# La diagnostica di laboratorio delle trombofilie

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# Quali alterazioni trombofiliche ricercare

- Difetto di Antitrombina
- Difetto di Proteina C
- Difetto di Proteina S
- Mutazione Fattore V Leiden
- Mutazione G20210A della protrombina
- Lupus Anticoagulant e anticorpi antifosfolipidi
- Disfibrinogenemia
- Aumento Fattore VIII (Fattori IX, XI e altri)
- Iperomocisteinemia

# Quando NON eseguire i test

- Trombosi acuta (eseguire la diagnostica per la HIT, se presente sospetto)
- Durante le terapie anticoagulanti
- Gravidanza o terapia ormonale
- In tutte le condizioni associate ad aumento delle proteine della fase acuta (post-chirurgia, mal. infiammatorie)

Se indispensabile, test genetici sempre eseguibili, ma in genere non sono consigliabili screening parziali

# Proteina C e gravidanza

- Donna di 36 anni esaminata alla 25° settimana di gravidanza
- Il padre in trattamento anticoagulante per 2 pregressi episodi di TEV (1 TVP prossimale a 30 anni dopo gesso e recidiva idiopatica di TVP a 62 anni)
- Livello della Proteina C durante la gravidanza = 66% (n.v. > 68%)
- 2 anni dopo il parto:
  Proteina C attività = 47%
  Proteina C antigene = 51%
- Conferma del difetto di Proteina C nello zio paterno

*Zsuzsanna Bereczky, Réka Gindele, Marianna Speker, Judit Kállai*

*Deficiencies of the natural anticoagulants – novel clinical laboratory aspects of thrombophilia testing*

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

**Table 2** Distribution of different mutations within the genes for antithrombin, protein C and protein S

| Mutation types | *SERPINC1* | *PROC* | *PROS1* |
|---|---|---|---|
| Missense/nonsense | 155 (56%) | 231 (74.5%) | 171 (64%) |
| Splicing | 17 (6%) | 25 (8%) | 27 (10%) |
| Regulatory | 0 (0%) | 12 (4%) | 3 (1%) |
| Small deletions | 52 (19%) | 24 (8%) | 28 (10%) |
| Small insertions | 23 (8%) | 13 (4%) | 15 (6%) |
| Small indels | 2 (0.8%) | 3 (1%) | 4 (1.5%) |
| Gross deletions | 24 (9%) | 2 (0.5%) | 14 (5%) |
| Gross insertions/ duplications | 1 (0.4%) | 0 (0%) | 4 (1.5%) |
| Complex rearragements | 2 (0.8%) | 0 (0%) | 2 (1%) |
| **Total** | **276** | **310** | **268** |

*According to HGMD database (The Human Gene Mutation Database* http://www.hgmd.cf.ac.uk/ac/search.php*), accessed on 14th December 2015.*

# Difetti eredofamiliari di Antitrombina, Proteina C e Proteina S

- Ereditarietà: autosomica dominante
- Livelli attesi: eterozigote ⇨ 50-60%
  omozigote ⇨ 0-15%
  o doppio eterozigote
- Tipo I ⇨ ridotto dosaggio funzionale e immunologico
- Tipo II ⇨ ridotto dosaggio funzionale normale dosaggio immunologico

# Classificazione dei difetti eredofamiliari di Antitrombina

| Tipo | Alterazione |
|---|---|
| I | Ridotta sintesi e/o aumentato turnover |
| II PE | Alterata funzionalità sito reattivo e sito di legame per eparina (Pleiotropic Effect) |
| II RS | Alterata funzione del solo sito reattivo (Reactive site) |
| II HBS | Alterata funzione del solo sito di legame per eparina (Heparin Binding Site) |

# Dosaggio dell'Antitrombina

Metodi funzionali

Test cromogenici che possono essere eseguiti:

- in presenza di eparina = attività di cofattore dell'eparina
- in assenza di eparina = attività antitrombinica progressiva
- con trombina (umana o bovina) o FXa

Metodi immunologici

- immunodiffusione radiale (RID), laser nefelometria, elettroimmunodiffusione (sec. Laurell)

# Test di laboratorio e difetti eredofamiliari di Antitrombina

| Tipo | Dosaggio funzionale (con trombina o FXa) | | Dosaggio immunologico |
|---|---|---|---|
| | Cofattore eparinico | Attività progressiva | |
| I | ↓ | ↓ | ↓ |
| II PE | ↓ | ↓ | ↔ |
| II RS | ↓ | ↓ | ↔ |
| II HBS | ↓ | ↔ | ↔ |

