

L'autoimmunità organo-specifica nella medicina clinica e di laboratorio

Ruolo della diagnostica auto-anticorpale in trapiantologia

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16132 GENOVA**

Diagnostica autoanticorpale in trapiantologia

Trapianto d'organo

- **Trapianto cardiaco**
- **Trapianto renale**

Trapianto midollo osseo

**Per malattie
oncoematologiche**

**Per malattie
autoimmuni
sistemiche**

Diagnostica autoanticorpale in trapiantologia

**Possibili
applicazioni**

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graph TD; A[Possibili applicazioni] --> B[Ricerca di autoanticorpi comparsi dopo il trapianto]; A --> C[Follow-up di autoanticorpi presenti prima del trapianto]; A --> D[Ricerca di autoanticorpi nel donatore];
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Ricerca di autoanticorpi
comparsi dopo il
trapianto

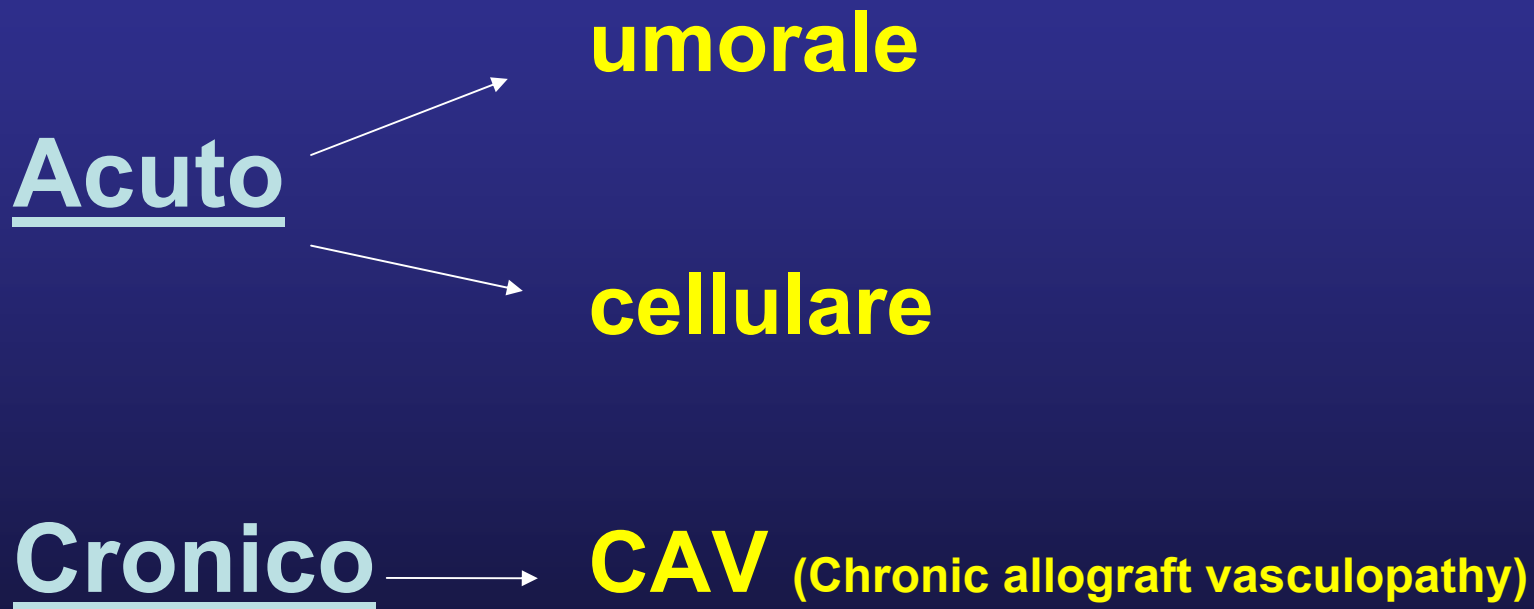
Follow-up di
autoanticorpi
presenti prima
del trapianto

Ricerca di
autoanticorpi nel
donatore

Diagnostica autoanticorpale in trapiantologia

- **Rigetto**
- **Recidiva/nuova malattia dell'organo trapiantato**
- **GVHD**
- **Scomparsa di malattie autoimmuni dopo trapianto**

Rigetto di trapianto cardiaco



Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection

2004		1990	
Grade 0 R₀	No rejection	Grade 0	No rejection
Grade 1 R, mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage	Grade 1, mild	
		A—Focal	Focal perivascular and/or interstitial infiltrate without myocyte damage
		B—Diffuse	Diffuse infiltrate without myocyte damage
		Grade 2 moderate (focal)	One focus of infiltrate with associated myocyte damage
Grade 2 R, moderate	Two or more foci of infiltrate with associated myocyte damage	Grade 3, moderate	
		A—Focal	Multifocal infiltrate with myocyte damage
Grade 3 R, severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis	B—Diffuse	Diffuse infiltrate with myocyte damage
		Grade 4, severe	Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ± hemorrhage + vasculitis

ISHLT Recommendations for Acute Antibody-Mediated Rejection (AMR)

	2004	1990
AMR 0	Negative for acute antibody-mediated rejection	
	No histologic or immunopathologic features of AMR	
AMR 1	Positive for AMR	Humoral rejection (positive immunofluorescence, vasculitis or severe edema in absence of cellular infiltrate) recorded as additional required information
	Histologic features of AMR	
	Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)	

Rigetto di trapianto renale

- **Iperacuto (Ab preformati, C')**
- **Accelerato (Ab, cell.)**
- **Acuto (Ab, cell.)**
- **Cronico (Ab, meccanismi non spec.)**



The NEW ENGLAND
JOURNAL of MEDICINE

Antibodies against MICA Antigens and Kidney-Transplant Rejection

***Yizhou Zou, M.D., Peter Stastny, M.D., Caner Süsal, M.D.,
Bernd Döhler, Ph.D., and Gerhard Opelz, M.D***

Activation of Autoimmune B Cells and Chronic Rejection

Marlene L. Rose

Allotransplantation into immunosuppressed individuals results in long-term survival of grafts. However, the grafts are damaged, probably at many stages before, during and after implantation. The hypothesis to be presented is that release of antigens and autoantigens from the chronically damaged graft results in breaking tolerance to self-antigens and an autoimmune response. There is experimental evidence that autoimmune responses following allotransplantation are damaging and cause accelerated graft rejection.

(Transplantation 2005;79: S22–S24)

An association between antibodies specific for endothelial cells and renal transplant failure

C Perre, PEC Brenchle, RWG Johnson and S Martin

Table 1 Association between IgG antibodies directed against EAHy.926 and renal transplant failure

	ECA positive (%) (<i>n</i>)	ECA negative (%) (<i>n</i>)
Failed transplants (105)	13.5 (14)	86.5 (91)
Successful transplants (94)	3 (3)	97 (91)

$p = 0.02$; Fisher's exact test.

Where ECA, endothelial cell reactive antibodies; *n*, number of patients.

Transplant Immunology 1998; 6: 101–106

THE CLINICAL SIGNIFICANCE OF ANTIBODIES TO HUMAN VASCULAR ENDOTHELIAL CELLS AFTER CARDIAC TRANSPLANTATION.

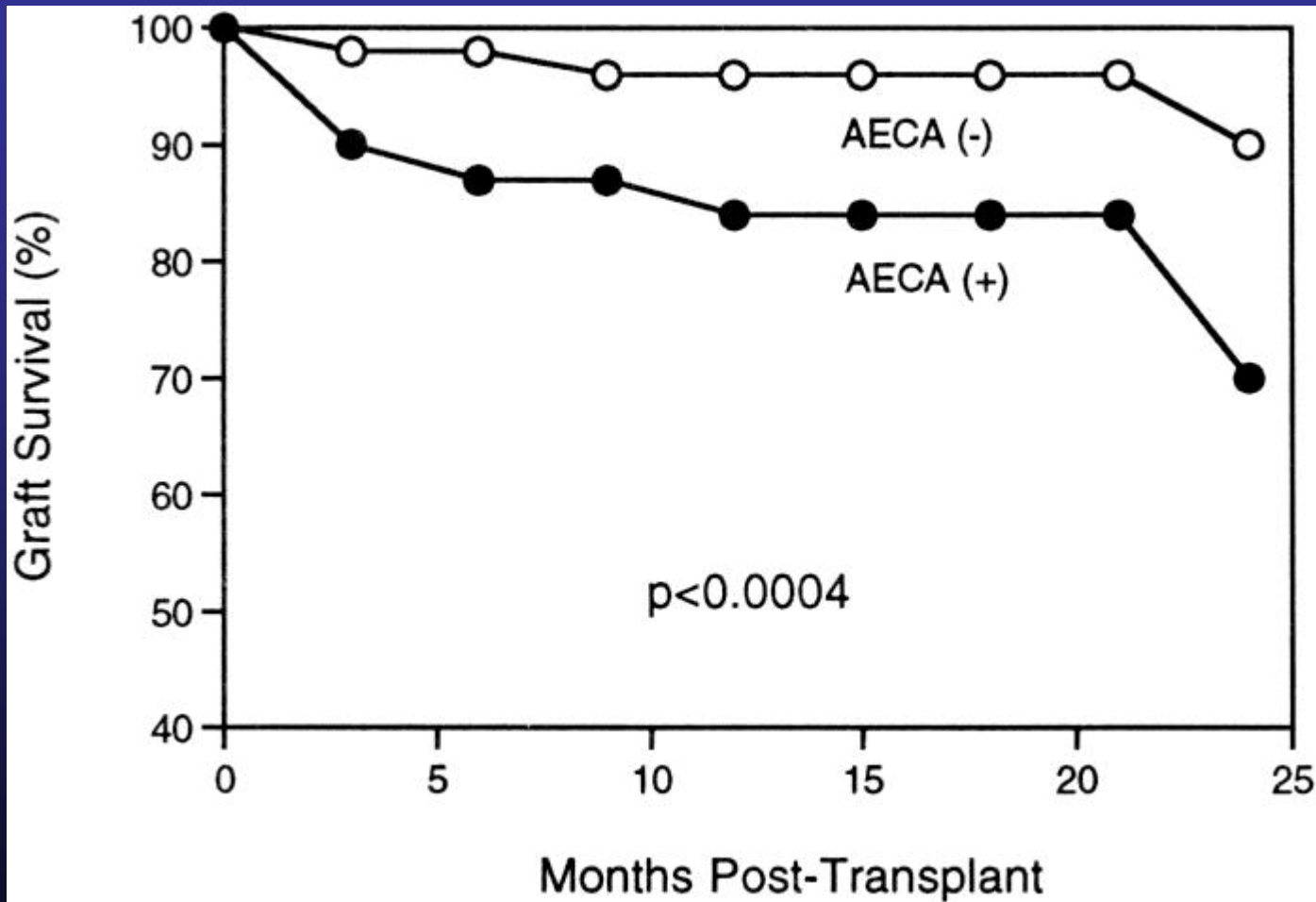
Fredrich, Ragini et al.

