y prospectivos con mayor tamaño de muestra para confirmar estos datos y evaluar el efecto de la anticoagulación en este contexto.

El trabajo descrito en el capitulo 6 surge de la necesidad de confirmar los hallazgos descritos en los capítulos anteriores en un estudio prospectivo multicéntrico con pacientes de otros hospitales y de otras zonas geográficas.

El este estudio multicéntrico se confirmó una prevalencia elevada de los IgA aB2GP1 en los pacientes en espera de trasplante renal. Es de destacar que la prevalencia en el conjunto de los hospitales estudiados es ligeramente más elevada que la observada en el Hospital 12 de Octubre.

En este estudio se confirmó que presencia pretrasplante de IgA aB2GP1 fue el más importante factor de riesgo independiente para la trombosis del injerto, la perdida precoz del injerto (en general) y el desarrollo de un cuadro de retraso en la función del injerto (Necrosis tubular aguda).

Estos hallazgos, ahora confirmados, plantean la necesidad de que se evalúe la conveniencia de instaurar un tratamiento profiláctico con anticoagulación a los pacientes con

mayor riesgo. A estos efectos sería necesario en el futuro plantear un ensayo clínico donde se evalúe la eficacia y la seguridad de la anticoagulación profiláctica para evitar estas graves complicaciones.

CONCLUSIONES

1. La presencia de los anticuerpos IgA aB2GP1 en pacientes con insuficiencia renal crónica no se asocia con polimorfismos genéticos predisponentes para la elaboración de anticuerpos antifosfolipídicos, con la causa de la insuficiencia renal ni con el tipo de tratamiento sustitutorio de la disfunción renal en nuestra serie de pacientes.

2. Se demuestra la elevada prevalencia de anticuerpos IgA aB2GP1 pretrasplante renal así como su asociación a pérdida precoz del injerto por trombosis en una cohorte de 1350 pacientes durante 12 años consecutivos de trasplantes renales y en un estudio multicéntrico prospectivo.

3. Los pacientes portadores de IgA aB2GP1 que también presentan inmunocomplejos de IgA unida a B2GP1 son los que acumulan el riesgo de eventos trombóticos tras el trasplante. Sin embargo, los que son negativos para inmunocomplejos, tienen el mismo riesgo de pérdida del injerto renal por trombosis que la población sin anticuerpos antifosfolipídicos.

4. Los inmunocomplejos de IgA aB2GP1 unidos a B2GP1 son un factor de riesgo independiente para pérdida precoz del injerto renal por trombosis.

5. La presencia de inmunocomplejos de IgA unida a B2GP1 se asocia con eventos trombóticos agudos,

6. La positividad aislada de anticuerpos IgA aB2GP1 en pacientes con insuficiencia cardíaca terminal representa un factor de riesgo independiente para mortalidad precoz tras el trasplante cardiaco y para la incidencia de eventos cardiovasculares los 3 primeros meses tras el trasplante.

7. De las conclusiones anteriores se puede deducir que posiblemente estemos describiendo una nueva forma de APS relacionada con la insuficiencia funcional de los órganos productores de B2GP1, como el riñón y el corazón, sin relación con otras enfermedades autoinmunes sistémicas y asociada a positividad de anti B2GP1 de isotipo IgA.

RESUMEN.

En las últimas décadas han mejorado los resultados del trasplante renal, a corto y largo plazo, gracias a la introducción de la inmunosupresión moderna, los avances en el control de infecciones, mejores metodologías quirúrgicas y los avances en el conocimiento de la respuesta inmune y las técnicas de histocompatibilidad. Sin embargo, las pérdidas del injerto en los primeros meses tras el trasplante apenas han registrado cambios (5-8% de los trasplantes), siendo la principal causa la trombosis del injerto.

Uno de los principales factores responsables del deterioro de la función del órgano transplantado después del trasplante es la respuesta inmune frente al aloinjerto.

Desde hace años se conoce que el principal objetivo de este ataque inmunitario es el complejo principal de histocompatibilidad (CPH) del donante (alorreactividad) aunque recientemente se ha informado que también respuestas autoinmunes basadas en anticuerpos dirigidos frente a otras moléculas distintas del CPH también pueden afectar el resultado del trasplante renal.

El síndrome antifosfolípido (APS) es un trastorno autoinmune caracterizado por la presencia de trombosis yo morbilidad gestacional en paciente que en su sangre tienen anticuerpos antifosfolipídícos (aPL). Los aPL están dirigidos frente a fosfolípidos, proteínas plasmáticas de unión a fosfolípidos, o el complejo formado por ambos unión de ambos.

No existen criterios diagnósticos establecidos para el APS, existen unos criterios de clasificación establecidos en Sidney en 2004. Para clasificar a un paciente como APS debe presentar simultáneamente un criterio clínico y un criterio de laboratorio. Los criterios clínicos consensuados son los eventos trombóticos y/o la morbimortalidad gestacional, fundamentalmente la pérdida recurrente de embarazo. Los criterios de laboratorio son la presencia de anticoagulante lúpico o aPL de isotipo IgG o IgM dirigidos ante la cardiolipina (aCL) o B2-Glicoproteína 1 (aB2GP1).

Además de la sintomatología descrita en los criterios clínicos de clasificación, el espectro clínico de APS abarca manifestaciones que pueden afectar a muchos órganos y no pueden ser explicadas exclusivamente por pacientes en estado protrombótico. Estas

manifestaciones clínicas no enumeradas en los criterios de clasificación (conocidas como manifestaciones de extra-criterios) incluyen manifestaciones neurológicas (corea, mielitis y migraña), hematológicas (trombocitopenia y anemia hemolítica), cutáneas como livedo reticularis, renales (nefropatía) y cardiacas (enfermedad valvular).

Recintements ha crecido el interes por describir nuevos marcadores que permitan identificar pacientes con APS entre aquellos que, pese a reunir los criterios clínicos de APS, no reúnen los de laboratorio (APS seronegativo). Entre los autoanticuerpos asociados con APS seronegativo y que no están incluidos en los criterios de clasificación destacan aB2GP1 de isotipo IgA, anti fosfatidilserina/protrombina, anti anexina A2, anti anexina A5 y anti S100A10.

Se han descrito tres formas bien definidas de APS. Enumerándolas por orden cronológico de su descripción son: 1) APS asociado a otras enfermedades autoinmunes sistémicas concomitantes (SAD-APS)fue la primera forma en ser descrita y la más conocida. La enfermedad autoinmune que más se asocia con el APS es el lupus eritematoso sistémico. 2) APS primario (PAPS), la enfermedad aparece de manera aislada sin asociarse a otras enfermedades autoinmunes. 3) APS catastrófico (CAPS), que se caracteriza por un fallo multiorgánico con microangiopatía trombótica con aparición súbita y una elevada tasa de mortalidad, afortunadamente es la menos frecuente de las tres.

En los últimos años los anticuerpos de isotipo IgA están adquiriendo gran relevancia en relación con la clínica del APS. Aunque no están incluido en los criterios de clasificación, en el XIV Congreso Internacional de Anticuerpos Antifosfolipídicos alebrado en Galveston 2014 se recalcó su utilidad en aquellos pacientes con característica clínicas de APS que sin embargo son seronegativos para los aPL de consenso.

La B2GP1 es una proteína que se sintetiza principalmente no solo en el hígado sino también en el riñón y el corazón. Se localiza en el plasma y en la membrana de las células endoteliales y plaquetas.

La prevalencia de anticuerpos anti B2GP1 del isotipo IgA (IgA aB2GP1) es mayor en pacientes con enfermedad renal crónica (ERC) que en la población general (30% vs. 1.5%). En los pacientes con ERC y tratamiento con hemodiálisis se demostró la

asociación entre la presencia de estos anticuerpos y la incidencia de eventos trombóticos y mortalidad.

Aunque desconoce el origen de estos anticuerpos, se planteó la hipótesis de que la unión de B2GPI a la membrana de diálisis podría inducir un cambio conformacional de la proteína, que expusiera epítopos crípticos.

Nuestro grupo demostró en un estudio prospectivo sobre la cohorte de "Grupo Forum Renal" en los pacientes trasplantados en el Hospital Doce de Octubre, la presencia pretrasplante de los anticuerpos IgA aB2GP1 es el principal factor de riesgo independiente para pérdida temprana del injerto renal, principalmente de trombosis, sin embargo esta última asociación no llegó a ser significativa debido al pequeño tamaño de la muestra de pacientes.

Para confirmar la hipótesis de la asociación de la presencia de los IgA a B2GP1 con la trombosis renal se decidió hacer un nuevo estudio prospectivo en un grupo de pacientes más numeroso. Se utilizó la cohorte histórica "Magnum 12+12" que consta de 1375 pacientes trasplantados de forma consecutiva durante 12 años en el Hospital 12 de Octubre. Tras un seguimiento durante 24 meses se confirmó la elevada prevalencia de los IgA aB2GP1 (cercana al 30%) de los pacientes con insuficiencia renal crónica (ERC) inmediatamente antes de ser trasplantados. En el análisis multivariable se demostró por primera vez que la presencia de anticuerpos IgA aB2GP1 constituye el principal factor de riesgo para la pérdida precoz del injerto por trombosis, seguido por la edad y la presencia de diabetes mellitus tipo 2.

Nuestro grupo describió la existencia de inmunocomplejos formados por IgA unida a B2GPI (B2A-CIC) en la sangre de pacientes con antecedentes de sintomatología de APS y positivos aislados para IgA aB2GP1 (negativos para isotipos IgG e IgM).

