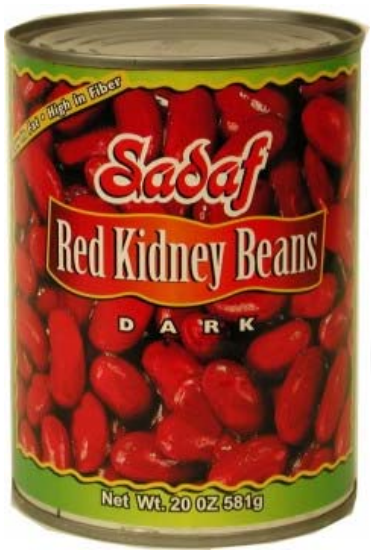
# CITOMETRIA DE FLUJO EN NEFROLOGIA

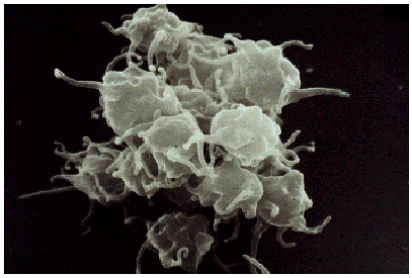





# ¿QUE ES LA CITOMETRIA DE FLUJO?

Método analítico por el que se mide la emisión de múltiples fluorescencias y la dispersión de luz de células o partículas microscópicas, alineadas secuencialmente mediante una corriente líquida laminar, cuando son presentadas de una en una y a gran velocidad (hasta miles de células/segundo) frente a un haz de luz láser de longitud de onda adecuada

# APLICACIONES CLINICAS DE LA CITOMETRIA DE FLUJO



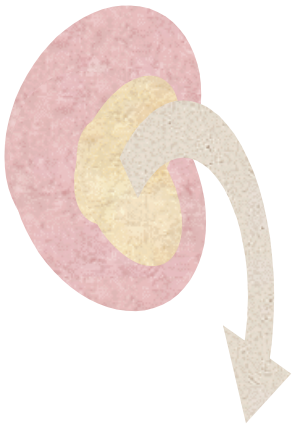


# APLICACIONES CLINICAS GENERALES

- DIAGNOSTICO BASADO EN EL ANALISIS CELULAR
- PRONOSTICO BASADO EN EL ANALISIS CELULAR
- EVALUACION Y MONITORIZACION DE TRATAMIENTO
- ANALISIS DE LA LESION Y MUERTE CELULAR

# APLICACIONES GENERALES DE LA CITOMETRIA EN NEFROLOGIA

**Enfermedad renal**

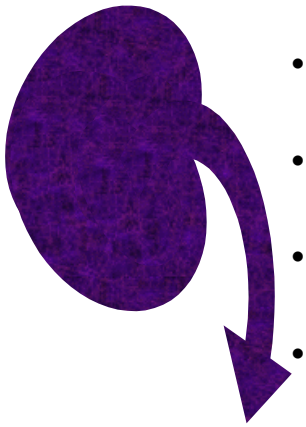


**Transplante renal**

**Dialisis**



# APLICACIONES DE LA CITOMETRIA EN LA ENFERMEDAD RENAL



- Diagnóstico y pronóstico de tumores renales
- Análisis de la función y lesión renal
- Evaluación funcional del Sistema Inmunitario
- Análisis funcional de la Hemostasia

# DIAGNOSTICO Y PRONOSTICO DE TUMORES

## Intratumoral Heterogeneity of DNA Content in Renal Cell Carcinoma and Its Prognostic Significance

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Miguel Hernández, Ph.D.[2]
Amparo Sempere, Ph.D.[3]
J. Enrique O'Connor, Ph.D.[4]
Bruce F. Kimler, Ph.D.[5]
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[5] Department of Radiation Oncology, University of Kansas Medical Center, Kansas City, Kansas.

**BACKGROUND.** A multiple sampling study was performed on 124 specimens of renal cell carcinomas to analyze the consistency and reliability of DNA measurements. The authors investigated intratumoral DNA heterogeneity and its role as a adverse prognostic factor for disease progression.

**METHODS.** DNA content was analyzed by flow cytometry on three different samples of the same tumor. The Cronbach $\alpha$ coefficient was used to assess the reliability and a Cox proportional hazards model was used to test the effect of DNA ploidy heterogeneity on time of disease progression.