# AT levels in different kindreds
## (Demers et al, 1993, modified)

| Pts | AT Def. type | AT anti-IIa UI/ml | AT anti-Xa UI/ml | AT:Ag UI/ml |
|---|---|---|---|---|
| 1 | I | 0.64 | 0.45 | 0.42 |
| 2 | I | 0.70 | 0.53 | 0.50 |
| 3 | I | 0.55 | 0.40 | 0.36 |
| 4 | II | 0.65 | 0.57 | 0.70 |

Minore probabilità di falsi negativi (valori normali in soggetti portatori del difetto) con l'uso FXa

# Influenza del Cofattore Eparinico II

- Il Cofattore Eparinico II inibisce la trombina umana, ha un debole effetto sulla trombina bovina e non inibisce il FXa
- Se il livello di Cofattore Eparinico II è particolarmente alto, l'uso di trombina umana nel test determina una sovrastima dei livelli di Antitrombina del 20-30%
- L'interferenza del Cofattore Eparinico II si può quindi evitare usando FXa e in parte usando trombina bovina

# Antitrombina Cambridge II (Ala384Ser)
## (Perry et al, 1998)

- Difetto di tipo II RS (dosaggio immunologico normale)
- Dosaggio cromogenico con FXa e trombina umana normale
- Dosaggio cromogenico con trombina bovina moderatamente ridotto

| FXa (n=105) | Trombina umana (n=25) | Trombina bovina (n=59) |
|---|---|---|
| 83% | 76% | 71% |

# Antithrombin Cambridge II (A384S): an underestimated genetic risk factor for venous thrombosis

Javier Corral,[1] David Hernandez-Espinosa,[1] Jose Manual Soria,[2] Rocio Gonzalez-Conejero,[1] Adriana Ordonez,[1] Jose Ramon Gonzalez-Porras,[3] Elena Perez-Ceballos,[1] Ramon Lecumberri,[4] Ignacio Sanchez,[1] Vanessa Roldan,[1] Jose Mateo,[2] Antonia Minano,[1] Marcos Gonzalez,[3] Ignacio Alberca,[3] Jordi Fontcuberta,[2] and Vicente Vicente[1]

**Table 1. Characteristics of patients with venous thrombosis and control subjects**

| Characteristic | Patients | | Controls | | Multivariate analysis P | Multivariate analysis OR (95% CI) |
|---|---|---|---|---|---|---|
| AT 1246 (A384S), no. | | | | | | |
| G/G | 1001 | 1.7% | 1016 | 0.2% | — | — |
| G/T | 17 | | 2 | | .002 | 9.75 (2.2-42.5) |

Prevalenza altri difetti di AT nei soggetti con precedente tromboembolismo venoso = 0.6%

4/17 pazienti = forma omozigote

BLOOD, 15 MAY 2007 • VOLUME 109, NUMBER 10

# Antithrombin Cambridge II (A384S) supports a role for antithrombin deficiency in arterial thrombosis

Vanessa Roldán[1]; Adriana Ordoñez[1]; Francisco Marín[2]; Esther Zorio[3]; José M. Soria[4]; Antonia Miñano[1]; Francisco España[3]; Rocio González-Conejero[1]; Javier Pineda[5]; Amparo Estellés[3]; Jordi Fontcuberta[4]; Vicente Vicente[1]; Javier Corral[1]

**Thromb Haemost 2009; 101: 483–486**

| | All Patients (n= 1224) | Controls (n= 1649) | Crude OR (95%CI) p value | Adjusted OR (95%CI) p value |
|---|---|---|---|---|
| Age | 51 ± 13 | 47 ± 16 | p<0.001 | |
| AT A384S<br>G/G (%)<br>G/T (%) | 1214 (99.2)<br>10 (0.8) | 1644 (99.7)<br>5 (0.3) | 2.71<br>(0.85–9.10)<br>0.059 | 5.66<br>(1.53–20.88)<br>0.009 |

| | Young Patients (n= 533) | Crude OR (95%CI) p value | Adjusted OR (95%CI) p value |
|---|---|---|---|
| Age | 40 ± 5 | p<0.001 | |
| AT A384S<br>G/G (%)<br>G/T (%) | 528 (99.1)<br>5 (0.9) | 3.11<br>(0.78–12.41)<br>0.059 | 9.98<br>(1.60–62.24)<br>0.009 |

# The phenotypic and genetic assessment of antithrombin deficiency

P. C. COOPER*, F. COATH†, M. E. DALY†, M. MAKRIS*,†

*Int. Jnl. Lab. Hem.* 2011, **33**, 227–237

| AT defect | AT level: bovine thrombin-based assay (IU/dl) | AT level: bovine FXa-based assay (IU/dl) | AT antigen level (IU/dl) |
|---|---|---|---|
| Cambridge II | 67 | 93 | 87 |
| Denver | 63 | 90 | 88 |
| Reference range | 86–132 | 83–135 | 83–124 |