	AECA (-) (n=49)	AECA (+) (n=31)	<i>P</i> ^a
Age	52.5 (33–69) ^b	55.5 (16–67) ^b	NS
Sex			
Male	44	29	NS
Female	5	2	
Original disease			
Ischemic	25	25	0.004
Idiopathic	24	6	
Ischemia time (min)	148 (86–234) ^b	171 (64–274) ^b	NS
Panel reactive antibodies (%)	3.0 (0–43) ^b	15.2 (0–100) ^b	0.0005
Crossmatch (+)			
T cell	4	5	NS
B cell	4	5	

^a *P*: AECA (-) vs. AECA (+).
^b Mean (range).

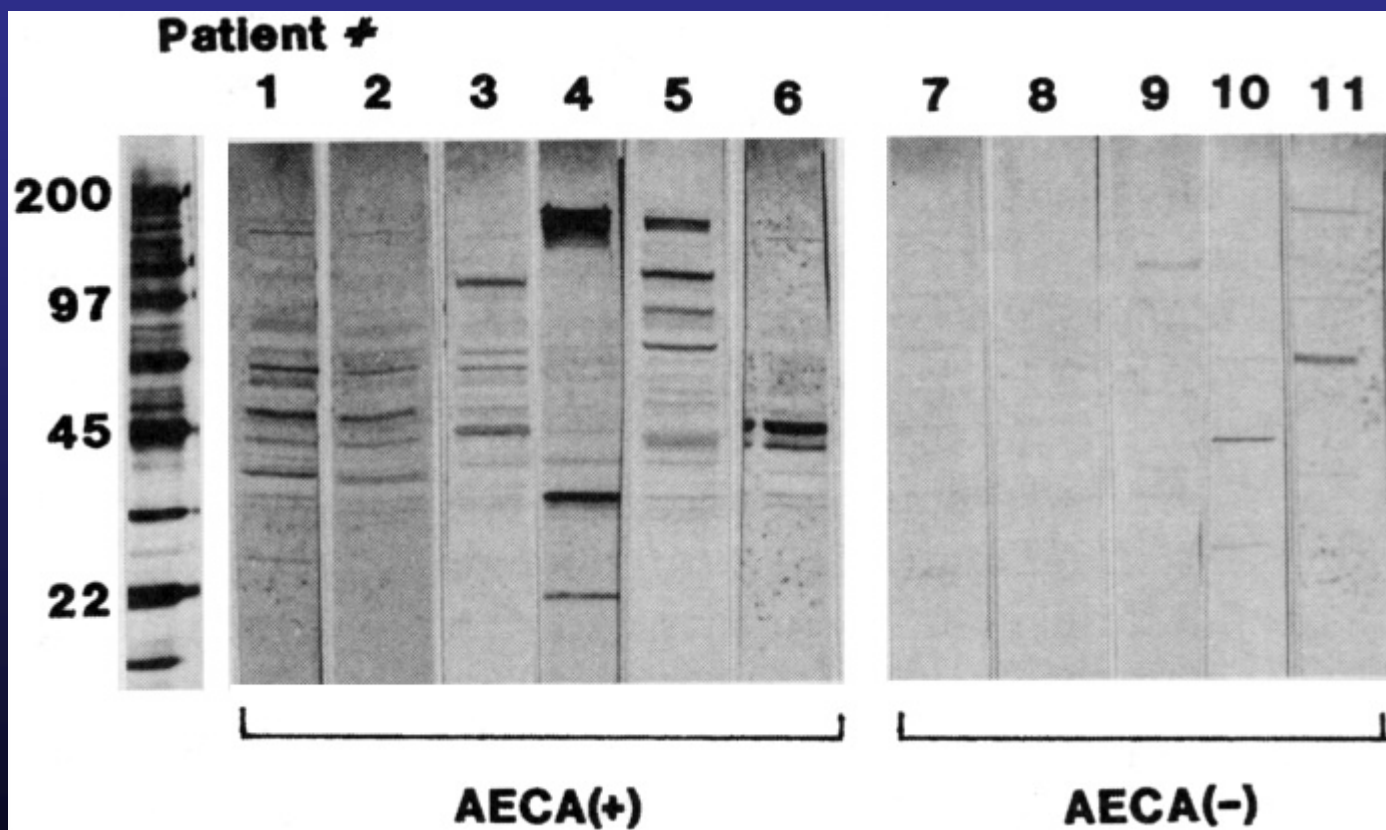
THE CLINICAL SIGNIFICANCE OF ANTIBODIES TO HUMAN VASCULAR ENDOTHELIAL CELLS AFTER CARDIAC TRANSPLANTATION

Fredrich, Ragini et al.



THE CLINICAL SIGNIFICANCE OF ANTIBODIES TO HUMAN VASCULAR ENDOTHELIAL CELLS AFTER CARDIAC TRANSPLANTATION

Fredrich, Ragini et al.



Anticorpi anti cellule endoteliali

- **IgG (IgM, IgA)**
- **Specificità antigeniche (non HLA) multiple**
- **Presenti anche in individui sani**
- **Ruolo patogenetico?**

Metodi di dosaggio

- **ELISA (cellule endoteliali fissate o non fissate)**
- **(IFI)**
- **Immunoblot**

Vimentina

- Proteina citoscheletrica (filamento intermedio)
- MW 57KD
- Espressa da cellule mesenchimali (e da cellule neoplastiche in coltura)

Determinazione autoanticorpi:

- IF
- ELISA

ANTIVIMENTIN ANTIBODIES ARE AN INDEPENDENT PREDICTOR OF TRANSPLANT-ASSOCIATED CORONARY ARTERY DISEASE AFTER CARDIAC TRANSPLANTATION

Jurcevic, Stipo, Ainsworth, Mark E.;
Pomerance, Ariala; Smith, John D.; Robinson, Derek R.;
Dunn, Michael J.; Yacoub, Magdi H.; Rose, Marlene L.
Transplantation 2001; 71:886-92

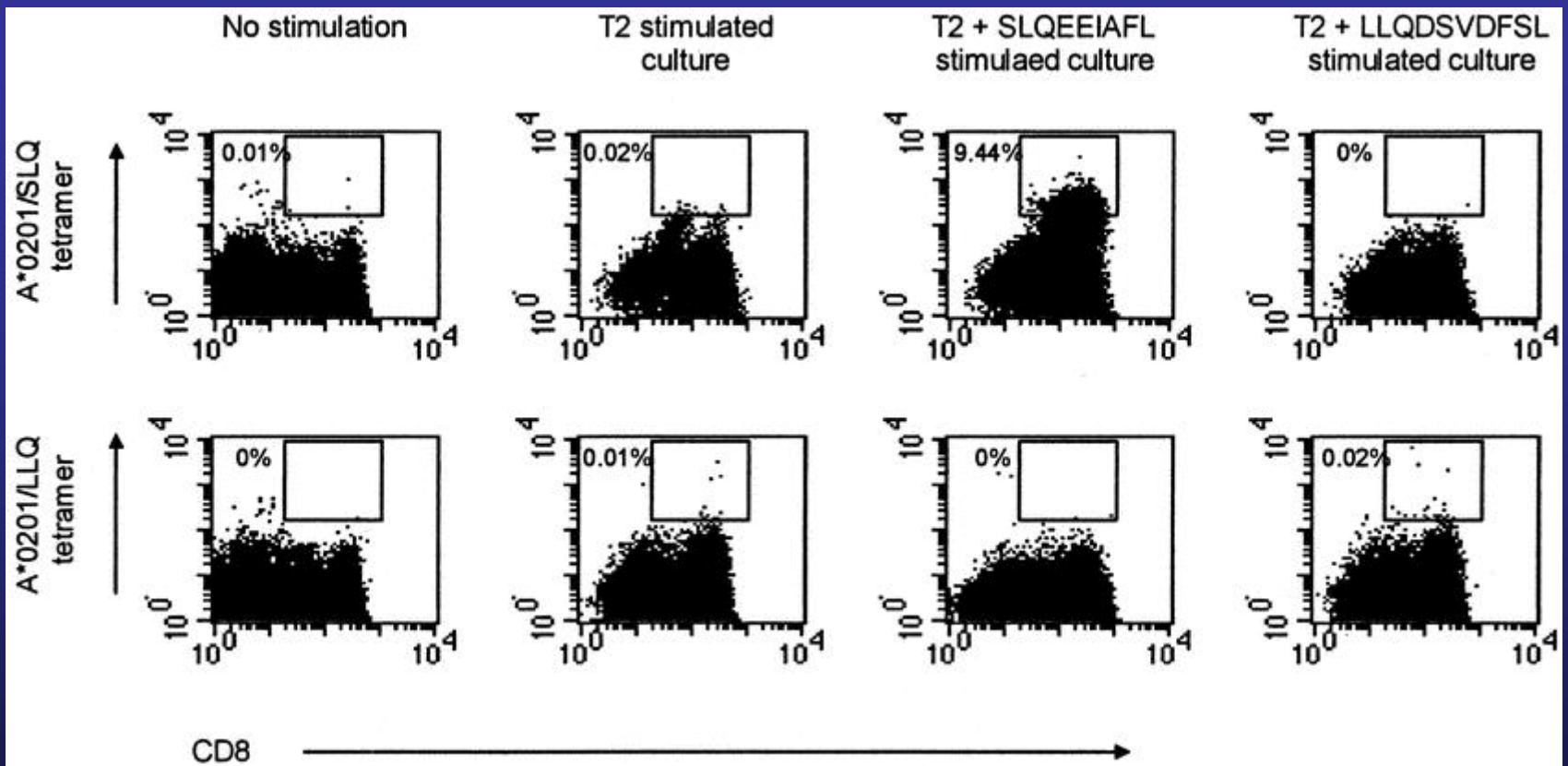
TABLE 2. Antivimentin titres in patients at 1 yr and combined 1 and 2 yr after transplantation

	Year 1 titre mean (SE)	Year 1 and 2 titre mean (SE)
TxCAD (n=38)	188 (30)	202 (23)
Non-CAD (n=71)	101 (16)	105 (14)
<i>P</i> value Mann-Whitney	0.0038	<0.0001

Autoantibodies to Vimentin Cause Accelerated Rejection of Cardiac Allografts

Balakrishnan Mahesh, Hon-Sing Leong, Ann McCormack, Padmini Sarathchandra, Angela Holder and Marlene L. Rose
Am J Pathol 2007; 171 1415-27

Immunohistochemical analysis of allografts from vimentin/complete Freund's adjuvant mice demonstrated increased numbers of T cells and enhanced microvascular deposition of C3d, CD41, and P-selectin compared with controls. Antibodies were necessary for accelerated rejection, shown by the fact that vimentin-immunized B-cell-deficient IgH6 mice did not show accelerated rejection of 129/sv allografts, but rejection was restored by adoptive transfer of serum containing anti-vimentin antibodies.