Asimismo nuestro grupo describió un nuevo procedimiento mucho más simplificado que los que venían siendo utilizados, para la detección de dichos inmunocomplejos. Gracias a esta metodología, en un trabajo posterior incluido en esta memoria, se ha visto que la presencia de B2A-CIC está fuertemente asociada con la ocurrencia de eventos trombóticos agudos.

Los niveles de plaquetas en los pacientes B2A-CIC positivos son más bajos que los negativos para B2A-CIC, lo que sugiere que los inmunocomplejos se forman en un

estado de hipercoagulabilidad. La presencia de B2A-CIC no está relacionada con el proceso de activación del complemento.

De este trabajo surgió la hipótesis de que la presencia de B2A-CIC podría utilizarse como un biomarcador para identificar a los pacientes IgA anti B2GPI positivos con mayor riesgo de desarrollar un evento trombótico. Sin embargo, aunque se ha descrito la relación de B2A-CIC con eventos trombóticos agudos, no se puede discernir si son causa o consecuencia de la trombosis, dado que en un paciente con un evento trombótico que es atendido en puerta de urgencias es prácticamente imposible obtener una muestra de sangre extraída en los días previos a la aparición de dicho evento trombótico.

Planteamos la evaluación de la presencia de B2A-CIC y la incidencia de eventos trombóticos postrasplante en la cohorte histórica Magnum 12+12, puesto que se dispone de muestra de suero pretrasplante, y un seguimiento prospectivo de todos ellos.

Tras evaluarlos se encontró que aquellos pacientes que fueron positivos para B2A-CIC tienen más riesgo de eventos APS en los primeros meses postrasplante, y más concretamente de pérdida del injerto por trombosis.

Se observó que los pacientes que positivos para IgA aB2GP1 solo están en riesgo de experimentar trombosis si son B2A-CIC positivos, puesto que aquellos pacientes que son B2A-CIC-negativos, aunque tengan IgA aB2GP1, tienen el mismo riesgo de desarrollar eventos trombóticos que el grupo de control (negativos para IgA aB2GP1).

Este hallazgo implica que los posibles tratamientos preventivos para evitar la aparición de eventos trombóticos agudos deberían focalizarse sobre los pacientes B2A-CIC-positivos.

Dada la alta prevalencia observada de IgA aB2GP1 en la población con ERC, y descartando en nuestra serie de pacientes que esto se debiera a factores genéticos o ambientales relacionados con la causa o el tratamiento de la ERC, hipotetizamos que la elaboración de B2GP1 en un órgano insuficiente por la disfunción crónica podría acarrear la elaboración de formas defectuosas de la proteína debidas a plegamientos defectuosos, lo que podría exponer epítopos inmunogénicos que previamente estaban ocultos.

Si esta hipótesis fuera correcta, la disfunción de otros órganos donde se elabore fisiológicamente B2GP1 como corazón e hígado implicaría también una inmunización aberrante frente a autoantígenos que supusiera la aparición de los autoanticuerpos.

Para comprobar esta hipótesis se realizó un estudio sobre una cohorte de 151 pacientes que, de forma consecutiva durante 8 años, recibieron un trasplante cardiaco. Estos pacientes fueron seguidos durante 2 años.

De nuevo se observó que alrededor de 30% presentaron positividad de los anticuerpos IgA aB2GP1. En un análisis multivariable se confirmó que la presencia de IgA aB2GP1 previa al trasplante se asoció de forma independiente con mayores tasas de mortalidad temprana y de aparición de eventos trombóticos después del trasplante cardiaco.

La asociación de la presencia antes del trasplante de los autoanticuerpos IgA anti B2GP1 con la pérdida temprana del injerto, especialmente debida a trombosis. fue descrita en los pacientes trasplantados en el Hospital 12 de octubre, y requería la confirmación mediante un estudio prospectivo multicéntrico.

Para ello se realizó un último estudio, con los pacientes de 5 hospitales de la cohorte histórica “Forum Renal” que incluye trasplantes renales de toda España.

En este estudio se confirmó la elevada prevalencia (30%) de los autoanticuerpos IgA en los pacientes con insuficiencia renal crónica que iban a recibir un trasplante renal.

Asimismo en este estudio prospectivo multicéntrico quedó confirmado que la presencia previa al trasplante de IgA aB2GP1 constituye el principal factor de riesgo para la pérdida precoz del injerto, la trombosis del órgano trasplantado y la necrosis tubular aguda (función retardada del injerto).

El análisis detallado de los datos y conclusiones descritos en estos trabajos nos permite responder a nuestras preguntas de investigación y a los objetivos de la tesis y también nos hace plantearnos la posibilidad de que podamos estar ante una nueva forma de APS primario relacionada con la insuficiencia funcional de los órganos productores de B2GP1, mediada por anticuerpos frente a B2GP1 de isotipo IgA.

La presencia de inmunocomplejos B2A-CIC en pacientes positivos para los autoanticuerpos IgA aB2GP1 se comportan como un biomarcador que permite

identificar mejor a la población con mayor riesgo de desarrollar eventos APS, puesto que la población negativa para estos inmunocomplejos, aunque san positivos para aB2GP1 tienen el mismo riesgo de trombosis que las personas que no presentan estos autoanticuerpos.

ABSTRACT

In recent decades, the short and long term outcomes of renal transplantation have improved, thanks to the introduction of modern immunosuppression, advances in infection control, better surgical methodologies, and advances in the knowledge of the immune response and the histocompatibility techniques.

However, graft losses in the first months after transplantation have barely registered changes (5-8% of transplants), the main cause is by graft thrombosis. One of the main factors responsible for the deterioration of graft function is the immune response against the allograft.

For years it has been known that the main target of this immune attack is the donor major histocompatibility complex (MHC), although it has recently been reported that autoimmune responses based on antibodies directed against molecules other than MHC can also affect the result of the kidney transplant.

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of thrombosis and / or gestational morbidity in patients who have antiphospholipid antibodies (aPL) in the blood. The aPL are directed against phospholipids, plasma proteins binding to phospholipids, or the complex formed by union of both.

There are no established diagnostic criteria for APS, however classification criteria were established in Sydney in 2004. To classify a patient as APS must simultaneously present a clinical and a laboratory criteria. The clinical criteria are thrombotic events and / or gestational morbidity and mortality, mainly the recurrent loss of pregnancy. The laboratory criteria are the presence of lupus anticoagulant or aPL of isotype IgG or IgM directed against cardiolipin (aCL) or B2-glycoprotein 1 (aB2GP1).

In addition to the symptomatology described in the clinical classification criteria, the clinical spectrum of APS include manifestations that can affect many organs and can not be explained exclusively by patients in the prothrombotic state. These clinical manifestations not included in the classification criteria (known as manifestations of extra-criteria) encompass neurological manifestations (chorea, myelitis and migraine),

hematological (thrombocytopenia and hemolytic anemia), cutaneous as livedo reticularis, renal (nephropathy) and cardiac (valvular disease).

Recently there has been an increasengly interest in describing new markers that allow the identification of patients with APS who meet clinical criteria of APS, but do not meet the laboratory criteria (seronegative APS). Among the autoantibodies associated with seronegative APS and which are not included in the classification criteria include aB2GP1 of IgA isotype, anti phosphatidylserine / prothrombin, anti annexin A2, anti annexin A5 and anti S100A10.

Three well-defined forms of APS have been described. Listing them in chronological order of their description are: 1) APS associated with other concomitant systemic autoimmune diseases (SAPS) that is the best known. Systemic lupus erythematosus is the most associated autoimmune disease. 2) Primary APS (PAPS), the disease appears without other autoimmune diseases associated. 3) Catastrophic APS (CAPS), which is characterized by multiorganic failure with thrombotic microangiopathy with sudden onset and a high mortality rate, fortunately it is the least frequent of the three.

In recent years, the IgA isotype antibodies are becoming very relevant in relation to the APS clinic. Although are not included in the classification criteria, the XIV International Congress on Antiphospholipid Antibodies (Galveston 2014) emphasized its usefulness in those patients with suspiction of APS but seronegative for consensus aPL.

B2GP1 is a protein that is synthesized mainly in the liver but also in the kidney and heart. It is located in the plasma and in the membrane of endothelial cells and platelets. The prevalence of anti-B2GP1 antibodies of the IgA isotype (IgA aB2GP1) is higher in patients with chronic kidney disease (CKD) than in the general population (30% vs. 1.5%). In patients with CKD in hemodialysis treatment, the association between the presence of these antibodies and the incidence of thrombotic events and mortality was demonstrated.

Although the origin of these antibodies is unknown, it was hypothesized that the binding of B2GPI to the dialysis membrane could induce a conformational change of the protein, which would expose cryptic epitopes. Our group demonstrated in a unicentre prospective study on a cohort of patients transplanted at Hospital Doce de Octubre, that the pretransplant presence of IgA aB2GP1 is the main independent risk factor for early

renal graft loss, mainly of thrombosis, however this last association did not become significant due to the small size of the sample of patients.

To confirm the hypothesis of the association of the presence of IgA aB2GP1 with renal thrombosis, it was decided to perform a new prospective study in a larger group of patients. We used the historical cohort "Magnum 12 + 12" which consists of 1375 patients transplanted consecutively for 12 years in the Hospital 12 de Octubre. After 24-month follow-up, the high prevalence of IgA aB2GP1 (around 30%) in patients CKD was confirmed immediately before being transplanted. The multivariable analysis showed for the first time that the presence of IgA aB2GP1 antibodies constitutes the main risk factor for early graft loss due to thrombosis, followed by age and the presence of type 2 diabetes mellitus.

