In Memoriam

Silvia Pierangeli, PhD (1955–2013)E. Nigel Harris, MPhil, MD, DM¹ Rohan Willis, MBBS, MSc, DM²¹ Office of the Vice Chancellor, University of the West Indies, Kingston, Jamaica² Division of Rheumatology, Department of Internal Medicine, Antiphospholipid Standardization Laboratory, University of Texas Medical Branch, Galveston, Texas

Semin Thromb Hemost 2014;40:137–139.

Silvia Pierangeli was one of the most respected, prolific and well-liked investigators in the field of antiphospholipid antibody (aPL)/antiphospholipid syndrome (APS) research. In the course of a 25-year academic career, she combined passion for her work, talent, prodigious energy, an ability to pursue several tasks at once, a winning personality, an abiding spirit of generosity, and a strong association with colleagues across the globe.

Born in Buenos Aires, Argentina, to Dr. Hector and Mrs. Suzanna Pierangeli, who were themselves of scientific background and owned and operated a diagnostic laboratory in Buenos Aires, Silvia was educated at Buenos Aires National University where she received a PhD degree in Biochemistry. After working in her parents' laboratory for a few years, she went on to start one of her own. In 1986, she was awarded a Fulbright Scholarship and moved from Argentina with her husband and two young children to the University of Louisville in Louisville, Kentucky, where she received her second PhD degree in Microbiology and Immunology.

Dr. Pierangeli joined me (E.N.H.) as a postdoctoral fellow in 1987 to start what we named the Antiphospholipid Standardization Laboratory. As that name suggests, our major interest at the time was in popularizing and advocating a standard method for performance of the anticardiolipin antibody (aCL) test and investigation of the specificity of aCL antibodies and the lupus anticoagulant, with particular interest in the differential specificity of aCL antibodies produced in APS versus infectious diseases. By the mid-1990s, interest expanded to the mouse model of thrombosis and exploration of the pathogenesis of aPLs in APS using this *in vivo* model. By the first decade of the 21st century, Dr. Pierangeli's interests grew exponentially in using *in vivo* and *in vitro* strategies to probe cellular and intracellular effects of aPL as well as studies of biological agents that might ameliorate the clinical complications of APS. Her contributions fall into four major categories (1) standardization of aCL and anti- β_2 -glycoprotein I (anti- β_2 GPI) tests; (2) using mouse models of thrombosis and fetal loss to study the pathogenic

effects of aPLs and mechanisms by which these antibodies might be induced; (3) investigation of agents that ameliorate the effects of these antibodies so offering possible treatment options; and (4) development of a unique assay that distinguishes aPL in APS versus those produced in other disorders.

Dr. Pierangeli's work on standardization of the aCL and more recently, the anti- β_2 GPI test extended over 25 years. Recognizing the significant variability in performance of the tests and test results, she embarked on a tireless effort to promote standard test performance through multiple publications, workshops, membership in International Committees interested in standardization efforts, and making herself available to provide advice to countless laboratories and groups worldwide. Her efforts culminated in the assembly of experts 2 years ago to reach consensus on performance of the aCL and anti- β_2 GPI assays, which was published recently.¹ In addition, she was made chairman of the Clinical and Laboratory Standards Institute (CLSI) Document Development Committee on Antiphospholipid Antibodies, which is engaged in formalizing standard methods for the aCL and anti- β_2 GPI enzyme-linked immunosorbent assays (ELISA).

Focusing on the anti- β_2 GPI test, Dr. Pierangeli assembled another group of experts to prepare reference calibrators for the assay (using an approach similar to that used in 1986 to prepare aCL calibrators) and to develop units of measurement for levels of anti- β_2 GPI antibodies, which are expected to be adopted universally. Of special note for this journal were several contributions in specially aPL/APS themed issues in both 2012 and 2008.^{2–6}

Dr. Pierangeli's second area of work involved mouse models of thrombosis and fetal loss. The model entails the use of anesthetized mice whose femoral veins can be isolated and a thrombus induced by a standardized pinch injury, the vein is transilluminated and thrombus image projected on to a screen enabling the dynamics of thrombus formation and

Dr. Silvia Pierangeli, PhD

Address for correspondence
E. Nigel Harris, MPhil, MD, DM,
Office of the Vice Chancellor,
University of the West Indies,
Kingston, Jamaica
(e-mail: vcoffice@uwimona.edu.jm).

Issue Theme Quality in Hemostasis
and Thrombosis, Part III; Guest
Editors, Emmanuel J. Favaloro, PhD,
FFSc (RCPA), Giuseppe Lippi, MD,
and Mario Plebani, MD.

Copyright © 2014 by Thieme Medical
Publishers, Inc., 333 Seventh Avenue,
New York, NY 10001, USA.
Tel: +1(212) 584-4662.