**Discrepancy between antithrombin activity methods revealed in Antithrombin Stockholm: do factor Xa–based methods overestimate antithrombin activity in some patients?**

BLOOD, 15 MARCH 2002 • VOLUME 99, NUMBER 6

**Table 1. Antithrombin levels with antigen and activity methods**

| | Antigen (Lia test), % | Thrombin-based activity test, % | Factor Xa–based activity test, % |
|---|---|---|---|
| Proband (our results) | 93 | 60-64 | 110 |
| Proband (Canadian results) | NA | 72-74 | 53-56 |
| Sister (our results) | 86 | 62 | 92 |
| Brother (our results) | 92 | 55 | 89 |

# Great discrepancy in antithrombin activity measured using five commercially available functional assays

Kaija Javela *, Sari Engelbarth, Leena Hiltunen, Pirjo Mustonen, Marja Puurunen

Thrombosis Research 132 (2013) 132–137

These patients belonged to 94 different families.
100 patients were type I and 104 patients type II.

*Conclusions:* There was great inter-assay variability especially in type II deficient patients, but also in patients with type I deficiency. However, most of the patients defined as having normal AT activity by some methods had thrombotic symptoms. Most tested assays find type I AT deficient patients accurately. In our study population only methods A1 and C could find most patients with type II AT deficiency, whereas methods A2, B and D misdiagnosed the majority of patients as non-deficient.

| | Method | Principle | Incubation time (s) |
|---|---|---|---|
| A1 | STA-Stachrom AT III® | Thrombin-based | 60* |
| A2 | STA-Stachrom AT III® | Thrombin-based | 300 |
| B | Berichrom Antithrombin® | Thrombin-based | 180* |
| C | Innovance Antithrombin® | FXa-based | 180* |
| D | HemosIL® | FXa-based | 120* |

# Antithrombin heparin binding site deficiency: A challenging diagnosis of a not so benign thrombophilia

Christelle Orlando [a,*], Olivier Heylen [a], Willy Lissens [b], Kristin Jochmans [a]

Thrombosis Research 135 (2015) 1179–1185

*Objectives and Methods:* The study population consisted of 82 genetically confirmed HBS deficient patients sharing six different mutations. Plasma samples of 35 of them, including one homozygous patient, were used for the evaluation of 4 commercial activity assays in their ability to diagnose HBS deficiency. We assessed mutation-specific prevalence of venous and arterial thrombosis and the contribution of additional thrombophilic risk factors.

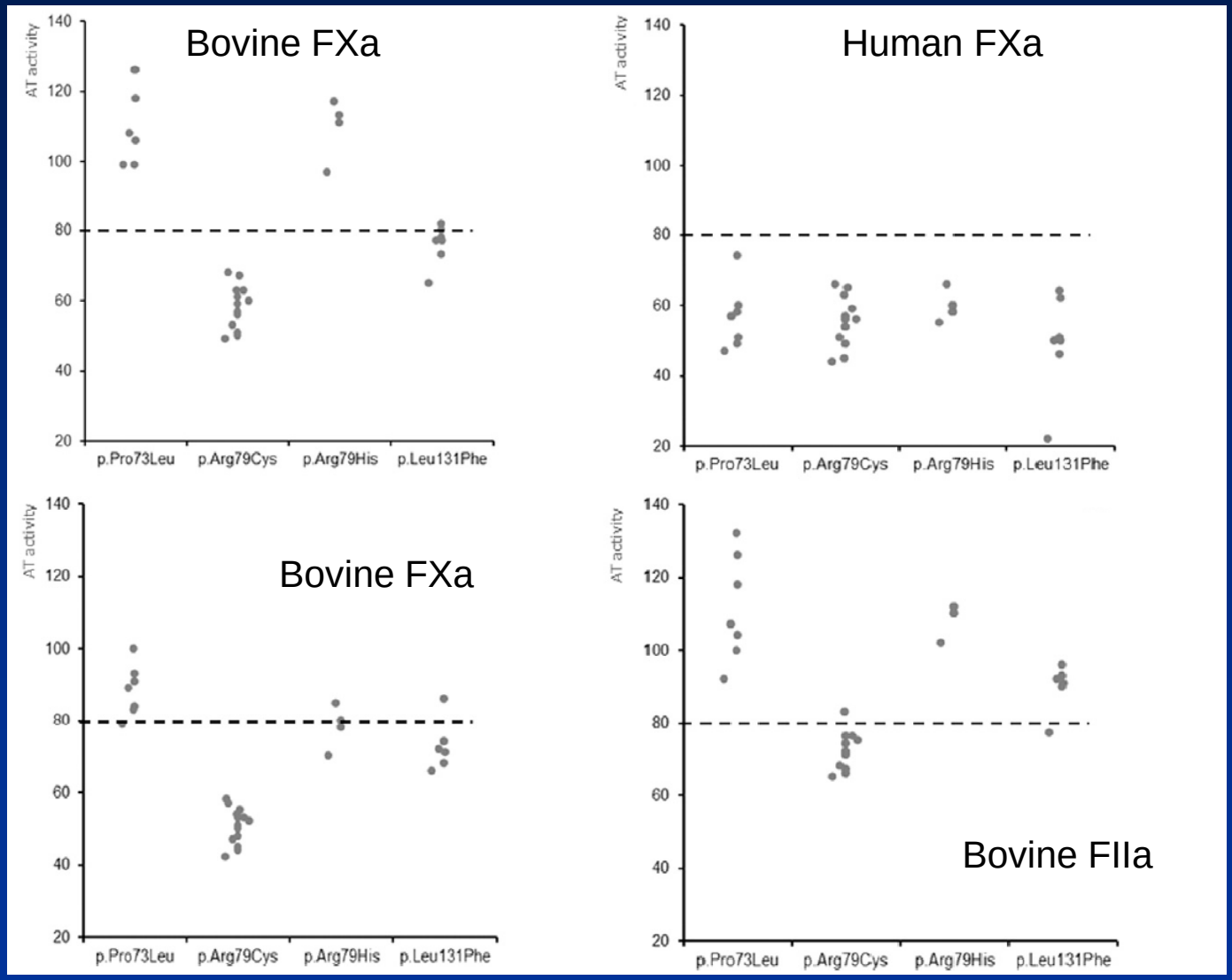
| | LIQ | COA | INN | BIO |
|---|---|---|---|---|
| **Substrate source** | Bovine FXa | Bovine FXa | Human FXa | Bovine FIIa |
| **Incubation time** | 100-140 s | 100-140 s | 180-190 s | 55-65 s |
| **Heparin concentration** | 3000U/mL | 3000U/mL | 1500U/mL | 5000U/mL |