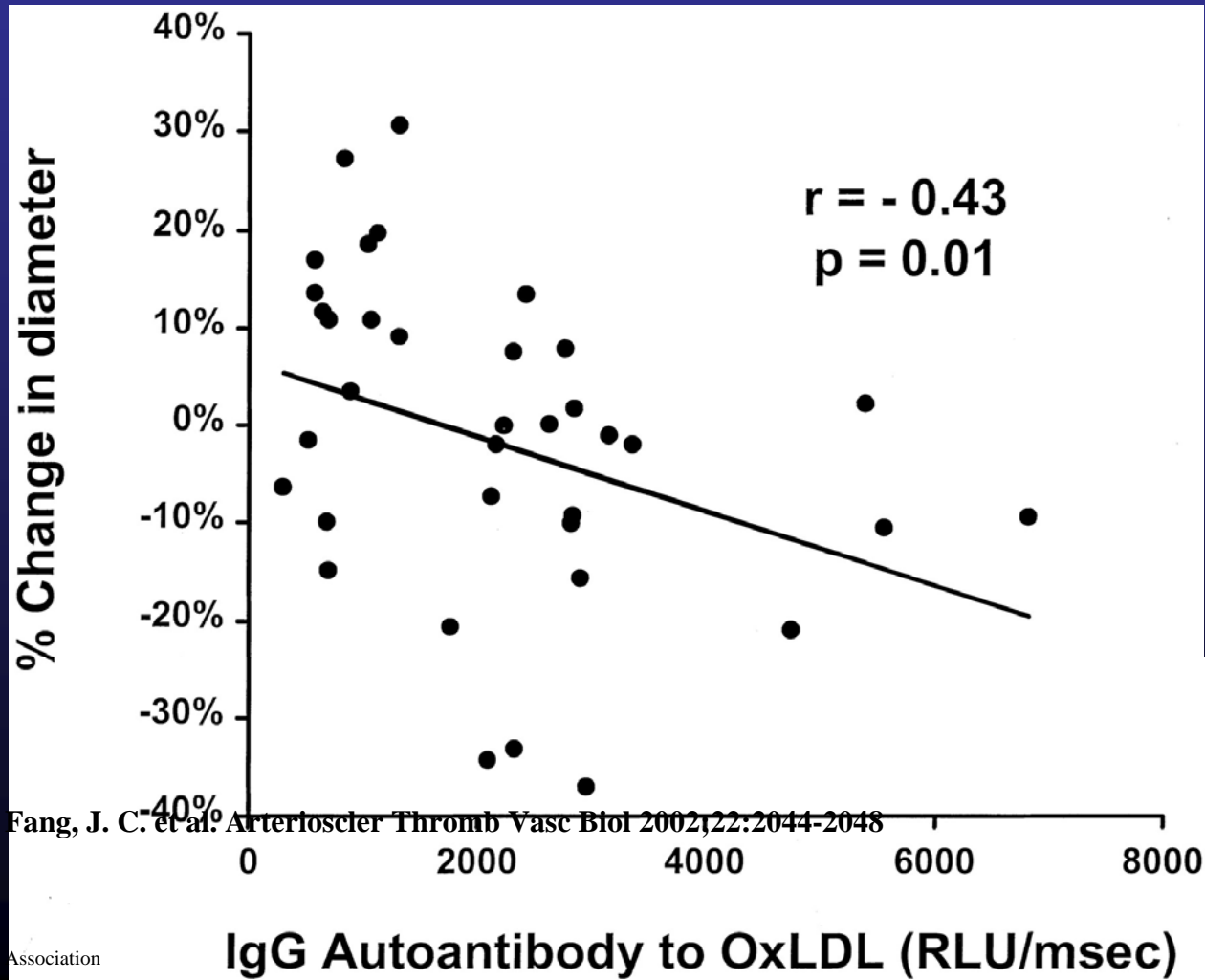


Barber LD et al. - Detection of vimentin specific autoreactive CD8 +Tcells in Cardiac transplant patients. Transplantation 2004; 77: 1604

Relationship between IgG autoantibodies to OxLDL and the coronary vasomotor response to acetylcholine (endothelium-dependent response)

Circulating Autoantibodies to Oxidized LDL Correlate With Impaired Coronary Endothelial Function After Cardiac Transplantation

James C. Fang; Scott Kinlay; Dominik Behrendt; Hiroyuki Hikita; Joseph L. Witztum; Andrew P. Selwyn; Peter Ganz



Fang, J. C. et al. Arterioscler Thromb Vasc Biol 2002;22:2044-2048

The Cardiac Tissue Antigens Identified as Targets of Autoantibodies in Human DCM

Types of Target Antigens	Autoantigens
1. Structural-intracellular	Myosin, actin, laminin, vimentin, desmin, carnitin, tubulin
2. Non-structural intracellular	ANT, BCKD, HSP 60, DnaseB, hyaluronidase, transaldolase, strptosyme sarcoplasmic ATPase
3. Cell surface receptors	BETA-1-adrenoreceptor. M2-cholinergic receptor

Myocardial autoantibodies and their clinical significance (Ansari AA et al., in Shoenfeld Y. et al., "Autoantibodies", 2007)

Cardiac myosin autoantibodies and acute rejection after heart transplantation in patients with dilated cardiomyopathy

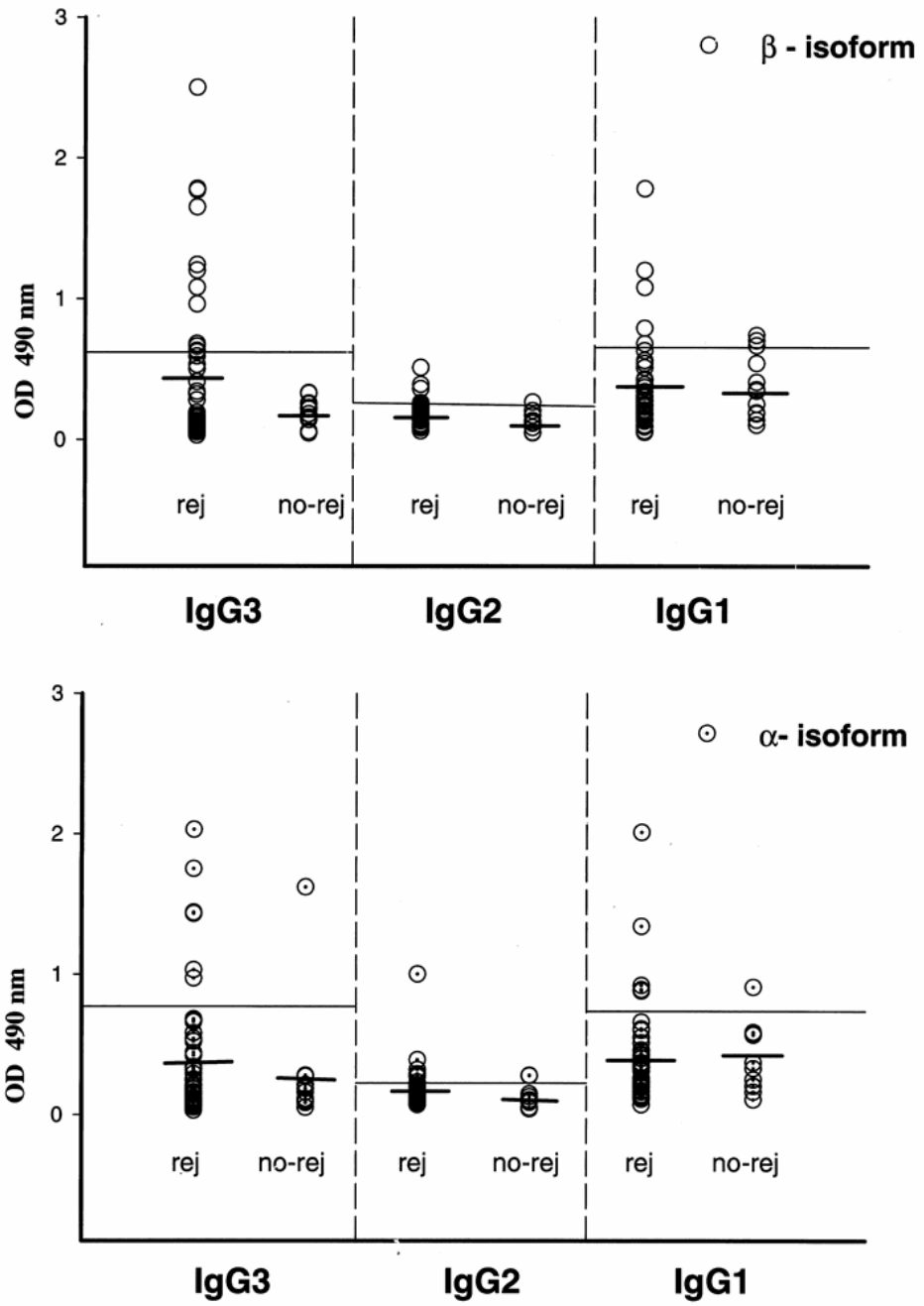
TABLE 3. Association of preformed antibody status to posttransplant clinical course in patients transplanted for idiopathic dilated cardiomyopathy