**RESULTS.** The agreement among the DNA ploidy samples was high. The number of aneuploid findings increased significantly with the number of samples analyzed. The presence of non-diploid cell populations was a significant adverse predictive value for disease progression. However, the authors were unable to demonstrate that intratumoral heterogeneity DNA content had any influence on the biological behavior of the tumor.

**CONCLUSIONS.** Determination of DNA ploidy based on single samples may be inaccurate. Spatial variation in DNA ploidy is a feature of renal cell carcinoma; however, its biologic significance remains to be demonstrated. *Cancer* 1999;86: 664–71. © *1999 American Cancer Society.*

***Cancer* 86:664–671, 1999**



# DIAGNOSTICO Y PRONOSTICO DE TUMORES

TABLE 1
Distribution of DNA Ploidy Patterns

| Pattern (%) | No. |
|---|---|
| Homogeneously diploid (49)[a] | 61 |
| Nondiploid (51) | 63 |
| Homogeneously nondiploid (29)[b] | 36 |
| One cell population (22) | 27 |
| Hypodiploid | 1 |
| Hyperdiploid | 21 |
| Tetraploid | 4 |
| Hypertetraploid | 1 |
| Two cell populations (7) | 9 |
| Hypertetraploid + hypertetraploid | 1 |
| Hyperdiploid + hyperdiploid | 3 |
| Hypodiploid + hyperdiploid | 5 |
| Heterogeneously nondiploid (22)[c] | 27 |
| 1 diploid + 1 nondiploid (17) | 22 |
| Hyperdiploid | 14 |
| Tetraploid | 6 |
| Hipodiploid | 2 |
| 1 diploid + 2 nondiploid (5) | 5 |
| Hypodiploid + hyperdiploid | 1 |
| Hyperdiploid + hyperdiploid | 1 |
| Hyperdiploid + tetraploid | 2 |
| Hyperdiploid + hypertetraploid | 1 |

[a] Tumor with all samples diploid.
[b] Tumor with all samples nondiploid.
[c] Tumor with diploid and nondiploid samples.

***Cancer* 86:664–671, 1999**

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# DIAGNOSTICO Y PRONOSTICO DE TUMORES

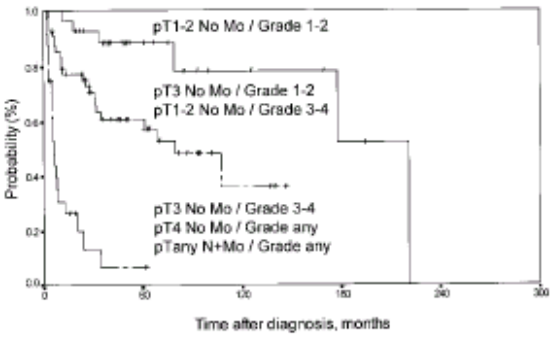
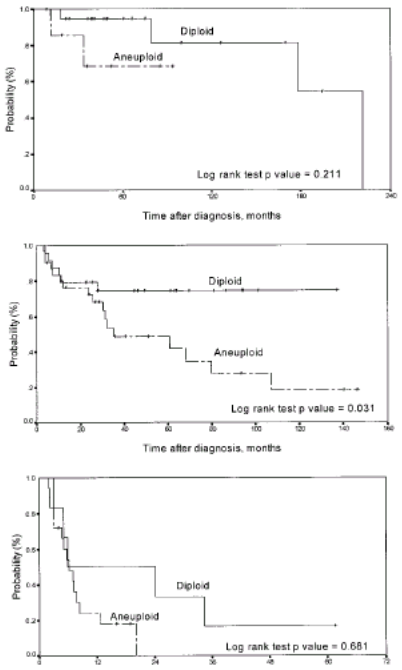



**FIGURE 1.** Kaplan–Meier plot of freedom from disease progression for 109 patients with renal cell carcinoma categorized by combined stage/grade classification. The survival distribution between the three groups is statistically significantly different by the log rank test ($P < 0.0001$).