LIQ = HemosIL ®Liquid AT, COA = Coamatic® AT, INN = Innovance®, BIO = Biophen® AT.

# Antithrombin heparin binding site deficiency: A challenging diagnosis of a not so benign thrombophilia

Christelle Orlando [a,*], Olivier Heylen [a], Willy Lissens [b], Kristin Jochmans [a]

Thrombosis Research 135 (2015) 1179–1185



# The phenotypic and genetic assessment of antithrombin deficiency

P. C. COOPER*, F. COATH†, M. E. DALY†, M. MAKRIS*,†

*Int. Jnl. Lab. Hem.* 2011, **33**, 227–237

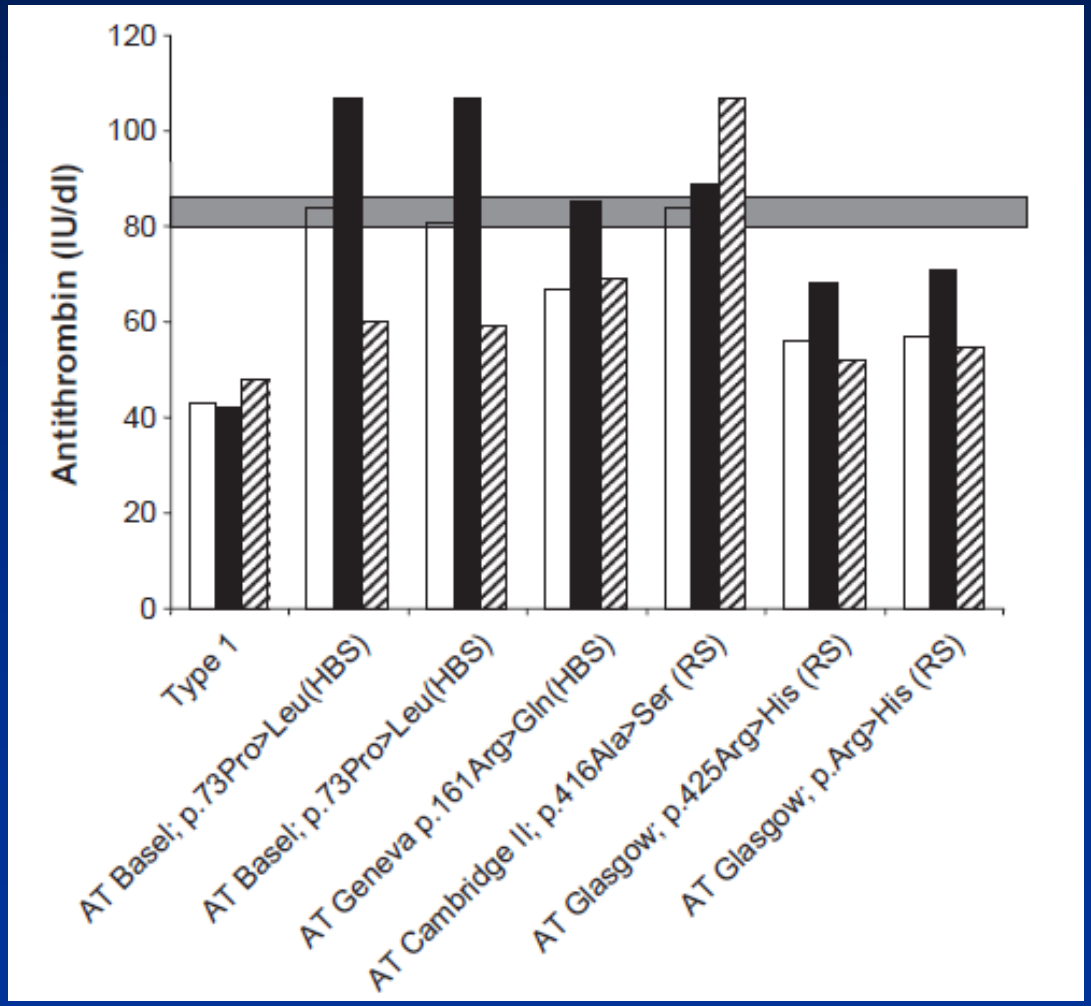



Figure 4. Antithrombin (AT) activity measured using a thrombin-based assay with 30-s incubation of sample dilution with bovine thrombin (□) or with a 180-s incubation with bovine thrombin (■) and assayed using a new human FXa-based assay (Innovance, Siemens) (▨). The broad horizontal bar indicates the lower limit of the normal range for AT in these assays.

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

This suggests that not only the source of enzyme (FIIa or FXa) is responsible for the difference in performance of AT functional tests. Table 1 demonstrates the assay characteristics of the most commonly used hc-anti-FXa AT assays. All but one reagent contains bovine FXa as enzyme in the reaction. The different tests differ in plasma dilution, in the added heparin concentration, in the source of chromogenic substrate and also in the incubation time. It can be concluded that clarification of the situation concerning heparin cofactor AT functional assays warrants future research to establish improved recommendation for AT testing.

Thrombosis Research 135 (2015) 1047–1048

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Editorial

# Puzzling questions on antithrombin: Diagnostic limitations and real incidence in venous and arterial thrombosis

In conclusion, despite of being an old molecule, there are still many interesting issues concerning antithrombin. We must improve the methods to diagnose antithrombin deficiency. And we should know the real incidence of antithrombin defects in atherothrombotic disorders.

# Principali domain funzionali della Proteina C

- Sito di attivazione
- Sito attivo
- Siti che legano i substrati (FVIII e FV)
- Sito di legame con Proteina S
- Siti di interazione con superfici fosfolipidiche, $Ca^{2+}$, EPCR

# Dosaggio della Proteina C

Metodi funzionali

Attivazione della PC con Protac e diluizione con plasma carente o tampone

- Metodi coagulativi
- Metodi cromogenici

Metodi immunologici

- ELISA
- Elettroimmunodiffusione (sec. Laurell)

# Dosaggio della Proteina C: metodi funzionali coagulativi

- Sensibili a tutte le possibili alterazioni della PC (interazione PC/$Ca^{2+}$,fosfolipidi, PS, FV e FVIII)
- Considerati a lungo i metodi di prima scelta, in quanto identificano tutti i possibili difetti
- Numerose interferenze

# Dosaggio della Proteina C: metodi funzionali cromogenici

- Evidenziano solo alterazioni del sito reattivo e del sito coinvolto nell'attivazione con Protac
- Nessuna interferenza

# Dosaggio della Proteina C:
## metodi funzionali coagulativi vs cromogenici

Metodi coagulativi

- Falsi valori ridotti per aumentati livelli FVIII, mutazione FV Leiden
- Falsi valori normali in presenza di LAC e eparina#

Metodi cromogenici

- Falsi valori normali in un raro difetto di tipo II (indicato come IIb; prevalenza 5% di tutti i difetti di tipo II) caratterizzato da una alterazione del sito di interazione con Ca2+ e fosfolipidi

# disponibili metodi insensibili fino a 1-2 U/ml

# Classificazione dei difetti eredofamiliari di Proteina C

| Tipo | Dosaggio funzionale | | Dosaggio immunologico |
|---|---|---|---|
| | coagulativo | cromogenico | |
| I | ↓ | ↓ | ↓ |
| II a | ↓ | ↓ | ↔ |
| II b | ↓ | ↔ | ↔ |