Our group described the existence of immunocomplexes formed by IgA bounded to B2GPI (B2A-CIC) in the blood of patients with APS antecedents and isolated positive for IgA aB2GP1 (negative for IgG and IgM isotypes). Also our group described a new procedure much more simplified than those used before, for the detection of immunocomplexes. Thanks to this methodology, in a later work included in this report, it has been seen that the presence of B2A-CIC is strongly associated with the occurrence of acute thrombotic events.

Platelet levels in positive B2A-CIC patients are lower than those negative for B2A-CIC, suggesting that immunocomplexes are formed in a state of hypercoagulability. The presence of B2A-CIC is not related to the process with the complement activation process. From this work, we hypothesized that the presence of B2A-CIC could be used as a biomarker to identify IgA aB2GPI positive patients with a higher risk of developing a thrombotic event. However, although the relationship of B2A-CIC with acute thrombotic events has been described, it is not possible to discern whether they are a cause or consequence of thrombosis, because it is impossible to take a sample before of the thrombotic event. We propose the evaluation of the presence of B2A-CIC and the incidence of thrombotic events after transplantation in the historical cohort Magnum 12 + 12, because there were available of pretransplant sera, and a prospective follow-up of all of them.

After evaluating them, it was found that those patients who were positive for B2A-CIC are more at risk of APS events in the first months after transplantation, and more specifically of graft loss by thrombosis. It was observed that patients who are positive for

IgA aB2GP1 are only at real risk thrombosis if they are B2A-CIC positive, bcause patients who are B2A-CIC-negative, although they have IgA aB2GP1, have the same risk of developing thrombotic events that the control group (negative for IgA aB2GP1). This finding implies that possible preventive treatments to avoid the occurrence of acute thrombotic events should be focused on B2A-CIC positive patients. Given the high prevalence of IgA aB2GP1 in the population with CKD, and discarding (in our series of patients) genetic or environmental factors related to the cause or treatment of CKD, we hypothesized that the elaboration of B2GP1 in an insufficient organ due to chronic dysfunction could lead to the elaboration of misfolding forms of the, which could expose immunogenic epitopes that were previously hidden. If this hypothesis were correct, the dysfunction of other organs where B2GP1 is elaborated physiologically by heart and liver would also imply an aberrant immunization against autoantigens that would suppose the appearance of the autoantibodies. To test this hypothesis, a study was conducted on a cohort of 151 patients for 8 years, who received a heart transplant. These patients were followed for 2 years. Again it was observed that around 30% presented positive IgA aB2GP1. In a multivariate analysis it was confirmed that the presence of IgA aB2GP1 prior to transplantation was independently associated with higher rates of early mortality and the appearance of thrombotic events after heart transplantation.

The association of the presence (before transplantation) of IgA aB2GP1 with early graft loss, especially due to thrombosis, was described in patients transplanted at the Hospital Doce de Octubre, and required confirmation through a prospective multicenter study. For this, a final study was carried out, with patients from 5 hospitals of the historical "Forum Renal" cohort that includes kidney transplants from all Spain. This study confirmed the high prevalence (30%) of IgA aB2GP1 in patients with CKD who were going to receive kidney transplantation. In this prospective multicenter study, it was confirmed that the presence of IgA aB2GP1 prior to transplant is the main risk factor for early graft loss, thrombosis of the transplanted organ and delayed graft function. The detailed analysis of the data and conclusions described in these works allows us to answer our questions about research and the objectives of the thesis and also makes us consider the possibility.

We believe that we can be faced with a new form of primary APS related to functional insufficiency of B2GP1-producing organs, mediated by antibodies against B2GP1 of the IgA isotype.

The presence of B2A-CIC immunocomplexes in patients positive for IgA aB2GP1 autoantibodies is they act as a biomarker that allows a better identification of the population with greater risk of developing APS events, since the negative population for these immune complexes, although positive for aB2GP1 have the same risk of thrombosis as people who do not have these autoantibodies.

BIBLIOGRAFIA

1. Willis R, Harris EN, Pierangeli SS. Pathogenesis of the antiphospholipid syndrome. Semin Thromb Hemost. 2012 Jun;38(4):305-21. PubMed PMID: 22510982. Epub 2012/04/19. eng.

2. Hughes GR. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. Br Med J (Clin Res Ed). 1983 Oct 15;287(6399):1088-9. PubMed PMID: 6414579. Pubmed Central PMCID: 1549319. Epub 1983/10/15. eng.

3. Triplett DA. Antiphospholipid antibodies. Arch Pathol Lab Med. 2002 Nov;126(11):1424-9. PubMed PMID: 12421152. Epub 2002/11/08. eng.

4. Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. Semin Nephrol. 2007 Jan;27(1):35-46. PubMed PMID: 17336687.

5. Pangborn MC. Cardiolipin and its application in a chemically purified antigen for the serodiagnosis of syphilis. Proc N Y State Assoc Public Health Lab. 1946;26(1):26-9. PubMed PMID: 20245941. Epub 1946/01/01. eng.

6. Hanly JG. Antiphospholipid syndrome: an overview. CMAJ. 2003 Jun 24;168(13):1675-82. PubMed PMID: 12821621. Pubmed Central PMCID: 161613. Epub 2003/06/25. eng.

7. Moore JE, Mohr CF. Biologically false positive serologic tests for syphilis; type, incidence, and cause. J Am Med Assoc. 1952 Oct 4;150(5):467-73. PubMed PMID: 14955455. Epub 1952/10/04. eng.

8. Bowie EJ, Thompson JH, Jr., Pascuzzi CA, Owen CA, Jr. Thrombosis in Systemic Lupus Erythematosus Despite Circulating Anticoagulants. J Lab Clin Med. 1963 Sep;62:416-30. PubMed PMID: 14061973. Epub 1963/09/01. eng.

9. Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet. 1983 Nov 26;2(8361):1211-4. PubMed PMID: 6139567. Epub 1983/11/26. eng.

10. Hughes GR. Hughes syndrome/APS. 30 years on, what have we learnt? Opening talk at the 14th International Congress on antiphospholipid antibodies Rio de Janiero, October 2013. Lupus. 2014 Apr;23(4):400-6. PubMed PMID: 24619623. Epub 2014/03/13. eng.

11. Mackworth-Young C, l D, S L, MV W. Primary antiphospholipid syndrome. Features in patients with raised anticardiolipin antibodies and not other disorders (Abstr.). Br J Rheumatol 1987;26:1.

12. Harris EN. Syndrome of the black swan. Br J Rheumatol. 1987 Oct;26(5):324-6. PubMed PMID: 3664156. Epub 1987/10/01. eng.

13. McNeil HP, Chesterman CN, Krilis SA. Anticardiolipin antibodies and lupus anticoagulants comprise separate antibody subgroups with different phospholipid binding characteristics. Br J Haematol. 1989 Dec;73(4):506-13. PubMed PMID: 2514785. Epub 1989/12/01. eng.

14. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. Lancet. 1990 Jul 21;336(8708):177-8. PubMed PMID: 1973491. Epub 1990/07/21. eng.

15. Machin SJ GJ, Greaves M, Hutton RA, Mackie IJ, Malia RG, Taberner DA. Guidelines on testing for the lupus anticoagulant. Lupus Anticoagulant Working Party on behalf of the BCSH Haemostasis and Thrombosis Task Force. J Clin Pathol. 1991 Nov;44(11):885-9. PubMed PMID: 1752976. Pubmed Central PMCID: 496623. Epub 1991/11/01. eng.

16. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome

(APS). J Thromb Haemost. 2006 Feb;4(2):295-306. PubMed PMID: 16420554. Epub 2006/01/20. eng.

17. Ho Y, Ahuja K, Körner H, Adams M. β2GP1, Anti-β2GP1 Antibodies and Platelets: Key Players in the Antiphospholipid Syndrome. Antibodies. 2016;5(12):16.

18. Agar C, de Groot PG, Morgelin M, Monk SD, van Os G, Levels JH, et al. beta(2)-glycoprotein I: a novel component of innate immunity. Blood. 2011 Jun 23;117(25):6939-47. PubMed PMID: 21454452. Epub 2011/04/02. eng.

19. Ninivaggi M, Kelchtermans H, Lindhout T, de Laat B. Conformation of beta2glycoprotein I and its effect on coagulation. Thromb Res. 2012 Oct;130 Suppl 1:S33-6. PubMed PMID: 22925530. Epub 2012/08/29. eng.

20. Harris EN, Pierangeli SS. Primary, secondary, and catastrophic antiphospholipid syndrome: what's in a name? Semin Thromb Hemost. 2008 Apr;34(3):219-26. PubMed PMID: 18720301. Epub 2008/08/23. eng.

21. Alarcon-Segovia D, Deleze M, Oria CV, Sanchez-Guerrero J, Gomez-Pacheco L, Cabiedes J, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. Medicine (Baltimore). 1989 Nov;68(6):353-65. PubMed PMID: 2811661. Epub 1989/11/01. eng.

22. Alarcon-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. J Rheumatol. 1989 Apr;16(4):482-8. PubMed PMID: 2746588. Epub 1989/04/01. eng.