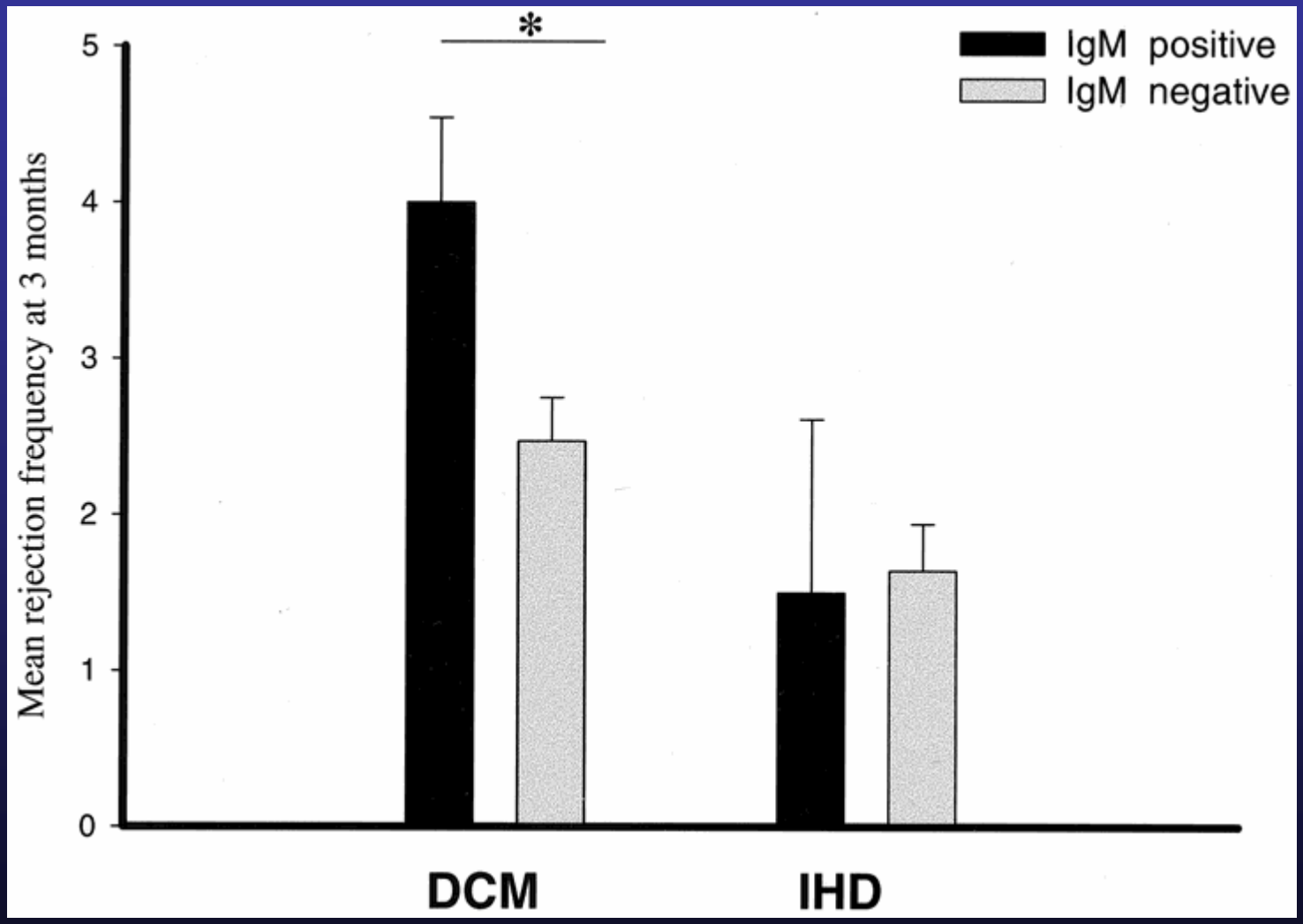
	IgG3		IgM	
	Ab+	Ab-	Ab+	Ab-
Time to initial rejection median (IQ range)	14.5 (6.5–28)	13.0 (7–23)	8.50 (5–13) ^a	14.0 (10–28)
Incidence of ISHLT grade 3 as initial rej: percentage and (frequency)	50 ^b (5/10)	15 (6/34)	15.4 (2/15)	33 (9/27)
Percentage and the (no. of patients with EI)	80 (8/10)	57 (31/54)	60 (9/15)	61 (30/49)
Frequency of EI/pt. Mean (\pm SEM)	2.88 (1.33) ^c	1.29 (0.55)	1.45 (1.08)	1.50 (0.6)

^a $P=0.009$.

^b $P<0.05$ (χ^2).

^c $P=0.006$.





De Novo Autoimmunity to Cardiac Myosin After Heart Transplantation and Its Contribution to the Rejection Process¹

Eugenia V. Fedoseyeva, et al

The Journal of Immunology, 1999, 162: 6836-6842.

Modulation of Tissue-Specific Immune Response to Cardiac Myosin Can Prolong Survival of Allogeneic Heart Transplants

Eugenia V. Fedoseyeva, et al

The Journal of Immunology, 2002, 169: 1168-1174.

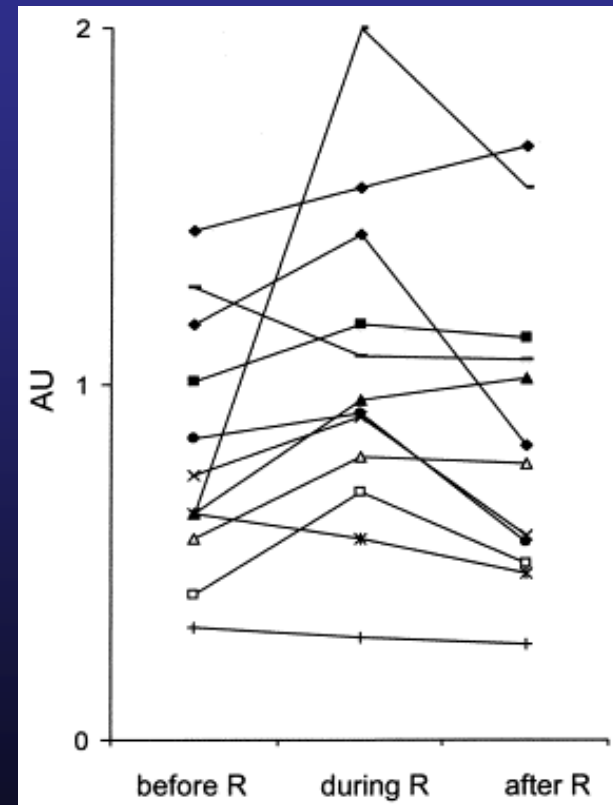
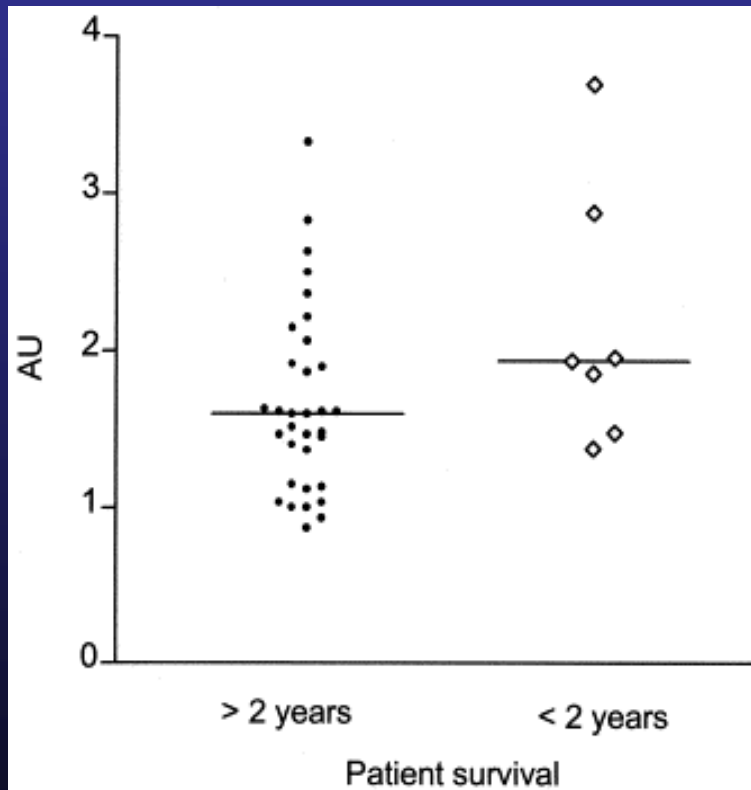
T-cell response to cardiac myosin persists in the absence of an alloimmune response in recipients with chronic cardiac allograft rejection.

Rolls, Hillary et al.

Transplantation 2002; 74; 1053-57

Pre- and post-transplant anti-myosin and anti-heat shock protein antibodies and cardiac transplant outcome

Andrey Morgun, et al.



Anti-phospholipid antibodies

Lupus Anticoagulant

Anti-cardiolipin antibodies

Antigens:

- β_2 GP1
- Prothrombin
- Annexin V

Isotype IgG, IgM IgA

Antiphospholipid antibodies in heart transplant recipients

McIntyre JA, Wagenknecht DR, Faulk P

Clin Cardiol. 1995 Oct;18(10):575-80

In our patient population, the incidence of IgG and/or IgA aPA was significantly higher ($p < 0.001$) than IgM. ...

..we investigated pretransplant serum samples which are available from 79 of the 105 recipients, and found aPA in 52 of 79 (66%) patients before transplantation. Longitudinal studies were done in three patients: two had increasing IgA aPA, beginning on Days 13 and 26 post transplant, whereas the third patient showed an increased aPA on Day 8 but a decrease on Day 23.

Cardiac transplantation in patients with anti-phospholipid antibodies.

Schofield RS, Aranda JM Jr, Shoemaker SB, Pauly DF, Hill JA, Klodell CT.

Department of Veterans Affairs Medical Center,
Gainesville, Florida, USA.

Patients with severe heart failure are known to have an increased incidence of thromboembolic events and frequently have a visible thrombus in the left ventricle. Thromboemboli in heart failure patients are usually attributed to the underlying heart failure, and alternative etiologies for thrombus formation are rarely sought. However, anti-phospholipid antibodies and other inherited or acquired clotting abnormalities may contribute to hypercoagulability in heart failure patients and can lead to a persistent high risk for clotting, even after heart transplantation has corrected the underlying heart failure. We report outcomes with heart transplantation in 3 young patients with anti-phospholipid antibodies and a history of pre-heart transplantation thromboembolic events, and demonstrate the importance of post-heart transplantation anti-coagulation in these patients.

Anticorpi antifosfolipidi e trapianto di rene

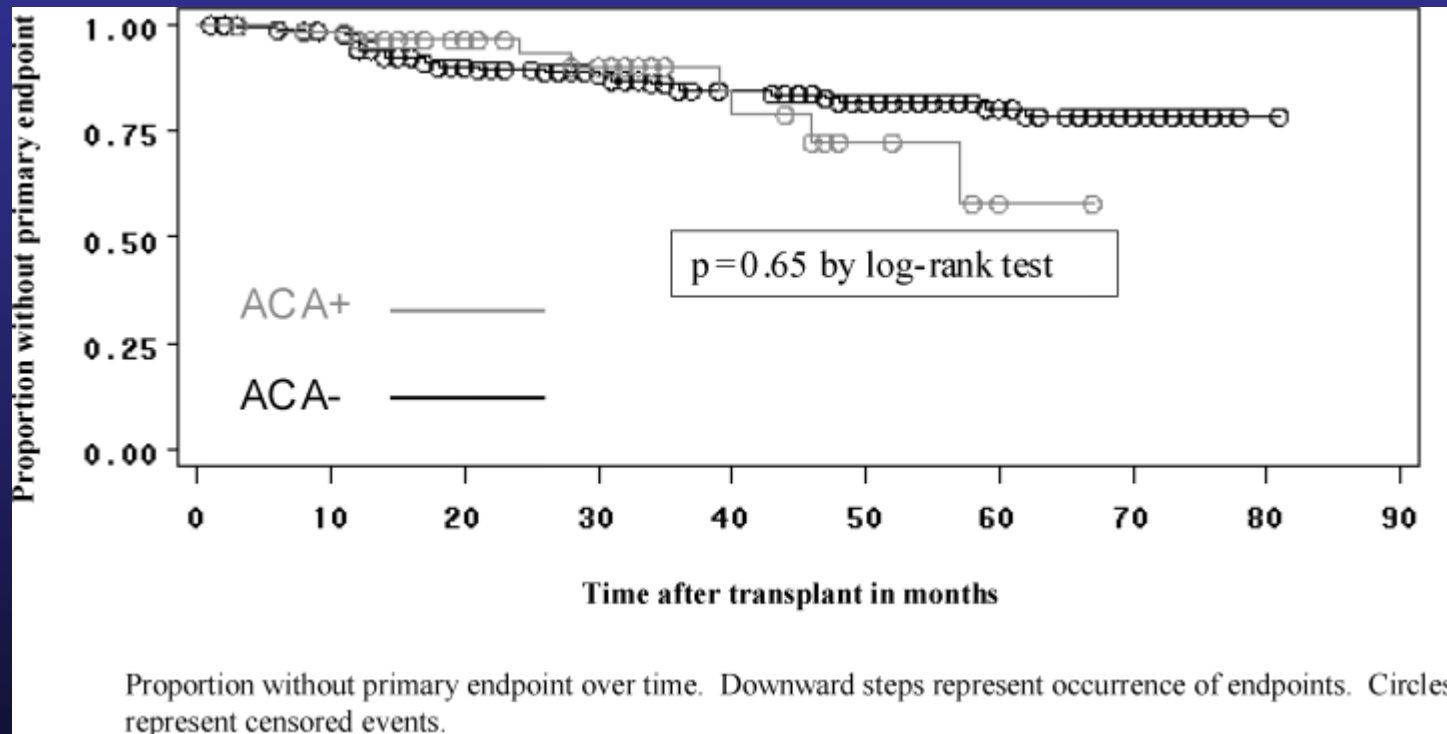
- **I pazienti con ESRD hanno anticorpi antifosfolipidi molto più frequentemente di soggetti sani, in particolare se dializzati**
- **La loro presenza pre-trapianto, o il loro sviluppo post trapianto si correla con il rigetto o con la patologia CV post trapianto?**
- **Uso della terapia anticoagulante?**