Altri fattori Vit-K dipendenti nella norma

# Dosaggio della Proteina C: scelta del metodo funzionale

- Le possibili interferenze sul test coagulativo sono numerose e si possono presentare una certa frequenza
- Il difetto di tipo IIb (alterato test coagulativo e normale test cromogenico) è molto raro
- L'uso di un metodo cromogenico è raccomandabile (pochi soggetti saranno non diagnosticati se si adotta questo metodo)
- L'uso di un metodo coagulativo è comunque indicato in casi selezionati in cui si sospetta la presenza di un difetto di tipo IIb

# Diagnostica dei difetti eredofamiliari di Proteina S

Complicata da:

- Presenza nel plasma di 2 forme di PS:
  libera (40%) = attiva come cofattore della PC
  legata al C4bBP (60%) = inattiva
- C4bBP è una proteina della fase acuta
  aumento del C4bBP = aumento della forma legata e riduzione della forma libera
- Nel plasma possono essere misurate attività, proteina totale e forma libera

# Dosaggio della Proteina S

Metodi funzionali

Per il dosaggio dell'attività

- Coagulativi (PT, aPTT)

Metodi immunologici

Per il dosaggio della proteina S totale e della frazione libera

- ELISA, nefelometrici, elettroimmunodiffusione (sec. Laurell)

# Classificazione dei difetti eredofamiliari di Proteina S

| Tipo | Dosaggio funzionale | Dosaggio immunologico | |
|---|---|---|---|
| | | Libera | Totale |
| I | ↓ | ↓ | ↓ |
| II | ↓ | ↔ | ↔ |
| III | ↓ | ↓ | ↔ |
| Altri fattori Vit-K dipendenti nella norma | | | |

# Dosaggio funzionale della Proteina S

Metodi coagulativi sensibili a:

- Mutazione FV Leiden
- Presenza LAC  e aumento di fattore VIII (metodi basati su PTT)
- Presenza di fattore VIIa (metodi basati su PT)

# Dosaggio della Proteina S: test di primo livello

- Raccomandabile utilizzare come test di primo livello un metodo immunologico per il dosaggio della frazione libera in sostituzione del metodo funzionale coagulativo
- Il dosaggio della frazione libera correla bene con il dosaggio funzionale nei difetti di tipo I e III, ma non in quelli di tipo II

TEST OF THE MONTH
AJH Educational Material
Am. J. Hematol. 89:1147–1150, 2014.

# Activated protein C resistance testing for factor V Leiden

■ Practical Considerations for Laboratories and Clinicians

The presence of $FV_{Leiden}$ can also be determined by DNA testing, which is not affected by any of the interferences described above. However, APC-R assays are advantageous as they are easily automated, cost effective [37,38], and may detect rare causes of APC resistance other than $FV_{Leiden}$. They are also necessary for detecting pseudohomozygous $FV_{Leiden}$ [11] and for assessing phenotypic $FV_{Leiden}$ thrombophilia in bone marrow or liver transplant patients [39]. APC-R assays that dilute patient plasma into factor V-deficient plasma (or the relevant equivalent for the assay) are much more accurate for detecting $FV_{Leiden}$ than are assays that do not. Although concordance of APC-R assays with DNA analysis is extremely high when the APC-R assay includes a dilution step, it is reasonable for laboratories to perform DNA analysis on patients with abnormal APC-R results, as this practice helps confirm that there has been no specimen mix-up during testing [39]. With most APC-R assays, lupus anticoagulants can cause falsely normal results (Table I). Therefore, if DNA analysis is not available, any patient with an abnormal result should be tested for a lupus anticoagulant.

*Journal of Thrombosis and Haemostasis*, **4**: 295–306

SPECIAL ARTICLE

# International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)

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R. H. W. M. DERKSEN,†† P. G. DE GROOT,†† T. KOIKE,‡ P. L. MERONI,‡‡ G. REBER,§§
Y. SHOENFELD,¶¶ A. TINCANI,*** P. G. VLACHOYIANNOPOULOS††† and S. A. KRILIS*

APS diagnosis is possible only if one clinical and one laboratory criterion are present

## Laboratory criteria

- Lupus Anticoagulant (LA) present in plasma, detected according to the guidelines of the ISTH (SSC on LA/phospholipid dependent antibodies)
- Anti phospholipid antibodies
  - Anticardiolipin antibodies (IgG and/or IgM) and
  - Anti ß2 glycoprotein I antibodies (IgG and/or IgM)

  in serum or plasma, present in medium or high titer (> 40 U or > the 99th percentile), measured by a standardized ELISA

Test positivity must be confirmed on two or more occasions at least 12 weeks apart

*Journal of Thrombosis and Haemostasis*, **4**: 295–306

- LA and solid-phase aPL (Ab aCL and Ab antiß2GPI) coexist in a limited number of patients with the APS syndrome
- Diagnosis must be based on both LA and solid-phase aPL detection