23. Asherson RA. A "primary" antiphospholipid syndrome? J Rheumatol. 1988 Dec;15(12):1742-6. PubMed PMID: 14552307. Epub 1988/12/01. eng.

24. Asherson RA, Khamashta MA, Ordi-Ros J, Derksen RH, Machin SJ, Barquinero J, et al. The "primary" antiphospholipid syndrome: major clinical and serological features. Medicine (Baltimore). 1989 Nov;68(6):366-74. PubMed PMID: 2509856. Epub 1989/11/01. eng.

25. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum. 2002 Apr;46(4):1019-27. PubMed PMID: 11953980. Epub 2002/04/16. eng.

26. Asherson RA, Cervera R, Piette JC, Font J, Lie JT, Burcoglu A, et al. Catastrophic antiphospholipid syndrome. Clinical and laboratory features of 50 patients. Medicine (Baltimore). 1998 May;77(3):195-207. PubMed PMID: 9653431. Epub 1998/07/08. eng.

27. Rodriguez-Pinto I, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: The current management approach. Best Pract Res Clin Rheumatol. 2016 Apr;30(2):239-49. PubMed PMID: 27886797. Epub 2016/11/26. eng.

28. Asherson RA. The catastrophic antiphospholipid syndrome, 1998. A review of the clinical features, possible pathogenesis and treatment. Lupus. 1998;7 Suppl 2:S55-62. PubMed PMID: 9814675. Epub 1998/11/14. eng.

29. Espinosa G, Rodriguez-Pinto I, Cervera R. Catastrophic antiphospholipid syndrome: an update. Panminerva Med. 2017 Sep;59(3):254-68. PubMed PMID: 28488841. Epub 2017/05/11. eng.

30. Felson DT, Anderson JJ. Methodological and statistical approaches to criteria development in rheumatic diseases. Baillieres Clin Rheumatol. 1995 May;9(2):253-66. PubMed PMID: 7656339. Epub 1995/05/01. eng.

31. Johnson SR, Goek ON, Singh-Grewal D, Vlad SC, Feldman BM, Felson DT, et al. Classification criteria in rheumatic diseases: a review of methodologic properties. Arthritis Rheum. 2007 Oct 15;57(7):1119-33. PubMed PMID: 17907227. Epub 2007/10/02. eng.

32. Gomez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. J Autoimmun. 2014 Feb-Mar;48-49:20-5. PubMed PMID: 24461539. Epub 2014/01/28. eng.

33. Aggarwal R, Ringold S, Khanna D, Neogi T, Johnson SR, Miller A, et al. Distinctions between diagnostic and classification criteria? Arthritis Care Res (Hoboken). 2015 Jul;67(7):891-7. PubMed PMID: 25776731. Pubmed Central PMCID: 4482786. Epub 2015/03/18. eng.

34. Sciascia S, Amigo MC, Roccatello D, Khamashta M. Diagnosing antiphospholipid syndrome: 'extra-criteria' manifestations and technical advances. Nat Rev Rheumatol. 2017 Sep;13(9):548-60. PubMed PMID: 28769114. Epub 2017/08/05. eng.

35. Alarcon-Segovia D, Perez-Vazquez ME, Villa AR, Drenkard C, Cabiedes J. Preliminary classification criteria for the antiphospholipid syndrome within systemic lupus erythematosus. Semin Arthritis Rheum. 1992 Apr;21(5):275-86. PubMed PMID: 1604324. Epub 1992/04/01. eng.

36. Vianna JL, Khamashta MA, Ordi-Ros J, Font J, Cervera R, Lopez-Soto A, et al. Comparison of the primary and secondary antiphospholipid syndrome: a European Multicenter Study of 114 patients. Am J Med. 1994 Jan;96(1):3-9. PubMed PMID: 8304360. Epub 1994/01/01. eng.

37. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med. 2008 Aug 28;359(9):938-49. PubMed PMID: 18753650. Epub 2008/08/30. eng.

38. Lijfering WM, Rosendaal FR, Cannegieter SC. Risk factors for venous thrombosis - current understanding from an epidemiological point of view. Br J Haematol. 2010 Jun;149(6):824-33. PubMed PMID: 20456358. Epub 2010/05/12. eng.

39. Sciascia S, Sanna G, Khamashta MA, Cuadrado MJ, Erkan D, Andreoli L, et al. The estimated frequency of antiphospholipid antibodies in young adults with cerebrovascular events: a systematic review. Ann Rheum Dis. 2015 Nov;74(11):2028-33. PubMed PMID: 24942381. Epub 2014/06/20. eng.

40. Cervera R. Antiphospholipid syndrome. Thromb Res. 2017 Mar;151 Suppl 1:S43-S7. PubMed PMID: 28262233. Epub 2017/03/07. eng.

41. Cummings KW, Bhalla S. Pulmonary Vascular Diseases. Clin Chest Med. 2015 Jun;36(2):235-48. PubMed PMID: 26024602. Epub 2015/05/31. Eng.

42. Moser KM, Fedullo PF, LitteJohn JK, Crawford R. Frequent asymptomatic pulmonary embolism in patients with deep venous thrombosis. JAMA. 1994 Jan 19;271(3):223-5. PubMed PMID: 8277550. Epub 1994/01/19. eng.

43. Rodriguez-Pinto I, Espinosa G, Cervera R. Catastrophic APS in the context of other thrombotic microangiopathies. Curr Rheumatol Rep. 2015 Jan;17(1):482. PubMed PMID: 25604575. Epub 2015/01/22. eng.

44. Kurosawa S, Stearns-Kurosawa DJ. Complement, thrombotic microangiopathy and disseminated intravascular coagulation. J Intensive Care. 2014;2(1):65. PubMed PMID: 25705421. Pubmed Central PMCID: 4336180. Epub 2015/02/24. eng.

45. Warwicker P, Goodship TH, Donne RL, Pirson Y, Nicholls A, Ward RM, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998 Apr;53(4):836-44. PubMed PMID: 9551389. Epub 1998/04/29. eng.

46. Dragon-Durey MA, Sethi SK, Bagga A, Blanc C, Blouin J, Ranchin B, et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. J Am Soc Nephrol. 2010 Dec;21(12):2180-7. PubMed PMID: 21051740. Pubmed Central PMCID: 3014031. Epub 2010/11/06. eng.

47. Wallace K, Harris S, Addison A, Bean C. HELLP Syndrome: Pathophysiology and Current Therapies. Curr Pharm Biotechnol. 2018 Jul 11. PubMed PMID: 29998801. Epub 2018/07/13. eng.

48. Rosove MH. Thrombotic microangiopathies. Semin Arthritis Rheum. 2014 Jun;43(6):797-805. PubMed PMID: 24360024. Epub 2013/12/24. eng.

49. Shatzel JJ, Taylor JA. Syndromes of Thrombotic Microangiopathy. Med Clin North Am. 2017 Mar;101(2):395-415. PubMed PMID: 28189178. Epub 2017/02/13. eng.

50. Amigo MC. What do we know about the cardiac valve lesion in the antiphospholipid syndrome (APS)? Lupus. 2014 Oct;23(12):1259-61. PubMed PMID: 25228720. Epub 2014/09/18. eng.

51. Abreu MM, Danowski A, Wahl DG, Amigo MC, Tektonidou M, Pacheco MS, et al. The relevance of "non-criteria" clinical manifestations of antiphospholipid syndrome: 14th International Congress on Antiphospholipid Antibodies Technical Task Force Report on Antiphospholipid Syndrome Clinical Features. Autoimmun Rev. 2015 May;14(5):401-14. PubMed PMID: 25641203. Epub 2015/02/03. eng.

52. Abreu MM, Danowski A, Wahl DG, Amigo MC, Tektonidou M, Pacheco MS, et al. The relevance of "non-criteria" clinical manifestations of antiphospholipid syndrome: 14th International Congress on Antiphospholipid Antibodies Technical Task Force Report on Antiphospholipid Syndrome Clinical Features. Autoimmun Rev. 2015 Jan 29. PubMed PMID: 25641203. Epub 2015/02/03. Eng.

53. Kozora E, Ulug AM, Erkan D, Vo A, Filley CM, Ramon G, et al. Functional Magnetic Resonance Imaging of Working Memory and Executive Dysfunction in Systemic Lupus Erythematosus and Antiphospholipid Antibody-Positive Patients. Arthritis Care Res (Hoboken). 2016 Nov;68(11):1655-63. PubMed PMID: 26946337. Epub 2016/10/27. eng.

54. Toubi E, Shoenfeld Y. Livedo reticularis as a criterion for antiphospholipid syndrome. Clin Rev Allergy Immunol. 2007 Apr;32(2):138-44. PubMed PMID: 17916983. Epub 2007/10/06. eng.

55. Gibson GE, Su WP, Pittelkow MR. Antiphospholipid syndrome and the skin. J Am Acad Dermatol. 1997 Jun;36(6 Pt 1):970-82. PubMed PMID: 9204065. Epub 1997/06/01. eng.

56. Sciascia S, Baldovino S, Schreiber K, Solfietti L, Roccatello D. Antiphospholipid Syndrome and the Kidney. Semin Nephrol. 2015 Sep;35(5):478-86. PubMed PMID: 26573550. Epub 2015/11/18. eng.

57. Sciascia S, Cuadrado MJ, Khamashta M, Roccatello D. Renal involvement in antiphospholipid syndrome. Nat Rev Nephrol. 2014 May;10(5):279-89. PubMed PMID: 24642799. Epub 2014/03/20. eng.