- **“Antiphospholipid antibodies are a risk factor for early allograft failure” (Wagenknecht et al., Transplantation 1999; 68: 241-46)**
- **“Antiphospholipid antibodies as a risk factor for atherosclerotic events in renal transplant recipients” (Duchoux et al., Kidney Int, 2003; 64: 1065-70)**

Significance of anticardiolipin antibodies on short and long term allograft survival and function following kidney transplantation.

Forman JP, Lin J, Pascual M, Denton MD, Tolkoﬀ-Rubin N.

Am J Transplant 2004; 4: 1786-91



Clinical significance of antiphospholipid antibodies on allograft and patient outcome after kidney transplantation.

Fernández-Fresnedo G, López-Hoyos M, Segundo DS, Crespo J, Ruiz JC, De Francisco AL, Arias M.

Transplantation Proceedings 2005; 37: 3710-3711

CONCLUSIONS: The detection of APA is not an independent risk factor for CVD after kidney transplantation. The inflammatory phenomena secondary to an ARE may be responsible for the de novo production of postransplant APA, which may be associated with the development of postransplant CVD. The control of cardiovascular risk factors should be intensified in this special group of patients.

Significance of Anticardiolipin Antibodies on Short- and Long-Term Allograft Survival and Function Following Kidney Transplantation

John Vella

Am J Transplant 2004; 4: 1731-2 (editorial)

The key question is whether all patients with demonstrable ACA benefit from anticoagulation.

It seems that some individuals are at extremely high risk:

Documented antiphospholipid syndrome

Hepatitis C combined with ACA (?)

High titer ACA (?)

that anticoagulation may not be necessary for all (low-risk) ACA-positive individuals *unless they have a history of thrombosis*

Trapianto renale in patologie con autoanticorpi

- **Nefrite lupica**
- **Anti – GBM GN**
- **Vasculiti ANCA-associate**

Trapianto renale in AntiGBM GN

- **La recidiva post-trapianto è più frequente in pazienti positivi per anticorpi anti GBM al momento del trapianto**
- **Probabile utilità di follow-up post trapianto**

(Segurnavan et al. 1990, Glasscock e al. 1997)

Relapse rate and outcome of ANCA-associated small vessel vasculitis after transplantation

**Abdurrezagh Elmedhem, Dwomoa Adu and Caroline O. S. Savage
Nephrol Dial Transplant (2003) 18: 1001-1004**

**The presence of ANCA at transplantation does not appear
to increase the rate of relapse post-transplantation**

**Both relapsers were ANCA-positive at the time of relapse, having
previously been negative, suggesting that monitoring presence of
ANCA post-transplant is beneficial for high-lighting patients at risk
of relapse**

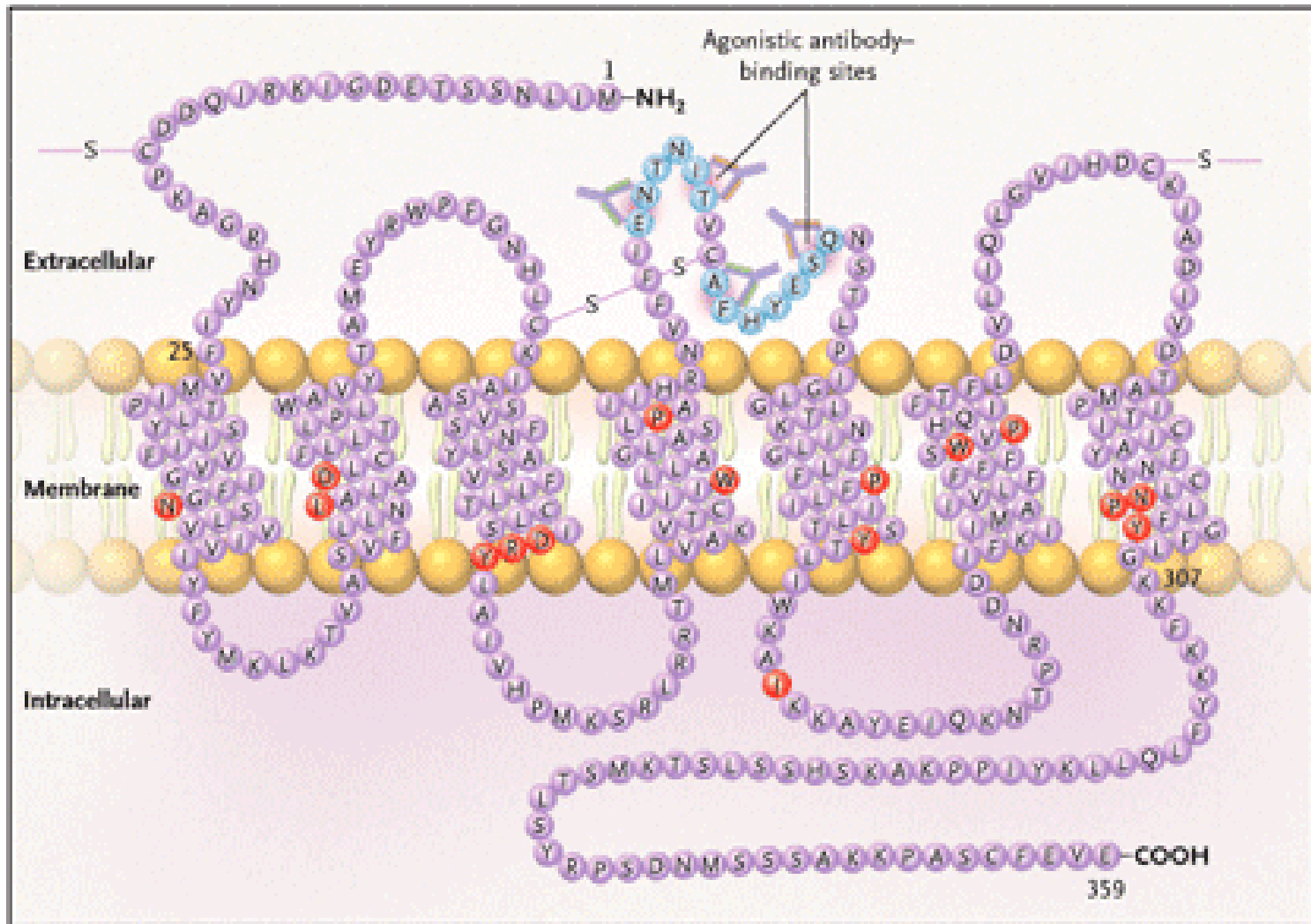
Recurrence of ANCA-associated vasculitis following renal transplantation in the modern era of immunosuppression

M Gera¹, M D Griffin¹, U Specks², N Leung¹, M D Stegall³ and F C Fervenza¹
Kidney International (2007) **71**, 1296–1301

No statistical difference was found between relapse rates in patients diagnosed with WG versus MPA, between patients treated with CsA versus those not receiving CsA, or between patients who were ANCA-positive at the time of transplantation versus those who were ANCA-negative.

ANCA testing was carried out in 18 patients around the time of transplantation, and positive ANCA by immunofluorescence was found in eight patients (8/18; 44%). Only one patient (3%) with WG developed an extrarenal relapse, with none of the patients developing a renal relapse.

The necessity for negative ANCA testing before proceeding with KTx is less well agreed upon, although most analyses conclude that the presence of ANCA at transplantation is not associated with an increased rate of relapse.



Dragon D et al. Angiotensin II type 1 receptor-activating antibodies in renal allograft rejection. *New Engl J Med* 2005; 352: 558-9

Autoimmunity following haematopoietic stem-cell transplantation.

Daikeler T, Tyndall A

**Department of Rheumatology, University of Basel,
University Hospital Basel, Petersgraben 4, CH-4058 Basel,
Switzerland.**

Best Pract Res Clin Haematol. 2007; 20: 349-60

Clin Lab Haematol. 2005 Aug;27(4):247-9.

Occurrence of autoantibodies in chronic graft vs. host disease after allogeneic stem cell transplantation.