***Cancer* 86:664–671, 1999**



# ANALISIS DE LA FUNCION Y LESION RENAL

ARTHRITIS & RHEUMATISM
Vol. 46, No. 3, March 2002, pp 735–740
DOI 10.1002/art.10112


## Selective Accumulation of CCR4+ T Lymphocytes Into Renal Tissue of Patients With Lupus Nephritis

Masato Yamada,[1] Hideo Yagita,[1] Hideko Inoue,[1] Tsuyoshi Takanashi,[1] Hironori Matsuda,[1] Eiko Munechika,[1] Yutaka Kanamaru,[1] Isao Shirato,[1] Yasuhiko Tomino,[1] Kouji Matushima,[2] Ko Okumura,[1] and Hiroshi Hashimoto[1]

ARTHRITIS & RHEUMATISM 46: 735–740, 2002

# ANALISIS DE LA FUNCION Y LESION RENAL

**Transpl Int. 1992;5 Suppl 1:S8-12.**

**Flow cytometry evaluation of urinary sediment in renal transplantation**

**Nanni-Costa A, Iannelli S, Vangelista A, Buscaroli A, Liviano G, Raimondi C, Todeschini P, Lamanna G, Stefoni S, Bonomini V.**

Lymphocyte surface-marker evaluation made it possible to differentiate lymphocyte populations observed during acute rejection episodes (cytotoxic T-cell, CD8 and HLA class II and NK cells) from those detected during bacterial infection (T-cell CD4 positive).
**These results suggest that urinary flow cytometry may be a reliable diagnostic tool in clinical renal transplantation.**



# ANALISIS DE LA FUNCION Y LESION RENAL

**Acta Univ Palacki Olomuc Fac Med. 1999;142:19-22.**

**Selected prognostic factors of long-term renal graft function.**

Krejci K.

We use several methods in order to make diagnosis of acute rejection. Urine cytology and urine flow cytometry have been found highly sensitive specific for the early diagnosis of acute rejection, provide us useful information in differentiation from others causes of graft dysfunction.

# ANALISIS DE LA FUNCION Y LESION RENAL

**Urol Int. 1999;62(3):143-6.**

**Flow-cytometric measurement of cellular changes in urine: a simple and rapid method for perioperatively monitoring patients after kidney transplantation.**

Yu DS, Sun GH, Lee SS, Wu CJ, Ma CP, Chang SY.

When 10% lymphocytes and 15% granulocytes in urine were set as the cutoff point of a normal ratio threshold, the flow cytometric analysis presented the highest sensitivity and the highest negative predictive rate for acute tubular necrosis.



# ANALISIS FUNCIONAL DE LA HEMOSTASIA

**Am J Kidney Dis. 2004 Feb;43(2):244-53.**

**Inflammation, endothelial dysfunction, and platelet activation in patients with chronic kidney disease: the chronic renal impairment in Birmingham (CRIB) study.**

Landray MJ, Wheeler DC, Lip GY, Newman DJ, Blann AD, McGlynn FJ, Ball S, Townend JN, Baigent C.

Clinical Trial Service Unit, University of Oxford, Oxford, UK. martin.landray@ctsu.ox.ac.uk

CONCLUSION: This cross-sectional analysis demonstrates that chronic kidney disease is associated with low-grade inflammation, endothelial dysfunction, and platelet activation, even among patients with moderate renal impairment.

# APLICACIONES DE LA CITOMETRIA EN LA DIALISIS

- Análisis de la compatibilidad de biomateriales
- Análisis funcional de la Hemostasia
- Evaluación funcional del Sistema Inmunitario



# ANALISIS FUNCIONAL DE LA HEMOSTASIA

**Blood Purif. 2003;21(2):176-82.**

**Comparison of the effects of cellulose triacetate and polysulfone membrane on GPIIb/IIIa and platelet activation.**

Kuragano T, Kuno T, Takahashi Y, Yamamoto C, Nagura Y, Takahashi S, Kanmatsuse K.

CONCLUSION: The characterization of changes in platelet membrane receptor (GPIIb/IIIa) may be a useful marker for studying the biocompatibility of dialysis membranes. On platelet aggregation, CTA might be more biocompatible membrane than PS.

# COMPATIBILIDAD DE BIOMATERIALES

## Flow cytometric study of *in vitro* neutrophil activation by biomaterials

M. B. Gorbet,[1] E. L. Yeo,[2] M. V. Sefton[1]
[1]*Department of Chemical Engineering and Applied Chemistry, Centre for Biomaterials, University of Toronto, Ontario, M5S 3E5, Canada*
[2]*Department of Medicine, University of Toronto, Ontario, M5S 3E5, Canada*

Biomed Mater Res, 44, 289–297, 1999.