58. Krause I, Blank M, Fraser A, Lorber M, Stojanovich L, Rovensky J, et al. The association of thrombocytopenia with systemic manifestations in the antiphospholipid syndrome. Immunobiology. 2005;210(10):749-54. PubMed PMID: 16325493. Epub 2005/12/06. eng.

59. Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol. 1987 Apr;68(1):215-22. PubMed PMID: 3652514. Pubmed Central PMCID: 1542695. Epub 1987/04/01. eng.

60. Giannakopoulos B, Krilis SA. How I treat the antiphospholipid syndrome. Blood. 2009 Sep 3;114(10):2020-30. PubMed PMID: 19587374. Epub 2009/07/10. eng.

61. Biggioggero M, Meroni PL. The geoepidemiology of the antiphospholipid antibody syndrome. Autoimmun Rev. 2010 Mar;9(5):A299-304. PubMed PMID: 19932199. Epub 2009/11/26. eng.

62. Villalta D, Bizzaro N, Corazza D, Tozzoli R, Tonutti E. Evaluation of a new automated enzyme fluoroimmunoassay using recombinant plasmid dsDNA for the detection of anti-dsDNA antibodies in SLE. J Clin Lab Anal. 2002;16(5):227-32. PubMed PMID: 12357451. Epub 2002/10/03. eng.

63. Bentow C, Lakos G, Rosenblum R, Bryant C, Seaman A, Mahler M. Clinical performance evaluation of a novel, automated chemiluminescent immunoassay, QUANTA Flash CTD Screen Plus. Immunol Res. 2015 Feb;61(1-2):110-6. PubMed PMID: 25420962. Epub 2014/11/26. eng.

64. Tozzoli R, Villalta D. Autoantibody profiling of patients with antiphospholipid syndrome using an automated multiplexed immunoassay system. Autoimmun Rev. 2014 Jan;13(1):59-63. PubMed PMID: 24075882. Epub 2013/10/01. eng.

65. Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. Lupus. 2002;11(5):269-75. PubMed PMID: 12090560. Epub 2002/07/02. eng.

66. Favaloro EJ, Wheatland L, Jovanovich S, Roberts-Thomson P, Wong RC. Internal quality control and external quality assurance in testing for antiphospholipid antibodies: Part I--

Anticardiolipin and anti-beta2-glycoprotein I antibodies. Semin Thromb Hemost. 2012 Jun;38(4):390-403. PubMed PMID: 22570184. Epub 2012/05/10. eng.

67. Audrain MA, Colonna F, Morio F, Hamidou MA, Muller JY. Comparison of different kits in the detection of autoantibodies to cardiolipin and beta2glycoprotein 1. Rheumatology (Oxford). 2004 Feb;43(2):181-5. PubMed PMID: 14585922. Epub 2003/10/31. eng.

68. de Laat B, Derksen RH, van Lummel M, Pennings MT, de Groot PG. Pathogenic anti-beta2-glycoprotein I antibodies recognize domain I of beta2-glycoprotein I only after a conformational change. Blood. 2006 Mar 1;107(5):1916-24. PubMed PMID: 16269621. Epub 2005/11/05. eng.

69. Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. Prog Hemost Thromb. 1972;1:75-95. PubMed PMID: 4569725. Epub 1972/01/01. eng.

70. Laurell AB, Nilsson IM. Hypergammaglobulinemia, circulating anticoagulant, and biologic false positive Wassermann reaction; a study in two cases. J Lab Clin Med. 1957 May;49(5):694-707. PubMed PMID: 13416728. Epub 1957/05/01. eng.

71. Lee SL, Sanders M. A disorder of blood coagulation in systemic lupus erythematosus. J Clin Invest. 1955 Dec;34(12):1814-22. PubMed PMID: 13271567. Pubmed Central PMCID: 438765. Epub 1955/12/01. eng.

72. Chighizola CB, Raschi E, Banzato A, Borghi MO, Pengo V, Meroni PL. The challenges of lupus anticoagulants. Expert Rev Hematol. 2016;9(4):389-400. PubMed PMID: 26789237. Epub 2016/01/21. eng.

73. Sciascia S, Khamashta MA, Bertolaccini ML. New tests to detect antiphospholipid antibodies: antiprothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies. Curr Rheumatol Rep. 2014 May;16(5):415. PubMed PMID: 24609824. Epub 2014/03/13. eng.

74. Mehdi H, Nunn M, Steel DM, Whitehead AS, Perez M, Walker L, et al. Nucleotide sequence and expression of the human gene encoding apolipoprotein H (beta 2-glycoprotein I). Gene. 1991 Dec 15;108(2):293-8. PubMed PMID: 1748314. Epub 1991/12/15. eng.

75. Okkels H, Rasmussen TE, Sanghera DK, Kamboh MI, Kristensen T. Structure of the human beta2-glycoprotein I (apolipoprotein H) gene. Eur J Biochem. 1999 Jan;259(1-2):435-40. PubMed PMID: 9914524. Epub 1999/01/23. eng.

76. Sodin-Semrl S, Rozman B. Beta2-glycoprotein I and its clinical significance: from gene sequence to protein levels. Autoimmun Rev. 2007;6(8):547-52. Epub Mar 2.

77. Wang HH, Chiang AN. Cloning and characterization of the human beta2-glycoprotein I (beta2-GPI) gene promoter: roles of the atypical TATA box and hepatic nuclear factor-1alpha in regulating beta2-GPI promoter activity. Biochem J. 2004 Jun 1;380(Pt 2):455-63. PubMed PMID: 14984368. Pubmed Central PMCID: 1224180. Epub 2004/02/27. eng.

78. Weber M, Hayem G, DeBandt M, Palazzo E, Roux S, Kahn MF, et al. The family history of patients with primary or secondary antiphospholipid syndrome (APS). Lupus. 2000;9(4):258-63. PubMed PMID: 10866096. Epub 2000/06/24. eng.

79. Cleve H. [Genetic studies on the deficiency of beta 2-glycoprotein I of human serum]. Humangenetik. 1968;5(4):294-304. PubMed PMID: 5670608. Epub 1968/01/01. Genetic Studies on the Deficiency of beta 2-Glycoprotein I of Human Serum. ger.

80. Roychoudhury AK, Nei M. Human polymorphic genes : world distribution. New York: Oxford University Press; 1988. viii, 393 p. p.

81. Kamboh MI, Sanghera DK, Mehdi H, Nestlerode CS, Chen Q, Khalifa O, et al. Single nucleotide polymorphisms in the coding region of the apolipoprotein H (beta2-glycoprotein I) gene and their correlation with the protein polymorphism, anti-beta2glycoprotein I antibodies and cardiolipin binding: description of novel haplotypes and their evolution. Ann Hum Genet. 2004 Jul;68(Pt 4):285-99. PubMed PMID: 15225155. Epub 2004/07/01. eng.

82. Prieto GAC, A.R.; Cabiedes, J. Polimorfismo de la Beta 2 Glucoproteina I. Relevancia en el síndrome antifosfolípidos. Rev Esp Reumatol. 2002;29(8):8. spanish.

83. Chen Q, Kamboh MI. Complete DNA sequence variation in the apolipoprotein H (beta-glycoprotein I) gene and identification of informative SNPs. Ann Hum Genet. 2006 Jan;70(Pt 1):1-11. PubMed PMID: 16441253. Epub 2006/01/31. eng.

84. Vlachoyiannopoulos PG, Routsias JG. A novel mechanism of thrombosis in antiphospholipid antibody syndrome. J Autoimmun. 2010 Nov;35(3):248-55. PubMed PMID: 20638238. Epub 2010/07/20. eng.

85. Yasuda S, Atsumi T, Matsuura E, Kaihara K, Yamamoto D, Ichikawa K, et al. Significance of valine/leucine247 polymorphism of beta2-glycoprotein I in antiphospholipid syndrome: increased reactivity of anti-beta2-glycoprotein I autoantibodies to the valine247 beta2-glycoprotein I variant. Arthritis Rheum. 2005 Jan;52(1):212-8. PubMed PMID: 15641049. Epub 2005/01/11. eng.

86. von Scheven E, Elder ME. Association between beta2-glycoprotein I gene polymorphisms and pediatric SLE and antiphospholipid antibodies. Lupus. 2005;14(6):440-4. PubMed PMID: 16038107. Epub 2005/07/26. eng.

87. Prieto GA, Cabral AR, Zapata-Zuniga M, Simon AJ, Villa AR, Alarcon-Segovia D, et al. Valine/valine genotype at position 247 of the beta2-glycoprotein I gene in Mexican patients with primary antiphospholipid syndrome: association with anti-beta2-glycoprotein I antibodies. Arthritis Rheum. 2003 Feb;48(2):471-4. PubMed PMID: 12571857. Epub 2003/02/07. eng.

88. Pernambuco-Climaco JM, Brochado MJ, Freitas MV, Roselino AM, Louzada-Junior P. Val/Leu247 polymorphism of beta2-glycoprotein I in Brazilian patients with antiphospholipid syndrome--a genetic risk factor? Ann N Y Acad Sci. 2009 Sep;1173:509-14. PubMed PMID: 19758193. Epub 2009/09/18. eng.

89. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci U S A. 1990 Jun;87(11):4120-4. PubMed PMID: 2349221.