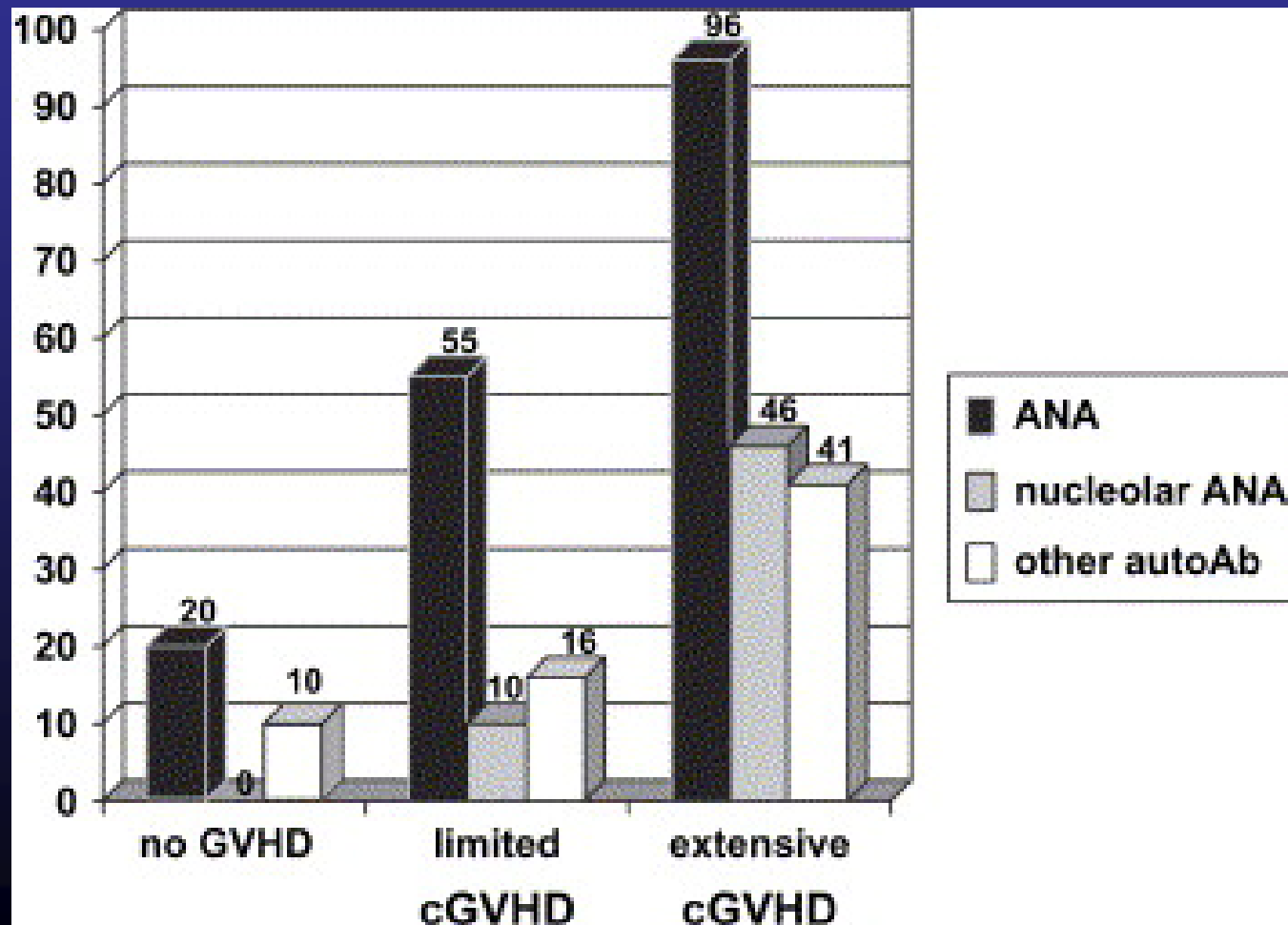
Wechalekar A, Cranfield T, Sinclair D, Ganzckowski M.

All the patients with antibodies had cGVHD where as none of the patients without cGVHD had any autoantibodies (P = 0.025). Three (23%) patients had only one autoantibody and three (23%) of them had more than one autoantibody. ANA was positive in three (23.3%) patients, double stranded DNA in four (30.7%) patients, RF in one (7.6%) and Anti Sm muscle in two (15.3%) patients. In the present study, autoantibodies were detected predominantly in patients with presence of cGVHD. They also appeared to be more frequent in an unmanipulated graft and so less in patients with a T-cell depleted allograft. In two of 13 patients only there appeared to be an association between the antibody titre and flare up in skin symptoms. In conclusion, this small series raises interesting questions about the presence and role of autoantibodies after SCT and their association with cGVHD.

Exp Hematol. 2006 Mar;34(3):389-96.

The development of autoantibodies after allogeneic stem cell transplantation is related with chronic graft-vs-host disease and immune recovery.

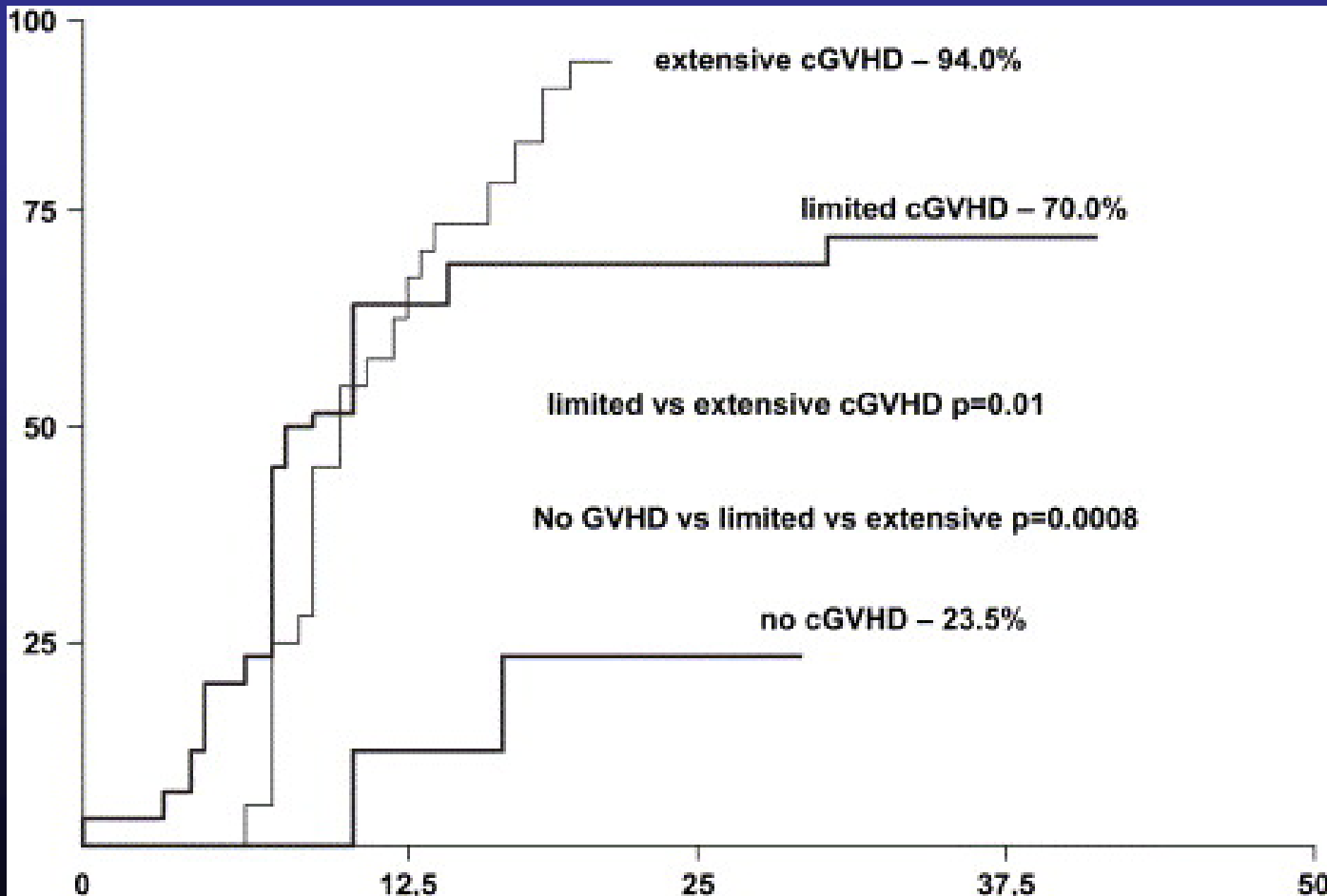
Patriarca F, Skert C, Sperotto A, Zaja F, Falletti E, Mestroni R, Kikic F, Calistri E, Fili C, Geromin A, Cerno M, Fanin R.



Exp Hematol. 2006 Mar;34(3):389-96.

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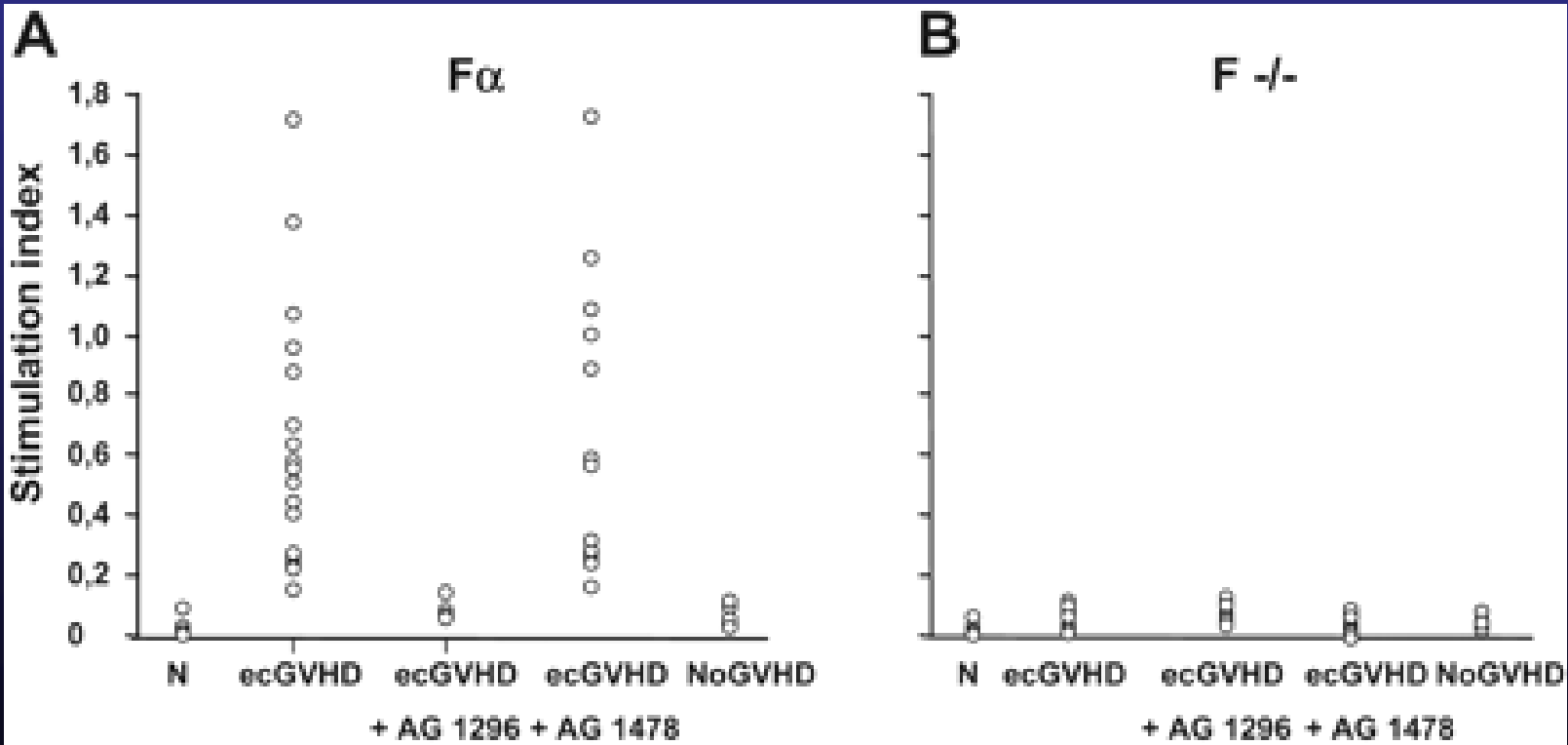
Patriarca F, Skert C, Sperotto A, Zaja F, Falletti E, Mestroni R, Kikic F, Calistri E, Fili C, Geromin A, Cerno M, Fanin R.



Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease

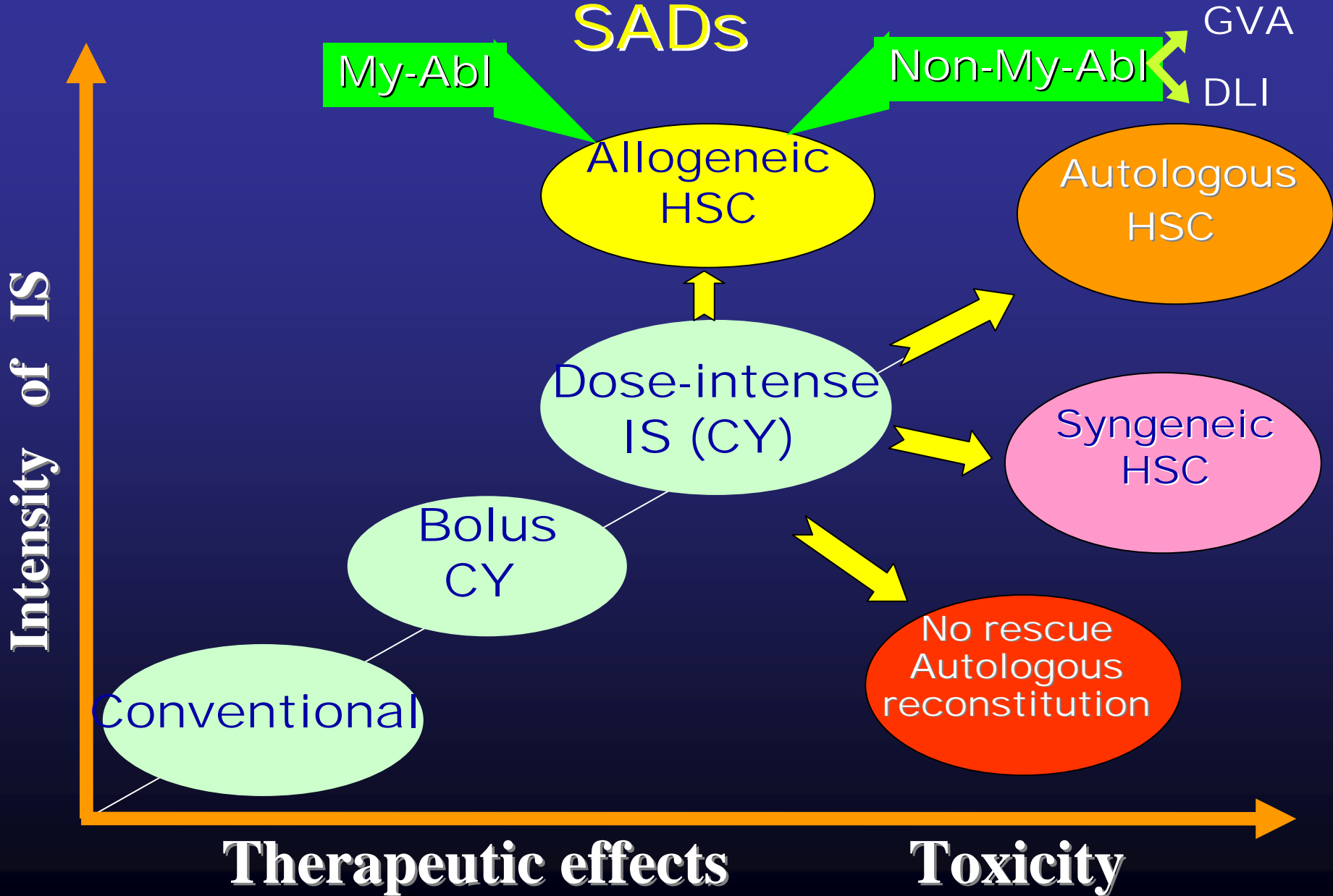
Silvia Svegliati¹, Attilio Olivieri², Nadia Campelli¹, Michele Luchetti¹, Antonella Poloni², Silvia Trappolini², Gianluca Moroncini¹, Andrea Bacigalupo³, Pietro Leoni², Enrico V. Avvedimento⁴, and Armando Gabrielli¹

Blood, 1 July 2007, Vol. 110, No. 1, pp. 237-241



NEW APPROACHES FOR TREATING

SADs



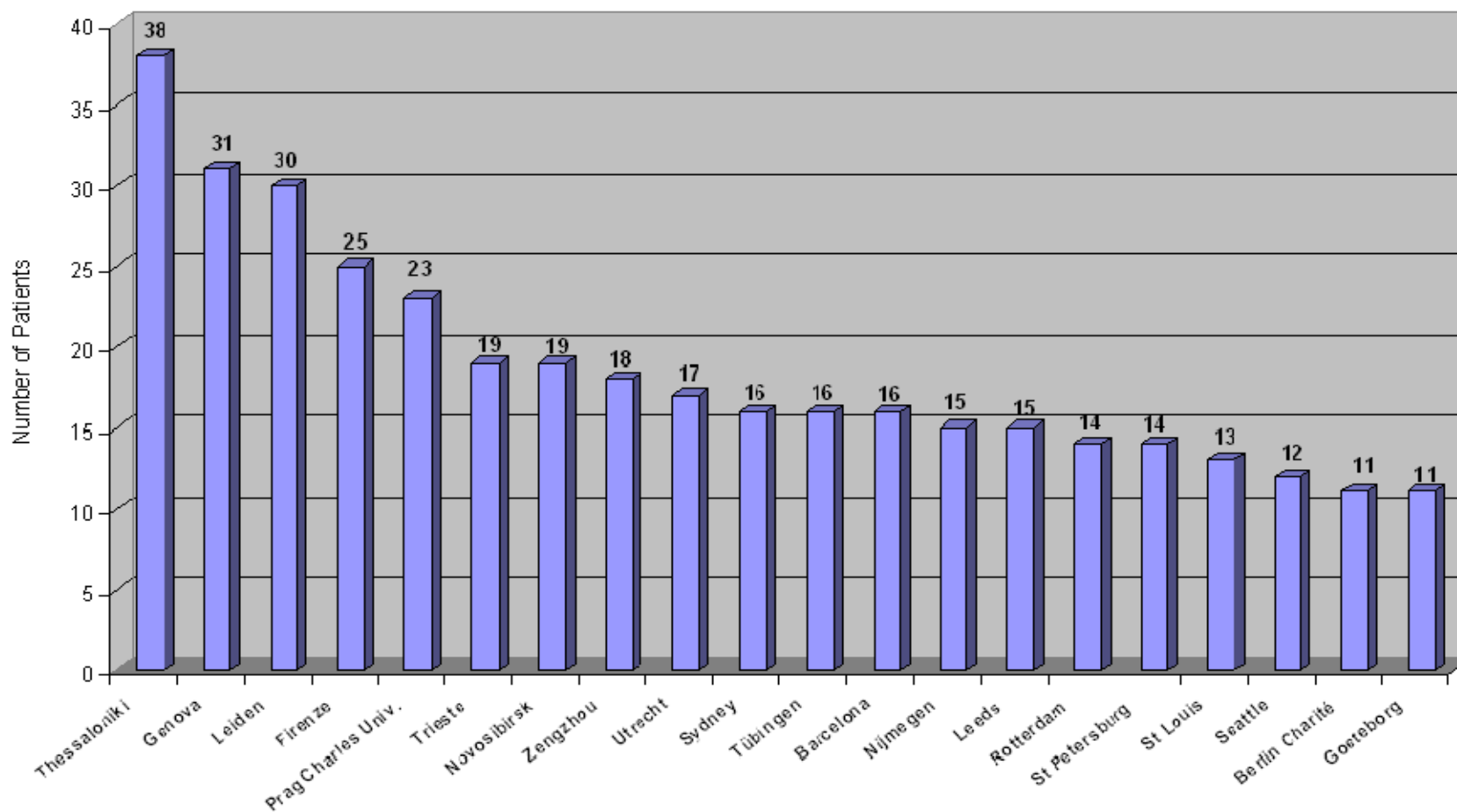
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR SLE

Clinical studies with over 5 patients

Patients

- The EBMT multicentric study
Jayne D et al: Lupus 2004 53
- The NWU Chicago study
Burt RK et al JAMA 2006 50
- The U. of Korea study
Kim JA et al BMT 2005 7
- The Novosibirsk study
Lisukov JA et al.: Lupus 2004 6
- The Genoa S.Martino study
Marmont A et al.: Lupus 1997
Lupus 2006 6

Patients by Centre (20 Major Centres)



