SISET

Workshop SISET

TROMBOFILIA

Post-ISTH: Novità dal meeting di Toronto 2015

Domenico Prisco
DMSC Università di Firenze
SOD Medicina Interna Interdisciplinare
AOU Careggi Firenze

Bergamo 29-30 Gennaio 2016

Thrombophilia is defined as a condition predisposing to the development of venous thromboembolism on the basis of a hypercoagulable state

Inherited Disorder
Acquired disorder

Inherited disorder

Loss-of-function mutations of the genes encoding natural anticoagulant proteins (protein C, protein S and antithrombin)

Gain-of-function polymorphism factor V Leiden and prothrombin G20210A

Inherited Marker

non-O blood group

Acquired disorder

Antiphospholipid Antibodies

Thrombophilia classification

Thrombophilia	Arterial/Venous Thrombosis	Inherited/Acquired	Relative Risk of Initial Thrombosis
Factor V Leiden	Venous	Inherited	↑ Heterozygous ↑↑ Homozygous
Prothrombin gene mutation	Venous	Inherited	↑ Heterozygous ↑↑ Homozygous
Protein C deficiency	Venous	Inherited	↑↑
Protein S deficiency	Venous	Inherited	↑↑
Antithrombin deficiency	Venous	Inherited	↑↑
Hyperhomocysteinemia	Arterial/venous	Rarely inherited/most often acquired	↑ If mild to moderate ↑↑ If severe
Antiphospholipid antibodies	Arterial/venous	Acquired	↑↑

Piazza G, Circulation 2015

Prevalence of thrombophilia abnormalities and relative risk of venous thromboembolism

Thrombophilia abnormality	Prevalence (%)		Relative risk	
	General population	Patients with VTE	First VTE	Recur-rent VTE
Antithrombin deficiency	0.02–0.2	1	50	2.5
Protein C deficiency	0.2–0.4	3	15	2.5
Protein S deficiency	0.03–0.1	2	10	2.5
Factor V Leiden (heterozygous)	5	20	7	1.5
Factor V Leiden (homozygous)	0.02	1.5	80	-
Prothrombin G20210A (heterozygous)	2	6	3–4	1.5
Prothrombin G20210A (homozygous)	0.02	<1	30	-
Non-O blood group	55–57	75	2	2

Mannucci PM, TH 2015

Inherited disorders

The FUTURE

1.

From SINGLE Thrombophilic alteration

Through COMBINED Defects

To ALGORITMS including Thrombophilic and Acquired risk factors

Genetic

From A single mutation

To Haplotype

The FUTURE …new hereditary thrombophilic markers….

2.

Family and twin studies indicate that genetics accounts for about 60% of the risk for VTE.

Deficiencies of antithrombin, protein C and S are rare and explain only about 1% of all DVTs. Heterozigosity for factor V Leiden and prothrombin G20210A are more common, but still only explain a fraction of VTE events.

A large proportion of the heritability of VTE remains unexplained.

Inherited disorders: news

- Genetic studies on AT heparin binding site deficiency, condition at higher risk than previously thought, not detectable with common tests
- Several new candidate loci as risk factors for thrombosis
- Role of non ABO group for hormone –related VTE
- Five case of thrombophilic afibrinogenemia patients treated with fibrinogen (restoration of antithrombin activity of fibrinogen?)

OR079

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

Orlando C[1], Heylen O[1], Lissens W[2] and Jochmans K[1]

[1]Department of Haematology; [2]Centre for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel), Vrije Universiteit Brussel (VUB), Brussel, Belgium

Aims: This study aimed to investigate the genetic background of AT HBS deficiency, to assess sensitivity of commercial AT activity assays for HBS mutations and to study the associated clinical picture.

Results: Six different mutations were identified in the studied patients. In one patient, a novel mutation, p.Asn77His, was identified, a quite exceptional finding given the restricted number of causal mutations in AT HBS deficiency. Only one assay (Xa-based) showed 100% sensitiv-

Conclusion: Our data proof the limited sensitivity of commercial assays in the identification of patients with AT HBS deficiency, while this subtype seems more prevalent and less benign than previously assumed.