90. Koike T, Ichikawa K, Kasahara H, Atsumi T, Tsutsumi A, Matsuura E. Epitopes on beta2-GPI recognized by anticardiolipin antibodies. Lupus. 1998;7 Suppl 2:S14-7. PubMed PMID: 9814665. Epub 1998/11/14. eng.

91. Kamboh MI, Manzi S, Mehdi H, Fitzgerald S, Sanghera DK, Kuller LH, et al. Genetic variation in apolipoprotein H (beta2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. Lupus. 1999;8(9):742-50. PubMed PMID: 10602447. Epub 1999/12/22. eng.

92. Tripodi A, de Groot PG, Pengo V. Antiphospholipid syndrome: laboratory detection, mechanisms of action and treatment. J Intern Med. 2011 Aug;270(2):110-22. PubMed PMID: 21323768. Epub 2011/02/18. eng.

93. Urbanus RT, Siegerink B, Roest M, Rosendaal FR, de Groot PG, Algra A. Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. Lancet Neurol. 2009 Nov;8(11):998-1005. PubMed PMID: 19783216. Epub 2009/09/29. eng.

94. Erkan D, Yazici Y, Peterson MG, Sammaritano L, Lockshin MD. A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. Rheumatology (Oxford). 2002 Aug;41(8):924-9. PubMed PMID: 12154210. Epub 2002/08/03. eng.

95. Tarr T, Lakos G, Bhattoa HP, Shoenfeld Y, Szegedi G, Kiss E. Analysis of risk factors for the development of thrombotic complications in antiphospholipid antibody positive lupus patients. Lupus. 2007;16(1):39-45. PubMed PMID: 17283584. Epub 2007/02/08. eng.

96. Hereng T, Lambert M, Hachulla E, Samor M, Dubucquoi S, Caron C, et al. Influence of aspirin on the clinical outcomes of 103 anti-phospholipid antibodies-positive patients. Lupus. 2008 Jan;17(1):11-5. PubMed PMID: 18089677. Epub 2007/12/20. eng.

97. Erkan D, Merrill JT, Yazici Y, Sammaritano L, Buyon JP, Lockshin MD. High thrombosis rate after fetal loss in antiphospholipid syndrome: effective prophylaxis with aspirin. Arthritis Rheum. 2001 Jun;44(6):1466-7. PubMed PMID: 11407709. Epub 2001/06/16. eng.

98. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med. 2000 Mar 2;342(9):605-12. PubMed PMID: 10699159. Epub 2000/03/04. eng.

99. Hamed MO, Chen Y, Pasea L, Watson CJ, Torpey N, Bradley JA, et al. Early graft loss after kidney transplantation: risk factors and consequences. Am J Transplant. 2015 Jun;15(6):1632-43. PubMed PMID: 25707303. Epub 2015/02/25. eng.

100. Ponticelli C, Moia M, Montagnino G. Renal allograft thrombosis. Nephrol Dial Transplant. 2009 May;24(5):1388-93. PubMed PMID: 19182239. Epub 2009/02/03. eng.

101. Morales JM, Marcen R, Andres A, Molina MG, Castillo DD, Cabello M, et al. Renal transplantation in the modern immunosuppressive era in Spain: four-year results from a multicenter database focus on post-transplant cardiovascular disease. Kidney Int Suppl. 2008 Dec;74(111):S94-9. PubMed PMID: 19034336. Epub 2008/11/27. eng.

102. Martinez-Flores JA, Serrano M, Perez D, Camara AG, Lora D, Morillas L, et al. Circulating Immune Complexes of IgA Bound to Beta 2 Glycoprotein are Strongly Associated with the Occurrence of Acute Thrombotic Events. J Atheroscler Thromb. 2016 Oct 1;23(10):1242-53. PubMed PMID: 27063992. Epub 2016/04/12. Eng.

103. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med. 1969 Apr 3;280(14):735-9. PubMed PMID: 4886455. Epub 1969/04/03. eng.

104. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med. 2010 Oct 7;363(15):1451-62. PubMed PMID: 20925547. Epub 2010/10/12. eng.

105. Sanchez-Zapardiel E, Castro-Panete MJ, Castillo-Rama M, Morales P, Lora-Pablos D, Valero-Hervas D, et al. Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. Transplantation. 2013 Jul 15;96(1):70-8. PubMed PMID: 23624543. Epub 2013/04/30. eng.

106. Sigdel TK, Sarwal MM. Moving beyond HLA: a review of nHLA antibodies in organ transplantation. Hum Immunol. 2013 Nov;74(11):1486-90. PubMed PMID: 23876683. Epub 2013/07/24. eng.

107. Serrano M, Martinez-Flores JA, Castro MJ, Garcia F, Lora D, Perez D, et al. Renal transplantation dramatically reduces IgA anti-beta-2-glycoprotein I antibodies in patients with endstage renal disease. J Immunol Res. 2014;2014:641962. PubMed PMID: 24818167. Pubmed Central PMCID: 4003762. Epub 2014/05/13. eng.

108. Serrano A, Garcia F, Serrano M, Ramirez E, Alfaro FJ, Lora D, et al. IgA antibodies against beta2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. Kidney Int. 2012 Jun;81(12):1239-44. PubMed PMID: 22358146. Epub 2012/02/24. eng.

109. Hadhri S, Rejeb MB, Belarbia A, Achour A, Skouri H. Hemodialysis duration, Human platelet antigen HPA-3 and IgA Isotype of anti-beta2glycoprotein I antibodies are associated with native arteriovenous fistula failure in Tunisian hemodialysis patients. Thromb Res. 2013 May;131(5):e202-9. PubMed PMID: 23538147. Epub 2013/03/30. eng.

110. Morales JM, Martinez-Flores JA, Serrano M, Castro MJ, Alfaro FJ, Garcia F, et al. Association of Early Kidney Allograft Failure with Preformed IgA Antibodies to beta2-Glycoprotein I. J Am Soc Nephrol. 2015 Mar;26(3):735-45. PubMed PMID: 25071084. Epub 2014/07/30. eng.

111. Branch DW. Summary of the 11th International Congress on antiphospholipid autoantibodies, Australia, November 2004. J Reprod Immunol. 2005 Jun;66(1):85-90. PubMed PMID: 16184635. Epub 2005/09/27. eng.

112. Ruiz-Garcia R, Serrano M, Angel Martinez-Flores J, Mora S, Morillas L, Martin-Mola MA, et al. Isolated IgA Anti- beta 2 Glycoprotein I Antibodies in Patients with Clinical Criteria for

Antiphospholipid Syndrome. J Immunol Res. 2014;2014:704395. PubMed PMID: 24741618. Pubmed Central PMCID: 3987939. Epub 2014/04/18. eng.

113. Murthy V, Willis R, Romay-Penabad Z, Ruiz-Limon P, Martinez-Martinez LA, Jatwani S, et al. Value of isolated IgA anti-beta2 -glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. Arthritis Rheum. 2013 Dec;65(12):3186-93. PubMed PMID: 23983008. Epub 2013/08/29. eng.

114. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC, et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum. 2012 Jan;64(1):1-10. PubMed PMID: 21953634. Epub 2011/09/29. eng.

115. Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012 Aug;64(8):2677-86. PubMed PMID: 22553077. Pubmed Central PMCID: 3409311. Epub 2012/05/04. eng.

116. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. N Engl J Med. 2013 Mar 14;368(11):1033-44. PubMed PMID: 23484830. Epub 2013/03/15. eng.

117. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol. 2011 Jun;7(6):330-9. PubMed PMID: 21556027. Epub 2011/05/11. eng.

118. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramon E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis. 2015 Jun;74(6):1011-8. PubMed PMID: 24464962. Epub 2014/01/28. eng.

119. Tortosa C, Cabrera-Marante O, Serrano M, Martinez-Flores JA, Perez D, Lora D, et al. Incidence of thromboembolic events in asymptomatic carriers of IgA anti ss2 glycoprotein-I antibodies. PLoS One. 2017;12(7):e0178889. PubMed PMID: 28727732. Epub 2017/07/21. eng.

120. Devreese KM. Antiphospholipid antibodies: evaluation of the thrombotic risk. Thromb Res. 2012 Oct;130 Suppl 1:S37-40. PubMed PMID: 23026657. Epub 2012/10/03. eng.

121. Martinez-Flores JA, Serrano M, Perez D, Lora D, Paz-Artal E, Morales JM, et al. Detection of circulating immune complexes of human IgA and beta 2 glycoprotein I in patients with antiphospholipid syndrome symptomatology. J Immunol Methods. 2015 Jul;422:51-8. PubMed PMID: 25865263. Epub 2015/04/14. eng.

122. Banzato A, Frasson R, Acquasaliente L, Bison E, Bracco A, Denas G, et al. Circulating beta2 glycoprotein I-IgG anti-beta2 glycoprotein I immunocomplexes in patients with definite antiphospholipid syndrome. Lupus. 2012 Jun;21(7):784-6. PubMed PMID: 22635233. Epub 2012/05/29. eng.

123. Biasiolo A, Rampazzo P, Brocco T, Barbero F, Rosato A, Pengo V. [Anti-beta2 glycoprotein I-beta2 glycoprotein I] immune complexes in patients with antiphospholipid syndrome and other autoimmune diseases. Lupus. 1999;8(2):121-6. PubMed PMID: 10192506. Epub 1999/04/07. eng.

124. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant. 2015 Oct;34(10):1264-77. PubMed PMID: 26454740. Epub 2015/10/12. eng.