ALLO-SCT

HDCY alone

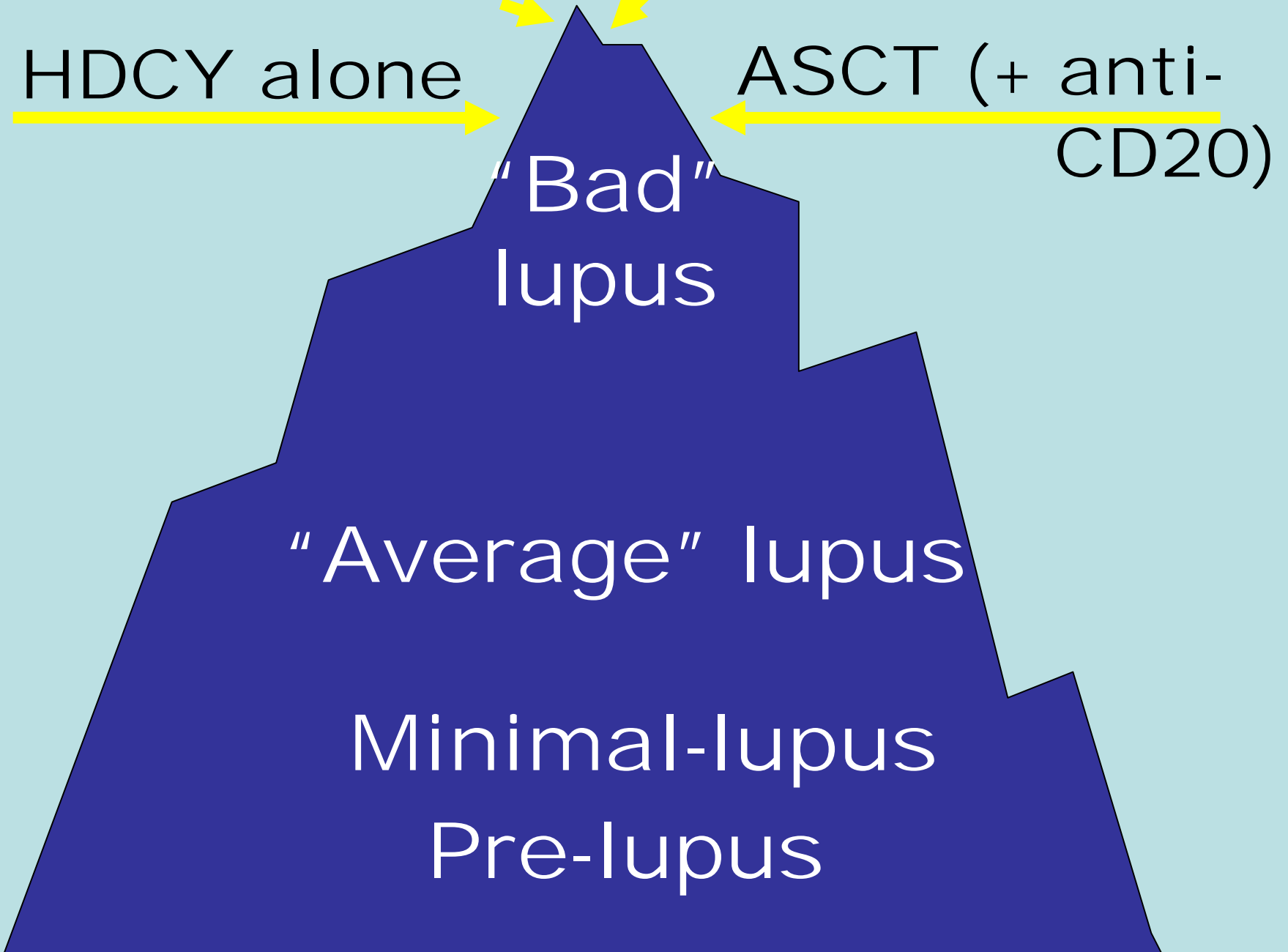
ASCT (+ anti-
CD20)

"Bad"
lupus

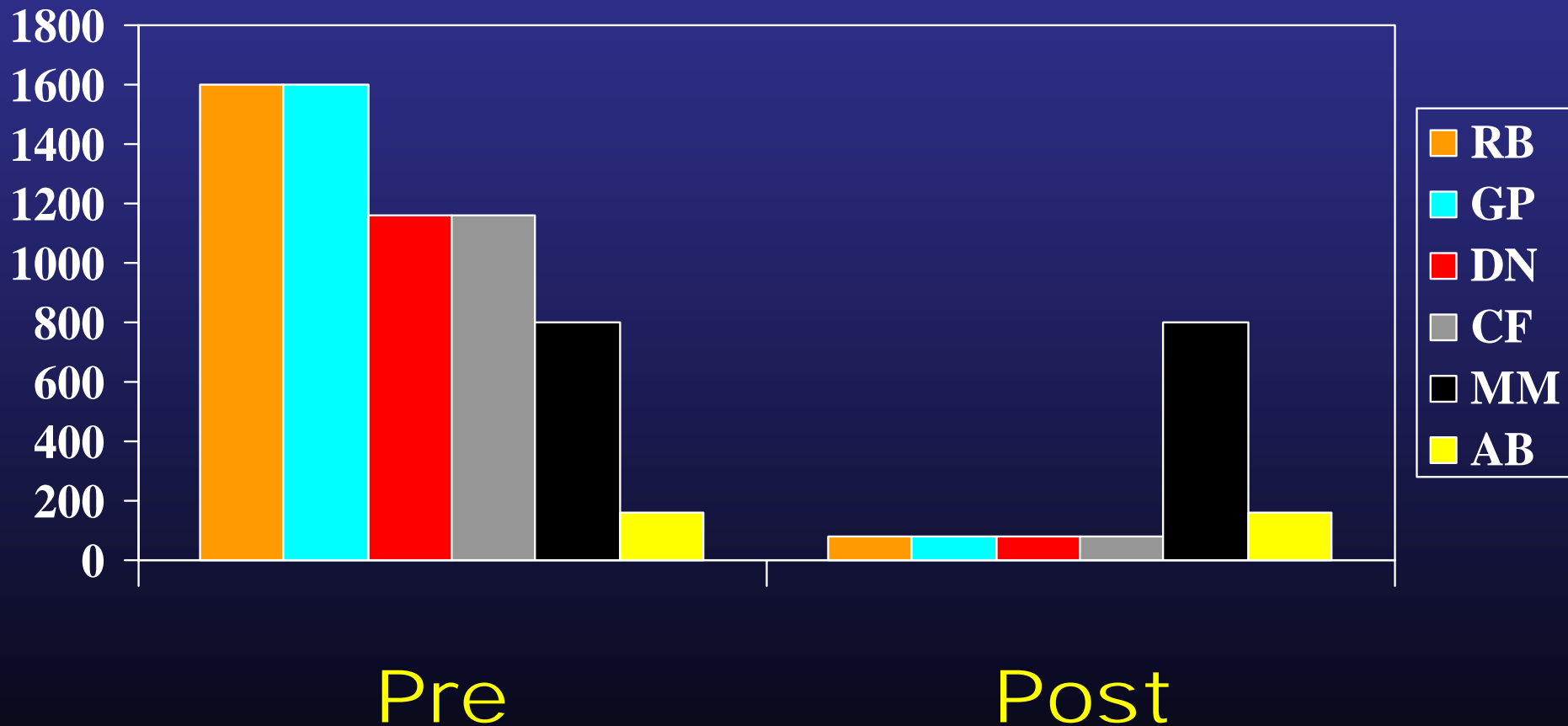
"Average" lupus

Minimal-lupus

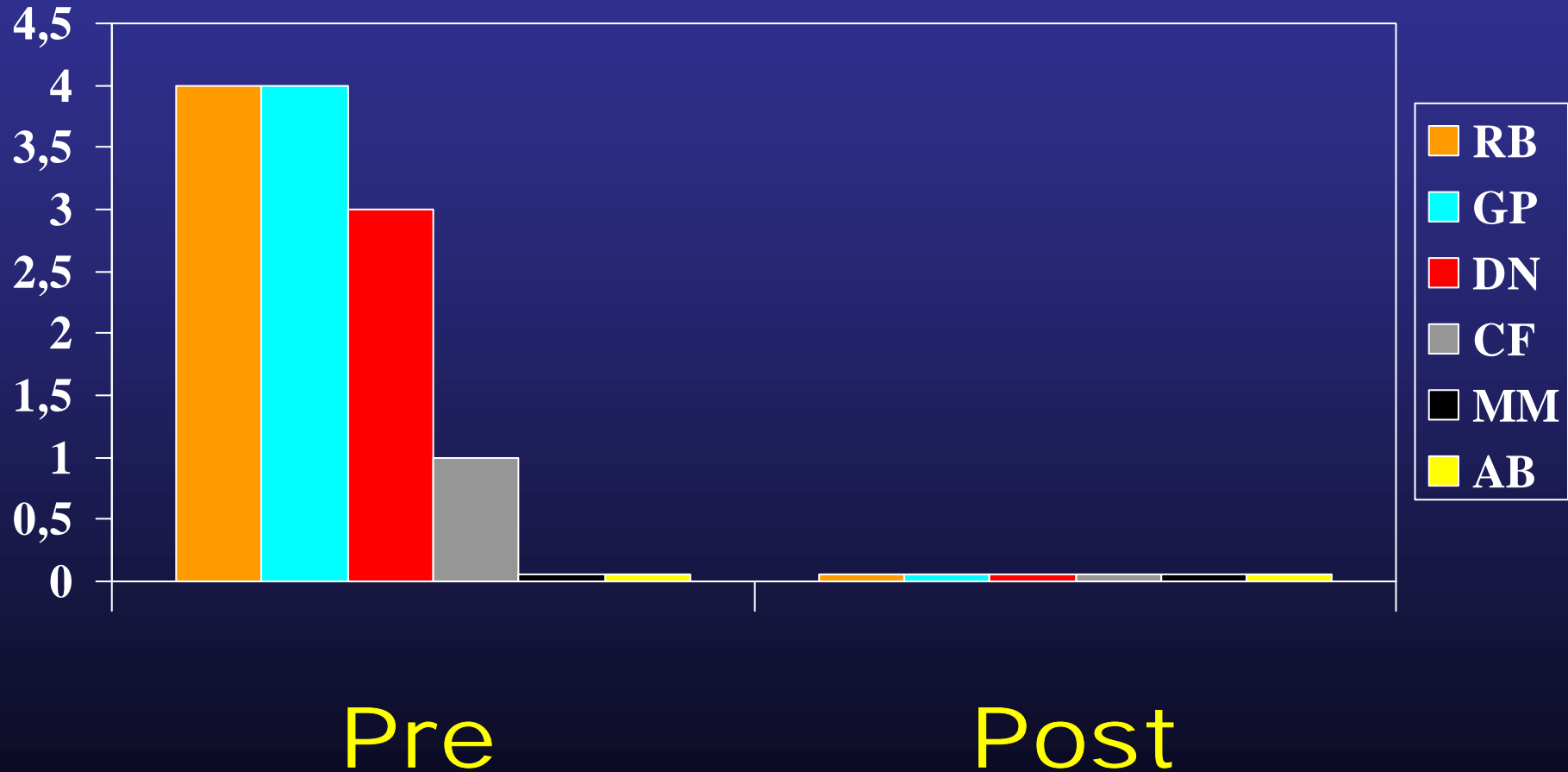
Pre-lupus



ANA pre and post -ASCT



ds-DNA pre and post-ASCT



Transfer of autoimmunity

Table 1. Case reports on autoimmune diseases transferred from donor to recipient.

Autoimmune disease	Graft source	Reference
Psoriasis	Syngeneic bone marrow	[21]
Psoriatic arthritis	Sibling bone marrow	[57]
Coeliac disease	Sibling bone marrow	[23]
Vitiligo	Sibling bone marrow	[26]
Type I diabetes mellitus	Sibling bone marrow	[24]
Autoimmune thyroiditis	Sibling peripheral stem cells	[22,39]
Crohn's disease	Peripheral stem cells	[27]
Myasthenia gravis		[25]

SEVERE RA

BP receipt

BG donor (healthy sister)

Basal CCP/Ab(*)

235 U/I

10 U/I

BMT

4 month CCP/Ab

44 U/I

--

12 month CCP/Ab

8 U/I

—

(*) cut-off 25 U/I, “grey zone” 25-50 U/I

Diagnostica autoanticorpale in trapiantologia

**Possibili
applicazioni**

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Ricerca di autoanticorpi
comparsi dopo il
trapianto

Follow-up di
autoanticorpi
presenti prima
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Ricerca di
autoanticorpi nel
donatore