DOI <http://dx.doi.org/10.1055/s-0034-1368795>.
ISSN 0094-6176.

disappearance to be quantified. Dr. Pierangeli and colleagues embarked on numerous studies using this model in an effort to probe thrombosis pathogenesis in APS.⁷ Some earlier findings were that mice passively infused with human immunoglobulin G (IgG), immunoglobulin M, or immunoglobulin A aPLs were subject to enhanced thrombosis size and duration.⁸ Likewise aPL antibodies induced in mice by immunization with β_2 GPI, human IgG aPL or viral peptides (homologous with select β_2 GPI regions) resulted also in enhanced thrombus size.^{9–11} Probing the mechanism of aPL-induced thrombosis, she along with her colleagues demonstrated in vivo activation of endothelial cells by aPL, and utilizing knockout mice, they showed that expression of endothelial cell markers intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (V-CAM 1) were required for thrombosis.^{12,13} More recently, she along with her colleagues showed the importance of tissue factor (TF),¹⁴ ApoER2¹⁵ (a splice variant of apolipoprotein endothelial receptor 2), annexin A₂,¹⁶ C5aR¹⁷ (complement 5a receptor), and C6¹⁸ (suggesting the membrane attack complex) in the pathophysiology of thrombosis in APS. These in vivo studies were accompanied by other in vitro work examining the intracellular molecular mechanism of aPL-induced endothelial and platelet activation.

Dr. Pierangeli also investigated how aPL antibodies might be induced in mice. In addition to earlier studies showing that human anti- β_2 GPI, human IgG aPL, and viral-derived peptides could induce aPL, she examined the role of major histocompatibility complex II (MHC II), different MHC II and Toll-like receptors in aPL formation. In addition, using TLR knockouts, Pierangeli and colleagues demonstrated the essential role of TLR7 and TLR 9.^{19,20}

Turning her attention to agents that would ameliorate the clinical complications of APS, Pierangeli demonstrated that hydroxychloroquine and fluvastatin decreased the thrombogenic effects of aPL in mice. In addition, she examined the ameliorative effects of the peptides—TIFI,²¹ Domain I,²² anti-NF κ B specific inhibitor,²³ anti-C5MoAb,²⁴ C5aR antagonist peptides,²⁵ and coversin (REV 576),²⁶ a novel C5 inhibitor. Her interest in these agents to “treat” aPL-induced thrombosis led to her becoming a major participant in the APS-ACTION group led by Dr. Doruk Erkan, which has gathered clinical and laboratory investigators from across the world to conduct clinical trials on patients with APS.

On the basis of a demonstration that aCL antibodies in patients with APS and infectious disease have different binding properties, Dr Pierangeli and colleagues developed and marketed the APHL ELISA test, which appears to be as sensitive as the aCL test for APS but more specific. For several years up to the time of her demise, she advocated replacement of the aCL test by this assay.

Dr. Pierangeli published more than 200 peer-review articles, reviews, chapters, and editorials. The crowning moment of her professional career was in 2010 when she organized the highly successful 13th International Congress on Antiphospholipid Antibodies, Galveston, Texas. This event attracted hundreds of participants from around the world and resulted in several significant publications, including a

comprehensive collection of articles from the conference in a book entitled *Antiphospholipid Syndrome: Insights and Highlights from the 13th International Congress on Antiphospholipid Antibodies*, coedited with Dr. Doruk Erkan.²⁷

While her investigative work was exceptional, people will remember her just as well for her winning personality. She was gregarious, caring, generous, and unafraid of challenges that life might have presented. She mentored dozens of fellows, residents, and students; she was a member of several committees (including NIH Study Sections) and on many editorial boards of peer-review journals. She was a wonderful mother to her daughters Cecilia and Eugenia and shared many treasured moments with her husband, Alvaro Schleh. Her goodwill and tremendous contributions to her field will undoubtedly inspire generations of young investigators to emulate her life.