125. Stehlik J, Feldman DS, Brown RN, VanBakel AB, Russel SD, Ewald GA, et al. Interactions among donor characteristics influence post-transplant survival: a multi-institutional analysis. J Heart Lung Transplant. 2010 Mar;29(3):291-8. PubMed PMID: 19804989.

126. Serrano M, Martínez JA, Castro MJ, García F, Lora D, Perez D, et al. Renal Transplantation Dramatically Reduces IgA Anti-beta-2-glycoprotein I Antibodies in Patients with Endstage Renal Disease J Immunol Res. 2014;2014(Article ID 641962):10 pages.

127. Lee YH, Choi SJ, Ji JD, Song GG. Association between the valine/leucine247 polymorphism of beta2-glycoprotein I and susceptibility to anti-phospholipid syndrome: a meta-analysis. Lupus. 2012 Jul;21(8):865-71. PubMed PMID: 22399073. Epub 2012/03/09. eng.

128. Hirose N, Williams R, Alberts AR, Furie RA, Chartash EK, Jain RI, et al. A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. Arthritis Rheum. 1999 Aug;42(8):1655-61. PubMed PMID: 10446865. Epub 1999/08/14. eng.

129. Harel M, Aron-Maor A, Sherer Y, Blank M, Shoenfeld Y. The infectious etiology of the antiphospholipid syndrome: links between infection and autoimmunity. Immunobiology. 2005;210(10):743-7. PubMed PMID: 16325492. Epub 2005/12/06. eng.

130. Keller AK, Jorgensen TM, Jespersen B. Identification of risk factors for vascular thrombosis may reduce early renal graft loss: a review of recent literature. J Transplant. 2012;2012:793461. PubMed PMID: 22701162. Pubmed Central PMCID: 3369524. Epub 2012/06/16. eng.

131. Amezquita Y, Mendez C, Fernandez A, Caldes S, Pascual J, Muriel A, et al. Risk factors for early renal graft thrombosis: a case-controlled study in grafts from the same donor. Transplant Proc. 2008 Nov;40(9):2891-3. PubMed PMID: 19010138. Epub 2008/11/18. eng.

132. Sadej P, Feld RI, Frank A. Transplant renal vein thrombosis: role of preoperative and intraoperative Doppler sonography. Am J Kidney Dis. 2009 Dec;54(6):1167-70. PubMed PMID: 19748716. Epub 2009/09/15. eng.

133. Bakir N, Sluiter WJ, Ploeg RJ, van Son WJ, Tegzess AM. Primary renal graft thrombosis. Nephrol Dial Transplant. 1996 Jan;11(1):140-7. PubMed PMID: 8649623. Epub 1996/01/01. eng.

134. Phelan PJ, O'Kelly P, Tarazi M, Tarazi N, Salehmohamed MR, Little DM, et al. Renal allograft loss in the first post-operative month: causes and consequences. Clin Transplant. 2012 Jul-Aug;26(4):544-9. PubMed PMID: 23050275. Epub 2012/10/11. eng.

135. Andres A, Morales JM, Herrero JC, Praga M, Morales E, Hernandez E, et al. Double versus single renal allografts from aged donors. Transplantation. 2000 May 27;69(10):2060-6. PubMed PMID: 10852597. Epub 2000/06/14. eng.

136. Tripodi A, Branchi A, Chantarangkul V, Clerici M, Merati G, Artoni A, et al. Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay. J Thromb Thrombolysis. 2011 Feb;31(2):165-72. PubMed PMID: 20640482. Epub 2010/07/20. eng.

137. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation. 2008 Jan 1;117(1):93-102. PubMed PMID: 18086925. Epub 2007/12/19. eng.

138. Li J, Kim K, Barazia A, Tseng A, Cho J. Platelet-neutrophil interactions under thromboinflammatory conditions. Cell Mol Life Sci. 2015 Feb 4. PubMed PMID: 25650236. Epub 2015/02/05. Eng.

139. Meroni PL, Shoenfeld Y. Predictive, protective, orphan autoantibodies: the example of anti-phospholipid antibodies. Autoimmun Rev. 2008 Sep;7(8):585-7. PubMed PMID: 18706525. Epub 2008/08/19. eng.

140. Tanimura K, Jin H, Suenaga T, Morikami S, Arase N, Kishida K, et al. beta2-glycoprotein I / HLA class II complexes are novel autoantigens in antiphospholipid syndrome. Blood. 2015 Mar 2. PubMed PMID: 25733579. Epub 2015/03/04. Eng.

141. Erkan D, Aguiar CL, Andrade D, Cohen H, Cuadrado MJ, Danowski A, et al. 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. Autoimmun Rev. 2014 Jun;13(6):685-96. PubMed PMID: 24468415. Epub 2014/01/29. eng.

142. Pawlicki J, Cierpka L, Krol R, Ziaja J. Risk factors for early hemorrhagic and thrombotic complications after kidney transplantation. Transplant Proc. 2011 Oct;43(8):3013-7. PubMed PMID: 21996213. Epub 2011/10/15. eng.

143. Lonze BE, Zachary AA, Magro CM, Desai NM, Orandi BJ, Dagher NN, et al. Eculizumab prevents recurrent antiphospholipid antibody syndrome and enables successful renal transplantation. Am J Transplant. 2014 Feb;14(2):459-65. PubMed PMID: 24400968. Epub 2014/01/10. eng.

144. Daha NA, Banda NK, Roos A, Beurskens FJ, Bakker JM, Daha MR, et al. Complement activation by (auto-) antibodies. Mol Immunol. 2011 Aug;48(14):1656-65. PubMed PMID: 21757235. Epub 2011/07/16. eng.

145. Canaud G, Bienaime F, Tabarin F, Bataillon G, Seilhean D, Noel LH, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. N Engl J Med. 2014 Jul 24;371(4):303-12. PubMed PMID: 25054716. Epub 2014/07/24. eng.

146. Sanchez-Fructuoso AI, Marques M, Prats D, Conesa J, Calvo N, Perez-Contin MJ, et al. Victims of cardiac arrest occurring outside the hospital: a source of transplantable kidneys. Ann Intern Med. 2006 Aug 1;145(3):157-64. PubMed PMID: 16880457. Epub 2006/08/02. eng.

147. Andres A, Polanco N, Cebrian MP, Sol Vereda M, Vazquez S, Nuno E, et al. Kidneys from elderly deceased donors discarded for transplantation. Transplant Proc. 2009 Jul-Aug;41(6):2379-81. PubMed PMID: 19715925. Epub 2009/09/01. eng.

148. Uthman I, Khamashta M. Antiphospholipid syndrome and the kidneys. Semin Arthritis Rheum. 2006 Jun;35(6):360-7. PubMed PMID: 16765713.

149. Pons-Estel GJ, Cervera R. Renal involvement in antiphospholipid syndrome. Curr Rheumatol Rep. 2014 Feb;16(2):397. PubMed PMID: 24357443. Epub 2013/12/21. eng.

150. Mattia E, Ruffatti A, Tonello M, Meneghel L, Robecchi B, Pittoni M, et al. IgA anticardiolipin and IgA anti-beta2 glycoprotein I antibody positivity determined by fluorescence enzyme immunoassay in primary antiphospholipid syndrome. Clin Chem Lab Med. 2014 Sep;52(9):1329-33. PubMed PMID: 24651022. Epub 2014/03/22. eng.

151. Sweiss NJ, Bo R, Kapadia R, Manst D, Mahmood F, Adhikari T, et al. IgA anti-beta2-glycoprotein I autoantibodies are associated with an increased risk of thromboembolic events in patients with systemic lupus erythematosus. PLoS One. 2010;5(8):e12280. PubMed PMID: 20808864. Pubmed Central PMCID: 2924386. Epub 2010/09/03. eng.

152. Andreoli L, Chighizola CB, Nalli C, Gerosa M, Borghi MO, Pregnolato F, et al. Antiphospholipid Antibody Profiling: The Detection of IgG Antibodies Against beta2glycoprotein I Domain 1 and 4/5 Offers Better Clinical Characterization: The ratio between anti-D1 and anti-D4/5 as a new useful biomarker for APS. Arthritis Rheumatol. 2015 May 4. PubMed PMID: 25939498. Epub 2015/05/06. Eng.

153. Oku K, Atsumi T, Bohgaki M, Amengual O, Kataoka H, Horita T, et al. Complement activation in patients with primary antiphospholipid syndrome. Ann Rheum Dis. 2009 Jun;68(6):1030-5. PubMed PMID: 18625630. Epub 2008/07/16. eng.

154. Lim W. Complement and the antiphospholipid syndrome. Curr Opin Hematol. 2011 Sep;18(5):361-5. PubMed PMID: 21730831. Epub 2011/07/07. eng.

155. Raschi E, Testoni C, Borghi MO, Fineschi S, Meroni PL. Endothelium activation in the anti-phospholipid syndrome. Biomed Pharmacother. 2003 Sep;57(7):282-6. PubMed PMID: 14499174. Epub 2003/09/23. eng.

156. Lutters BC, Derksen RH, Tekelenburg WL, Lenting PJ, Arnout J, de Groot PG. Dimers of beta 2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2'. J Biol Chem. 2003 Sep 5;278(36):33831-8. PubMed PMID: 12807892. Epub 2003/06/17. eng.

157. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. N Engl J Med. 2002 Mar 7;346(10):752-63. PubMed PMID: 11882732.