# ANALISIS FUNCIONAL DE LA HEMOSTASIA

*DEPRESSION AND ANXIETY 15:91–101 (2002)*

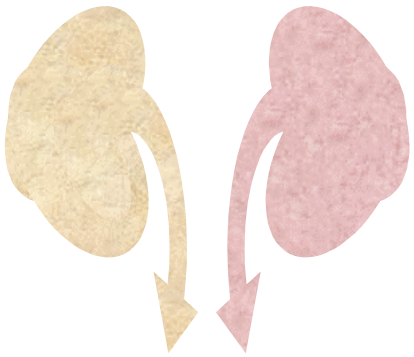
*Research Article*

## PLATELET ACTIVATION AND SECRETION IN PATIENTS WITH MAJOR DEPRESSION, THORACIC AORTIC ATHEROSCLEROSIS, OR RENAL DIALYSIS TREATMENT

Dominique L. Musselman, M.D., M.S.,[1*] Ulla Marzec, M.S.,[2] Madalyn Davidoff, M.D.,[4] Amita K. Manatunga, Ph.D.,[3] Feng Gao, M.D., M.P.H.,[3] Andrea Reemsnyder, B.A.,[1] Sasikanth Duggirala, B.B.A.,[1] Hannah Larsen,[1] Robert W. Taylor, M.D., Ph.D.,[4] Stephen Hanson, Ph.D.,[2] and Charles B. Nemeroff, M.D., Ph.D.[1]

DEPRESSION AND ANXIETY 15:91–101 (2002)

# APLICACIONES DE LA CITOMETRIA EN EL TRANSPLANTE RENAL



- Selección de receptor y donante
- Rechazo
- Supervivencia del transplante
- Complicaciones



# SELECCIÓN DE RECEPTOR Y DONANTE

**SHORT REPORT**

Laboratory investigations following an unexpectedly positive crossmatch result in a patient awaiting renal transplantation

J Cole, A Wortley, J Stoves, B Clark

*J Clin Pathol* 2002;**55**:627–628

# SELECCIÓN DE RECEPTOR Y DONANTE

In the preparation of patients for renal transplantation tests of human leucocyte antigen (HLA) sensitisation are performed to detect "unacceptable" HLA antigens that, if present on donor cells, would be expected to result in a positive crossmatch. Individuals bearing such specificities may then be excluded from consideration as donors. Unexpected positive crossmatch results are sometimes obtained when a serum specificity has not been detected on screening. Failure to identify a donor relevant HLA antibody in a recipient at the time of crossmatch may result in hyperacute rejection of the graft. This report describes laboratory investigations performed after a positive crossmatch result in a live donor situation. The pattern of crossmatch results indicated that reactivity resulted from HLA class I antibody. Previously performed serum screening using a standard complement dependent cytotoxicity technique had failed to identify donor relevant antibody specificities in the recipient. Retrospective flow cytometric screening of the same serum samples identified an HLA-A24 specificity of donor relevance. The lower sensitivity of methods used for routine serum screening compared with those used for crossmatching accounts for the findings in this case. The laboratory has amended its serum screening protocol to include flow cytometric analysis.

**Take home messages**

- The methods for the routine screening of serum should offer an equivalent or better degree of sensitivity than the methods used at final crossmatch
- Standard complement dependent cytotoxicity techniques should be complemented by more sensitive techniques, such as flow cytometric analysis