References

- Lakos G, Favaloro EJ, Harris EN, et al. International consensus guidelines on anticardiolipin and anti- β_2 -glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum* 2012;64(1):1–10
- Harris EN, Pierangeli SS. Primary, secondary, and catastrophic antiphospholipid syndrome: what's in a name? *Semin Thromb Hemost* 2008;34(3):219–226
- Pierangeli SS, Chen PP, Raschi E, et al. Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms. *Semin Thromb Hemost* 2008;34(3):236–250
- Pierangeli SS, Harris EN. A quarter of a century in anticardiolipin antibody testing and attempted standardization has led us to here, which is? *Semin Thromb Hemost* 2008;34(4):313–328
- Willis R, Harris EN, Pierangeli SS. Pathogenesis of the antiphospholipid syndrome. *Semin Thromb Hemost* 2012;38(4):305–321
- Willis R, Harris EN, Pierangeli SS. Current international initiatives in antiphospholipid antibody testing. *Semin Thromb Hemost* 2012;38(4):360–374
- Pierangeli SS, Barker JH, Stikovac D, et al. Effect of human IgG antiphospholipid antibodies on an in vivo thrombosis model in mice. *Thromb Haemost* 1994;71(5):670–674
- Pierangeli SS, Liu XW, Barker JH, Anderson G, Harris EN. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the antiphospholipid syndrome. *Thromb Haemost* 1995;74(5):1361–1367
- Pierangeli SS, Harris EN. Induction of phospholipid-binding antibodies in mice and rabbits by immunization with human β_2 glycoprotein 1 or anticardiolipin antibodies alone. *Clin Exp Immunol* 1993;93(2):269–272
- Pierangeli SS, Liu SW, Anderson G, Barker JH, Harris EN. Thrombogenic properties of murine anti-cardiolipin antibodies induced by β_2 glycoprotein 1 and human immunoglobulin G antiphospholipid antibodies. *Circulation* 1996;94(7):1746–1751
- Gharavi AE, Pierangeli SS. Origin of antiphospholipid antibodies: induction of aPL by viral peptides. *Lupus* 1998;7(Suppl 2):S52–S54
- Pierangeli SS, Liu X, Espinola R, et al. Functional analyses of patient-derived IgG monoclonal anticardiolipin antibodies using in vivo thrombosis and in vivo microcirculation models. *Thromb Haemost* 2000;84(3):388–395
- Pierangeli SS, Espinola RG, Liu X, Harris EN. Thrombogenic effects of antiphospholipid antibodies are mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin. *Circ Res* 2001;88(2):245–250
- Romay-Penabad Z, Carrera-Marin AL, Mackman N, Pierangeli SS. Pathogenic effects of antiphospholipid antibodies are ameliorated in tissue factor deficient mice. *Arthritis Rheum* 2011; 63(10):s5(abstract13)

- 15 Romay-Penabad Z, Aguilar-Valenzuela R, Urbanus RT, et al. Apolipoprotein E receptor 2 is involved in the thrombotic complications in a murine model of the antiphospholipid syndrome. *Blood* 2011; 117(4):1408–1414
- 16 Romay-Penabad Z, Montiel-Manzano MG, Shilagard T, et al. Annexin A2 is involved in antiphospholipid antibody-mediated pathogenic effects in vitro and in vivo. *Blood* 2009;114(14):3074–3083
- 17 Romay-Penabad Z, Liu XX, Montiel-Manzano G, Papalardo De Martínez E, Pierangeli SS. C5a receptor-deficient mice are protected from thrombophilia and endothelial cell activation induced by some antiphospholipid antibodies. *Ann N Y Acad Sci* 2007; 1108:554–566
- 18 Carrera-Marín AL, Romay-Penabad Z, Papalardo E, et al. C6 knockout mice are protected from thrombophilia mediated by antiphospholipid antibodies. *Lupus* 2012;21(14):1497–1505
- 19 Papalardo E, Romay-Penabad Z, Christadoss P, Pierangeli SS. Induction of pathogenic antiphospholipid antibodies in vivo are dependent on expression of MHC- II genes. *Lupus* 2010;19:496(abstract)
- 20 Aguilar-Valenzuela R, Nickerson K, Romay-Penabad Z, et al. Involvement of TLR7 and TLR9 in the production of antiphospholipid antibodies. *Arthritis Rheum* 2011;63(10):s281(abstract723)
- 21 Ostertag MV, Liu X, Henderson V, Pierangeli SS. A peptide that mimics the Vth region of beta-2-glycoprotein I reverses antiphospholipid-mediated thrombosis in mice. *Lupus* 2006;15(6):358–365
- 22 Ioannou Y, Romay-Penabad Z, Pericleous C, et al. In vivo inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept. *J Thromb Haemost* 2009;7(5):833–842
- 23 Montiel-Manzano G, Romay-Penabad Z, Papalardo de Martínez E, et al. In vivo effects of an inhibitor of nuclear factor-kappa B on thrombogenic properties of antiphospholipid antibodies. *Ann N Y Acad Sci* 2007;1108:540–553
- 24 Pierangeli SS, Girardi G, Vega-Ostertag ME, Liu X, Espinola RG, Salmon JE. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. *Arthritis Rheum* 2005;52(7):2120–2124
- 25 Carrera-Marín AL, Romay-Penabad Z, QuHC, Papalardo E, Lambris J, Reyes-Maldonado, García-Latorre E, Pierangeli SS. A C5a receptor antagonist ameliorates in-vivo effects of antiphospholipid antibodies. *Arthritis Rheum* 2009;60:s767(abstract)
- 26 Carrera-Marín AL, Romay-Penabad Z, Machin S, Cohen H, Pierangeli SS. C5 inhibitor REV576 ameliorates in vivo effects of antiphospholipid antibodies. *Arthritis Rheum* 2011;63(10):s5(abstract12)
- 27 Erkan D, Pierangeli SS, Eds. *Antiphospholipid Syndrome: Insights and Highlights from the 13th International Congress on Antiphospholipid Antibodies*. 1st ed. New York, NY: Springer Science and Business Media; 2012