JTH, 2015

OR148

Identification of new genetic risk factors for recurrent venous thrombosis

de Haan HG[1], Germain M[2], Baglin TP[3], Deleuze J-F[4], Trégouët D-A[2], Rosendaal FR[1,5,6] and van Hylckama Vlieg A[1]

Aims:
to identify genetic determinants of recurrent VT

Methods:
We performed a genome wide association scan in 1279 patients with venous thrombosis from the MEGA follow-up study

Conclusion:
Our results reconfirm the association of FV Leiden with recurrent VT. In addition, we identified **several new candidate loci** that may be associated with the risk of recurrent VT. For these loci replication and further studies are warranted

JTH, 2015

OR248

Characterization of a novel FV mutation (A512V, FV Bonn) associated with deep vein thrombosis and APC resistance

Pezeshkpoor B[1], Castoldi E[2], Hamedani NS[1], Biswas A[1], Oldenburg J[1] and Pavlova A[1]

Aims:

To characterise a novel F5 mutation (A512V, FVBonn), linked to APC resistance and clinically associated with deep vein thrombosis

Results

FVBonn conferred marked APC resistance to FV-deficient plasma

Conclusion:

We have identified and characterized a novel F5 mutation associated with APC resistance and deep vein thrombosis. Although the FVBonn mutation does not directly affect an APC-cleavage site, it is located close to the Arg506 cleavage site and causes APC resistance with a mechanism similar to that of FVLeiden. Decreased susceptibility to APC-catalysed inactivation and reduced APC-cofactor activity in APC-catalysedFVIIIa inactivation both contribute to FVBonn associated APC resistance.

JTH, 2015

OR394

Identification of new genetic risk factors for venous thrombosis by targeted sequencing: results of the MILES study

de Haan HG[1], van Hylckama Vlieg A[1], Lotta LA[2], Gorski MM[3], Bucciarelli P[2], Martinelli I[2], Baglin TP[4], Peyvandi F[2,3] and Rosendaal FR[1,5,6]

Aims:
We aimed to identify new genetic risk factors for VT using targeted Sequencing
Results
Single-variant association tests for 5218 common variants showed **123 associations with VT risk** (FDR < 0.20). **Most of these variants (89%) were located in F5, ABO, FGG/FGB/FGA, and the F11 region**. Gene-based analyses showed a burden of rare variants in 9 genes.
Conclusion:
Using a targeted sequencing strategy we were able to identify new genetic risk factors for venous thrombosis.

JTH, 2015

PO547-MON

The possible role of c.1824c>t prothrombin gene variant in pathogenesis of thrombophilia

Djordjevic V[1], Pruner I[1], Gvozdenov M[1], Tomić B[1], Kovac M[2,3], Miljic P[2,4] and Radojkovic D[1]

Aims:
In order to examine the possible role of FII c.1824C>T variant in pathogenesis of thrombophilia we performed case-control study and determined the plasma prothrombin level in carriers of this gene Variant

Results
We observed higher frequency of heterozygous carriers of FII c.1824C>T gene variant in the patient group compared to the controls (3.39% vs. 0.52%, P = 0.049). Presence of this variant leads to the increase of prothrombin level in the plasma

Conclusion:
Our results indicate that **FII c.1824C>T gene variant may be a potentially important thrombophilia marker**.

JTH, 2015

OR324

Risk factors for venous thromboembolism in women under combined oral contraceptive: the pill genetic risk monitoring (PILGRIM) study

Suchon P[1,2], Frouh FA[2], Henneuse A[1,2], Ibrahim M[1], Brunet D[1], Barthet M-C[1], Aillaud M-F[1,2], Alessi M-C[1,2], Tregouët DA[3,4,5] and Morange P-E[1,2]

Aims:

To identify determinants of VTE risk in a large cohort of women under COC and to evaluate the accuracy of a Family History Score (FHS) to detect inherited thrombophilia

Results

After adjusting for age, family history, type and duration of COC use, tobacco consumption and Body Mass Index (BMI) were associated with VTE. In addition, severe heritable thrombophilia (OR = 2.23 [1.39–3.66]) and non- O blood groups (OR = 1.85 [1.49–2.29]) were genetic risk factors for VTE

Conclusion:

This study shows for the first time **the impact of ABO blood group on the risk of VTE in women under COC** and confirms the inaccuracy of the family history to detect heritable thrombophilia, even using a family history score.

JTH, 2015

OR432

Hereditary afibrinogenemia – long-term observation of a highly thrombogenic condition and it's management

Nagler M[1,2,3], Kremer Hovinga JA[1,2], Alberio L[1,2,4], Peter-Salonen K[1,2,5], von Tengg-Kobligk H[6], Lottaz D[2] and Lämmle B[1,2,7]

Aims:

To report on long-term data of **five HA patients with severe thromboembolic complications, possibly due to lacking anti-thrombin activity of fibrinogen (fbg)/fibrin**

Results

After adjusting for age, family history, type and duration of COC use, tobacco consumption and Body Mass Index (BMI) were associated with VTE. In addition, severe heritable thrombophilia (OR = 2.23 [1.39–3.66]) and non- O blood groups (OR = 1.85 [1.49–2.29]) were genetic risk factors for VTE

Conclusion:

Our data suggest **regular fbg replacement therapy resulting in permanently measurable fbg levels to be a safe and effective treatment option in patients with HA and thromboembolic complications**.