*Journal of Thrombosis and Haemostasis*, **4**: 295–306

Arthritis & Rheumatism

An Official Journal of the American College of Rheumatology
www.arthritisrheum.org and wileyonlinelibrary.com

SPECIAL ARTICLE

# International Consensus Guidelines on Anticardiolipin and Anti–$\beta_2$-Glycoprotein I Testing

## Report From the 13th International Congress on Antiphospholipid Antibodies

Gabriella Lakos,[1] Emmanuel J. Favaloro,[2] E. Nigel Harris,[3] Pier Luigi Meroni,[4] Angela Tincani,[5] Richard C. Wong,[6] and Silvia S. Pierangeli[7]

Vol. 64, No. 1, January 2012, pp 1–10

*Journal of Thrombosis and Haemostasis*, **7**: 1737–1740 DOI: 10.1111/j.1538-7836.2009.03555.x

**OFFICIAL COMMUNICATION OF THE SSC**

# Update of the guidelines for lupus anticoagulant detection

V. PENGO,* A. TRIPODI,† G. REBER,‡ J. H. RAND,§ T. L. ORTEL,¶ M. GALLI** and P. G. DE GROOT††

**Clinical Cardiology, Thrombosis Center, University Hospital, Padova; †Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, University and IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy; ‡Haemostasis Unit, Division of Angiology and Haemostasis, University Hospital, Geneva, Switzerland; §Hematology and Advanced Coagulation Laboratory, Montefiore Medical Center, Bronx, NY; ¶Division of Hematology, Duke University Medical Center, Durham, NC, USA; **Department of Hematology, Ospedali Riuniti, Bergamo, Italy; and ††Department of Clinical Chemistry and Haematology, University Medical Centre, Utrecht, the Netherlands*