# SELECCIÓN DE RECEPTOR Y DONANTE

## The Detection and Definition of IgM Alloantibodies in the Presence of IgM Autoantibodies Using FlowPRA Beads

Naheed Khan, Amanda J. Robson, Judith E. Worthington, and Susan Martin

**ABSTRACT:** We have developed a flow cytometry–based screening method using FlowPRA (One Lambda) human leukocyte antigen (HLA) class I panel beads and FlowPRA (One Lambda) HLA class I specificity beads for the detection and definition of immunoglobulin (Ig)M HLA–specific antibodies in the presence of IgM autoantibodies. Forty-six autoantibody-positive patients who were on the waiting list for a renal transplant (56 sera) were tested in parallel with FlowPRA (One Lambda) HLA class I beads and FlowPRA (One Lambda) control beads. Sera that were positive for IgM HLA class I antibodies were subsequently tested with FlowPRA HLA class I specificity beads to determine the HLA specificities. Thirteen of the 46 patients were positive for IgM HLA class I–specific antibodies. Eleven of the 13 had previous failed transplants and 2 were awaiting a primary transplant. For 9 of the 13 positive patients, IgM HLA class I specificities were defined. We have demonstrated the presence of IgM HLA–specific antibodies in patients with IgM autoantibodies. This study demonstrates the value of FlowPRA HLA class I panel and specificity beads for the detection and definition of IgM HLA class I–specific antibodies. *Human Immunology 64, 593–599 (2003).* © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Inc.

**KEYWORDS:** IgM; flow cytometry; FlowPRA

Human Immunology 64, 593–599 (2003)

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# RECHAZO DEL TRANSPLANTE

ELSEVIER

**Relationship Between the Expression Levels of CD61, CD63, and PAC-1 on Platelet Surface in Peripheral Blood and the Transplanted Kidney Function**

Y.G. Zhang, D. Guan, C.Q. Xia, Z.Y. Han, J.J. Xu, J.Z. Gao, and K.R. Wu

Transplantation Proceedings, 35, 1360-1363 (2003)



# RECHAZO DEL TRANSPLANTE

Table 1. The Preoperative CD61, CD63, and PAC-1 Levels in Different Groups

| | Normal Graft Function Group | Acute Rejection Group | *P* |
|---|---|---|---|
| CD61 | 56.13 ± 10.73 | 79.28 ± 25.18 | <.05 |
| CD63 | 1.78 ± 0.69 | 15.24 ± 6.35 | <.01 |
| PAC-1 | 2.05 ± 1.38 | 9.71 ± 5.38 | <.01 |

Table 2. The Preoperative CD61, CD63, and PAC-1 Levels in Different Groups

| | Normal Graft Function Group | Acute Tubular Necrosis | *P* |
|---|---|---|---|
| CD61 | 56.13 ± 10.73 | 62.14 ± 29.27 | >.05 |
| CD63 | 1.78 ± 0.69 | 1.71 ± 1.38 | >.05 |
| PAC-1 | 2.05 ± 1.38 | 1.96 ± 0.89 | >.05 |

Transplantation Proceedings, 35, 1360-1363 (2003)

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# RECHAZO DEL TRANSPLANTE

ELSEVIER

**Diagnosis and Treatment of Acute Humoral Rejection After Kidney Transplantation: Preliminary Experience**

M. Crespo, M. Lozano, M. Sole, J. Mila, N. Esforzado, J. Martorell, and F. Oppenheimer

Transplantation Proceedings, 35, 1677–1678 (2003)



# RECHAZO DEL TRANSPLANTE

Background. Acute humoral rejection, or rejection associated with de novo production of anti-HLA donor-specific antibodies (DSA) after kidney transplantation (KTx), is a clinicopathologic entity that is not completely understood. Recent studies have proposed criteria for its diagnosis, including: (1) steroid-resistant acute dysfunction; (2) positive post-Tx donor-specific crossmatch (XM); and (3) widespread C4d deposits in peritubular capillaries (PTC) upon renal biopsy.

Methods. During 2002, prospective screening for AHR was established at our unit, seeking DSA post-KTx in selected cases of steroid-resistant acute rejection or acute dysfunction in high-risk sensitized or re-Tx patients. Frozen donor lymphocytes were used for post-Tx flow cytometry (FC) XM and high-definition flow PRA for patients with no frozen donor cells. We treated patients diagnosed with DSA using plasma exchange and polyclonal immunoglobulin.

Transplantation Proceedings, 35, 1677–1678 (2003)

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# RECHAZO DEL TRANSPLANTE

**Results.** Post-Tx DSA studies were performed in 9 of 94 patients transplanted during 2002. We detected DSA post-Tx in 3 of 9 recipients: 2 by FCXM and 1 using high-definition flow PRA. Two were highly sensitized pre-Tx, but the third patient was a 70-year-old woman receiving a first Tx (PRA = 0%). All 3 recipients presented with severe steroid-resistant acute renal dysfunction during the first 2 weeks post-Tx. Biopsies showed some features of AHR (neutrophils in PTC); 1 case showed no signs of concomitant cellular rejection. All rejection episodes were treated successfully (XM became negative and renal function recovered) by combining plasma exchange and polyclonal immunoglobulin.