158. Matsuura E, Shen L, Matsunami Y, Quan N, Makarova M, Geske FJ, et al. Pathophysiology of beta2-glycoprotein I in antiphospholipid syndrome. Lupus. 2010 Apr;19(4):379-84. PubMed PMID: 20353973. Epub 2010/04/01. eng.

159. Punnialingam S, Khamashta MA. Duration of anticoagulation treatment for thrombosis in APS: is it ever safe to stop? Curr Rheumatol Rep. 2013 Apr;15(4):318. PubMed PMID: 23494857. Epub 2013/03/16. eng.

160. Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, et al. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. Blood. 2005 Oct 1;106(7):2340-6. PubMed PMID: 15956288. Epub 2005/06/16. eng.

161. Barbhaiya M, Erkan D. Primary thrombosis prophylaxis in antiphospholipid antibody-positive patients: where do we stand? Curr Rheumatol Rep. 2011 Feb;13(1):59-69. PubMed PMID: 21104348. Epub 2010/11/26. eng.

162. Piazza G, Nguyen TN, Cios D, Labreche M, Hohlfelder B, Fanikos J, et al. Anticoagulation-associated adverse drug events. Am J Med. 2011 Dec;124(12):1136-42. PubMed PMID: 22114827. Pubmed Central PMCID: 3224344. Epub 2011/11/26. eng.

163. Bertero MT. Primary prevention in antiphospholipid antibody carriers. Lupus. 2012 Jun;21(7):751-4. PubMed PMID: 22635222. Epub 2012/05/29. eng.

164. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet. 2010 Oct 30;376(9751):1498-509. PubMed PMID: 20822807. Epub 2010/09/09. eng.

165. Morales JM, Serrano M, Martinez-Flores JA, Perez D, Castro MJ, Sanchez E, et al. The Presence of Pretransplant Antiphospholipid Antibodies IgA Anti-beta-2-Glycoprotein I as a Predictor of Graft Thrombosis After Renal Transplantation. Transplantation. 2017 May 2;101(3):597-607. PubMed PMID: 27140515. Epub 2016/05/04. Eng.

166. Verhave JC, Tagalakis V, Suissa S, Madore F, Hebert MJ, Cardinal H. The risk of thromboembolic events in kidney transplant patients. Kidney Int. 2014 Jun;85(6):1454-60. PubMed PMID: 24429408. Epub 2014/01/17. eng.

167. Remkova A, Remko M. The role of renin-angiotensin system in prothrombotic state in essential hypertension. Physiol Res. 2010;59(1):13-23. PubMed PMID: 19249905. Epub 2009/03/03. eng.

168. Chen FA, Chien CC, Chen YW, Wu YT, Lin CC. Angiotensin Converting-Enzyme Inhibitors, Angiotensin Receptor Blockers, and Calcium Channel Blockers Are Associated with Prolonged Vascular Access Patency in Uremic Patients Undergoing Hemodialysis. PLoS One. 2016;11(11):e0166362. PubMed PMID: 27832203. Pubmed Central PMCID: 5104366. Epub 2016/11/11. eng.

169. Moscarelli L, Zanazzi M, Bertoni E, Caroti L, Rosso G, Farsetti S, et al. Renin angiotensin system blockade and activated vitamin D as a means of preventing deep vein thrombosis in renal transplant recipients. Clin Nephrol. 2011 May;75(5):440-50. PubMed PMID: 21543024. Epub 2011/05/06. eng.

170. Erkan D, Harrison MJ, Levy R, Peterson M, Petri M, Sammaritano L, et al. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. Arthritis Rheum. 2007 Jul;56(7):2382-91. PubMed PMID: 17599766. Epub 2007/06/30. eng.

171. Al Marzooqi A, Leone A, Al Saleh J, Khamashta M. Current status and future prospects for the treatment of antiphospholipid syndrome. Expert Rev Clin Immunol. 2016 Sep;12(9):927-35. PubMed PMID: 27117597. Epub 2016/04/28. eng.

172. Belizna C. Hydroxychloroquine as an anti-thrombotic in antiphospholipid syndrome. Autoimmun Rev. 2015 Apr;14(4):358-62. PubMed PMID: 25534016. Epub 2014/12/24. eng.

173. Ragusa MA, Costa S, Cefalu AB, Noto D, Fayer F, Travali S, et al. RT-PCR and in situ hybridization analysis of apolipoprotein H expression in rat normal tissues. Int J Mol Med. 2006 Sep;18(3):449-55. PubMed PMID: 16865229. Epub 2006/07/26. eng.

174. Morales JM, Serrano M, Martinez-Flores JA, Perez D, Castro MJ, Sanchez E, et al. The Presence of Pretransplant Antiphospholipid Antibodies IgA Anti-beta-2-Glycoprotein I as a Predictor

of Graft Thrombosis After Renal Transplantation. Transplantation. 2016 May 2. PubMed PMID: 27140515. Epub 2016/05/04. Eng.

175. Cosio Carmena MD, Gomez Bueno M, Almenar L, Delgado JF, Arizon JM, Gonzalez Vilchez F, et al. Primary graft failure after heart transplantation: characteristics in a contemporary cohort and performance of the RADIAL risk score. J Heart Lung Transplant. 2013 Dec;32(12):1187-95. PubMed PMID: 24263021.

176. Almenar L, Vicente JL, Torregrosa S, Osa A, Martinez-Dolz L, Gomez-Plana J, et al. [Predictive variables of early mortality after orthotopic heart transplant in adults]. Rev Esp Cardiol. 1997 Sep;50(9):628-34. PubMed PMID: 9380932. Epub 1998/02/12. Variables predictoras de mortalidad precoz tras el trasplante cardiaco ortotopico en adultos. spa.

177. Muellner SK, Haut ER, Streiff MB, Holcomb JB, Cotton BA. ABO blood group as a potential risk factor for venous thromboembolism in acutely injured patients. Thromb Haemost. 2011 Jan;105(1):5-13. PubMed PMID: 21103665. Epub 2010/11/26. eng.

178. Dentali F, Sironi AP, Ageno W, Turato S, Bonfanti C, Frattini F, et al. Non-O blood type is the commonest genetic risk factor for VTE: results from a meta-analysis of the literature. Semin Thromb Hemost. 2012 Jul;38(5):535-48. PubMed PMID: 22740183. Epub 2012/06/29. eng.

179. Vasan SK, Rostgaard K, Majeed A, Ullum H, Titlestad KE, Pedersen OB, et al. ABO Blood Group and Risk of Thromboembolic and Arterial Disease: A Study of 1.5 Million Blood Donors. Circulation. 2016 Apr 12;133(15):1449-57; discussion 57. PubMed PMID: 26939588. Epub 2016/03/05. Eng.

Don Jose Angel Martínez Flores, Doctor en Farmacia, con NIF 47289211S,
Teléfono 699489571 y e-mail martinezfloresja@gmail.com

MANIFIESTO QUE:

Como coautor DOCTOR de los artículos integrados en la tesis doctoral que presenta Don Manuel Serrano Blanco, quedo informado de que tiene solicitada autorización de la Comisión Académica del Doctorado de Investigación Biomédica de la UCM para la presentación del ejemplar de su tesis en formato de compendio de publicaciones y, por lo tanto, autorizo la inclusión de los artículos de los que formo parte como coautor y que se relacionan a continuación:

1: Early mortality after heart transplantation related to IgA anti-β2-glycoprotein I antibodies.
J Heart Lung Transplant. 2017 Nov;36(11):1258-1265. PMID: 28579112.
doi: 10.1016/j.healun.2017.05.016.

2: β(2)-Glycoprotein I/IgA Immune Complexes: A Marker to Predict Thrombosis After Renal Transplantation in Patients With Antiphospholipid Antibodies.
Circulation. 2017 May 16;135(20):1922-1934. PMID: 28249878.
doi: 10.1161/CIRCULATIONAHA.116.025992.

3: Study of β(2)-Glycoprotein I Polymorphisms in Patients With Chronic Renal Failure as a Predisposing Factor for the Development of Anti-β(2)-Glycoprotein I Auto-Antibodies.
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Front. Immunol. 2018. 9: 468. Aceptado para publicación: 21 Feb 2018
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Madrid a 5 de Marzo de 2018

Don Jose María Morales Cerdán, Doctor en Medicin y Cirugia, con NIF 17832140X, Teléfono 915779548 y e-mail jmorales@h12o.es

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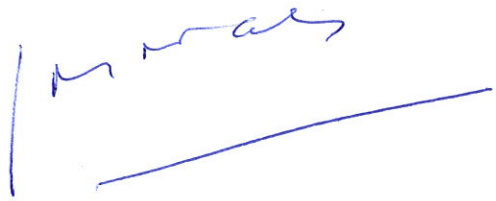

Don Antonio Serrano Hernández, Doctor en Medicina y Cirugía, con con NIF 50028413D , Teléfono 652085293 y e-mail aserrano@h12o.es

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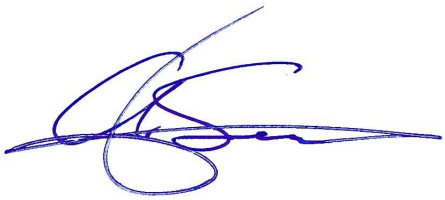

Don Juan Delgado Jiménez, Doctor en Medicina y Cirugía, con con NIF 5374416Y , Teléfono 639122454 y e-mail juan.f.delgado1@gmail.com

MANIFIESTO QUE:

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