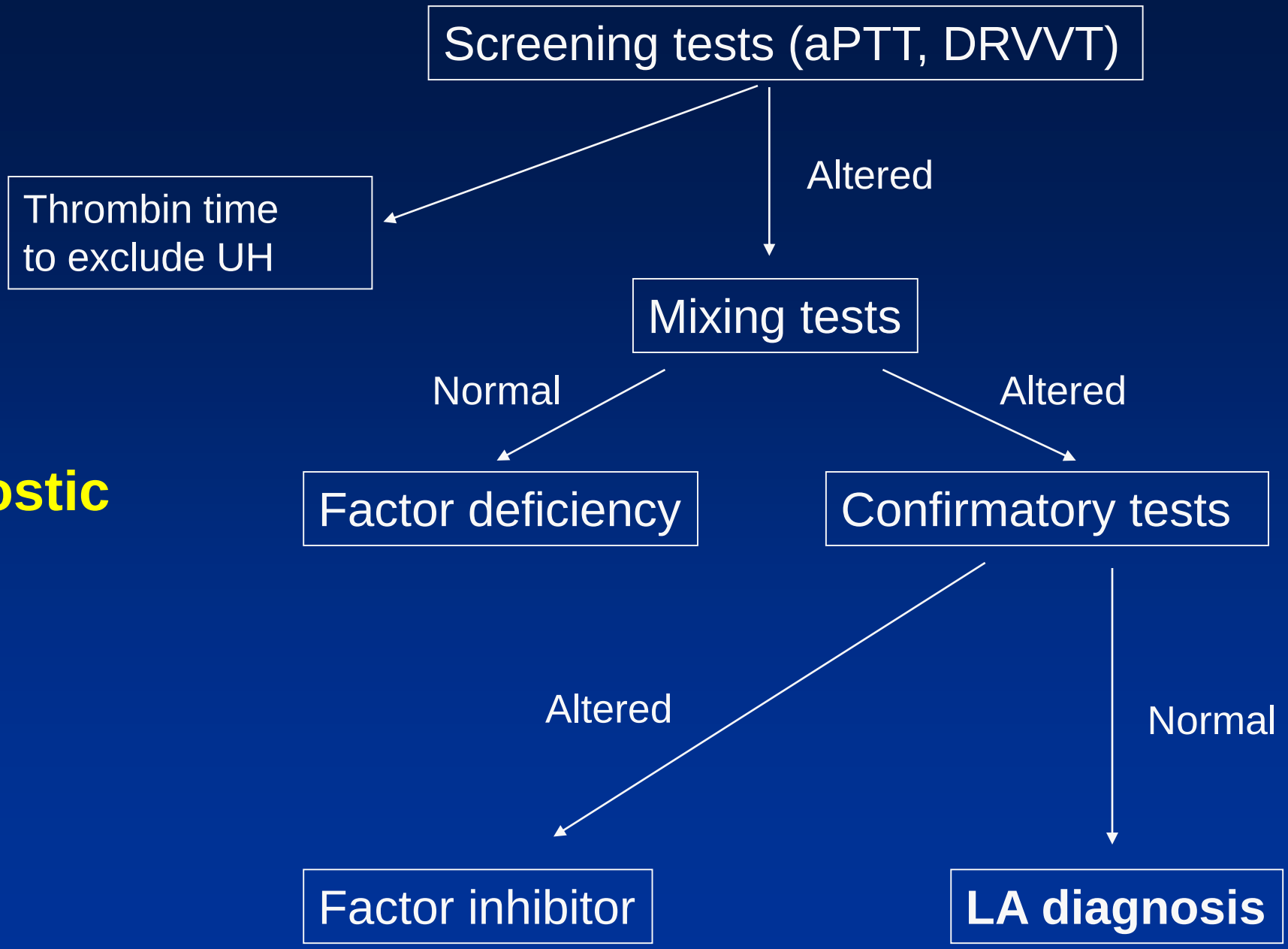
# LA laboratory detection

- Patients selection
- Blood collection
- Choice of tests
- Diagnostic steps
- Transmission results
- When testing

# Diagnostic steps

1. Screening tests
2. Mixing tests
3. Confirmatory tests

**LA diagnostic steps**



# Laboratory methods to detect antiphospholipid antibodies

*Steven A. Krilis[1,3] and Bill Giannakopoulos[1,2,3]*

*Hematology 2014*

**Summary of commonalities and contrasts between recent ISTH, BCSH, and CLSI guidelines for LAC detection**

| Area of recommendation | ISTH 2009 | CLSI 2014 |
|---|---|---|
| Sample preparation | Double centrifugation | Double centrifugation |
| Assays to use | dRVVT and aPTT | dRVVT and aPTT and/or others |
| Testing order | Screen-mix-confirm | Screen-confirm-mix |
| Ratio derivation | NPP denominator | RI mean denominator |
| RI/cutoffs | 99th percentile | 97.5th percentile (if Gaussian) |
| Calculations for phospholipid dependence | Percent correction of screen by confirm or LAC ratio (screen/confirm) | Percent correction of screen by confirm, or LAC ratio (screen/confirm) |
| Mixing test | Perform on 1:1 mixture with NPP; interpret with ICA or mixing test-specific cutoff | Perform on 1:1 mixture with NPP; interpret with ICA or mixing test-specific cutoff |
| Testing patients on VKAs | Undiluted plasma if INR $<1.5$; mix with NPP if INR $>1.5$ but $<3.0$ | Screen and confirm on 1:1 mixture with NPP; TSVT and ET or PNP |
| Testing patients on UFH | Interpret with caution | Can detect LAC in some cases where heparin neutralizer is effective |
| Interpretive reporting | Recommended | Recommended |

ET indicates Ecarin time; ICA, index of circulating anticoagulant; PNP, platelet neutralization procedure; RI, reference interval; TSVT, Taipan snake venom time; and UFH, unfractionated heparin.