**Conclusions.** The use of specific tools, like the crossmatch, in cases of acute, steroid-resistant renal graft dysfunction is important to identify and treat otherwise undetected humoral mechanisms of rejection.

Transplantation Proceedings, 35, 1677–1678 (2003)



# SUPERVIVENCIA A LARGO PLAZO

Cytometry (Communications in Clinical Cytometry) 34:103–112 (1998)

## Peripheral Blood Lymphoid Subsets and Long-Term Clinical Course of Kidney Recipients: A Longitudinal Study

J. Bas,[1] M. Mestre,[1]* J.M. Grinyó,[2] E. Massip,[1] J. Alsina,[2] A.M. Castelao,[2] M. Corominas,[1] and E. Buendia[1]

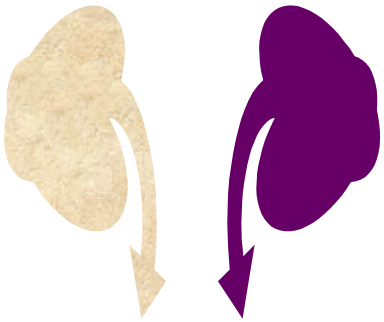
[1]Immunology Services, Ciutat Sanitària Universitària de Bellvitge, Barcelona, Catalonia, Spain

[2]Nephrology Services, Ciutat Sanitària Universitària de Bellvitge, Barcelona, Catalonia, Spain

Variations in the distribution of lymphocyte subsets are related with a long-term graft outcome. Within the first month after Tx, a rapid recovery of CD8+ lymphocytes, but not of CD4+ T cells, and a peak of HLA-DR expression, are associated with a good graft function. In contrast, long-term expression of activation markers is related with renal dysfunction.

# COMPLICACIONES DEL TRANSPLANTE

- Susceptibilidad a infecciones: HCMV
- Riesgo aumentado de enfermedades neoplásicas
- Riesgo aumentado de infarto de miocardio





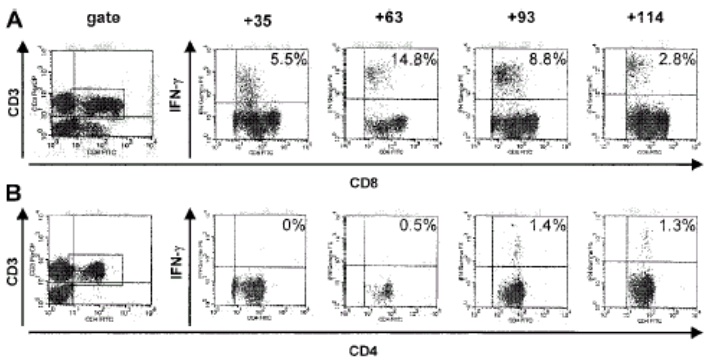
# COMPLICACIONES: INFECCION POR HCMV

TRANSPLANTATION

Sensitive detection of human cytomegalovirus peptide–specific cytotoxic T-lymphocyte responses by interferon-γ–enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation

Holger Hebart, Senay Daginik, Stefan Stevanovic, Ulrich Grigoleit, Andrea Dobler, Manuela Baur, Georg Rauser, Christian Sinzger, Gerhard Jahn, Juergen Loeffler, Lothar Kanz, Hans-Georg Rammensee, and Hermann Einsele

3834




Blood 99:3830-3837, 2002

# COMPLICACIONES: SINDROMES LINFOPROLIFERATIVOS

## Am J Clin Pathol. 2002 Jan;117(1):24-8.

**Flow cytometric immunophenotyping in posttransplant lymphoproliferative disorders.**

Dunphy CH, Gardner LJ, Grosso LE, Evans HL.

We studied the flow cytometric immunophenotyping (FCI) and genotypic data of 11 specimens from 10 transplant recipients and categorized them based on a scheme for posttransplant lymphoproliferative disorders (PTLDs). FCI is useful for identifying a clonal process in PTLDs with negative results by genotypic studies (GS). FCI and GS should be performed routinely in PTLDs to detect a clonal process.