# To Mix or Not to Mix in Lupus Anticoagulant Testing? That is the Question

Armando Tripodi, Ph.D. [1]

Semin Thromb Hemost 2012;38:385–389.

# ISTH guidelines on Lupus Anticoagulant testing

V. Pengo

Thrombosis Research 130 (2012) S76–S77

Diagnostic steps are:

a) screening step; b) mixing test; c) confirmatory test.

All these steps are needed and integrated tests where screening plasma is tested in the presence of low and high phospholipid concentration without a mixing test should be discouraged.

# More on: laboratory investigation of lupus anticoagulants: mixing studies are sometimes required

A. TRIPODI* and V. PENGO†

- Reporting a negative LA without having performed mixing tests may be dangerous
- The LA activity of some positive plasma samples is fully unveiled only when normal plasma is added, because of the so-called “lupus cofactor” phenomenon
- This unknown cofactor (prothrombin? β2-glycoprotein I?) which potentiates the inhibitory activity of LA is responsible for the paradoxical prolongation of the clotting time observed in the mixing test

*J Thromb Haemost* 2011; 9: 2126–7.

# Optimisation of lupus anticoagulant tests: should test samples always be mixed with normal plasma?

Maarten T. T. Pennings[1]; Philip G. de Groot[1]; Joost C. M. Meijers[2,3]; Albert Huisman[1]; Ronald H. W. M. Derksen[4]; Rolf T. Urbanus[1]

Thromb Haemost 2014; 112: 736–742

We conclude that mixing studies do not have an additional value in the exclusion of a single coagulation factor deficiency as the cause of a false positive screening assay. As a consequence, the merits of mixing during LA-assessment are questionable. Moreover, mixing tests might induce false negative LA results due to dilution effects. Since LA is readily detected with the combination of screening test and phospholipid confirm, we suggest to omit the mixing test during routine LA-assessment.