JTH, 2015

Acquired disorders

Acquired disorders: news

- Antiphospholipid antibodies:
  - The search of new lab and clinical markers for risk stratification
  - The validation of NOACs for thrombosis prevention and treatment in APS

OR093

Thrombin generation in patients with antiphospholipids antibodies

Valle PD[1], Novelli C[2], Pradella P[3], Morelli B[2] and D'Angelo A[1]

[1]Coagulation Service and Thrombosis Research Unit, Scientific Institute San Raffaele, Milano; [2]Transfusion Center and Haematology Laboratory, Legnano Hospital, Legnano; [3]Transfusion Center, Cattinara Hospital, Trieste, Italy

Aims:

To evaluate the sensitivity of the modified CAT according to aPL reactivity in patients with aPL

Results

In the overall aPL population, compared to HC, significant differences were observed for LT($P < 0.0001$), PK ($P = 0.02$), and ACC ratios ($P = 0.001$). In LA+ patients ($n = 77$), the LT ratio was the only abnormal parameter ($P < 0.0001$), irrespective of the association with either aCL-reactivity ($n = 2$), aß2-GPI-reactivity ($n = 8$) or both ($n = 27$; $P \geq 0.13$). LT wasalso prolonged in the 35 LA- patients (($P = 0.04$). ACC and PK ratios were increased only in LA- patients ($P < 0.0001$), irrespective of aPL reactivity ($P \geq 0.56$)

Conclusion:

In the modified CAT assay, an isolated, marked prolongation of the LT parameter is the hallmark of lupus anticoagulants.

JTH, 2015

APCr in antiphospholipid positive patients

Dr. Wahl aimed to determine the value of APC resistance measured by thrombin generation to predict thrombosis in platelet-rich plasma of APS patients. In a prospective multicenter study consisting of 137 persistent anti-phospholipid positive patients, the IC-50 APC concentration was determined. This concentration proved to be a significant predictor for thrombosis, both in asymptomatic patients as in patients that already showed clinical symptoms. He concluded that there is a need for standardized protocols and cut-off determinations.

Triple positivity now shows four main characteristics

1. High association with thromboembolic events;
2. No need for confirmation after 12 weeks;
3. Strong association with a single pathogenic autoantibody;
4. Method- and platform-independent detection.

Anti-domain I antibodies

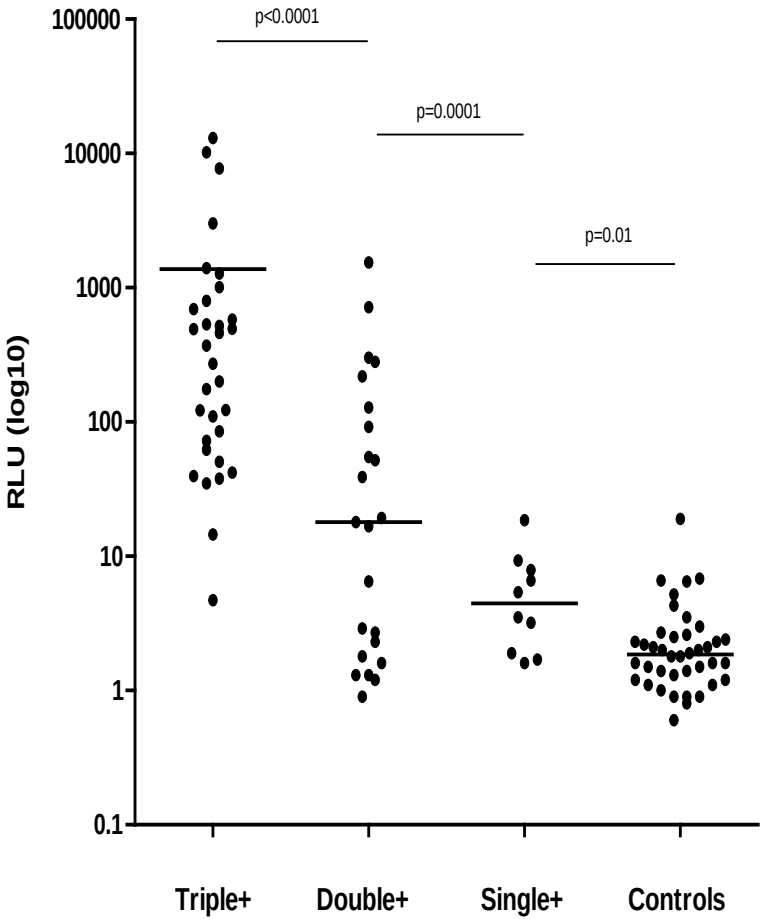
A competitive inhibition ELISA developed by Pengo to demonstrate the presence of anti-domain I antibodies.

Using this assay, a strong correlation was found between anti-domain I antibodies and triple positivity, as well as thrombosis. Interestingly, anti-domain I antibodies proved to be stable over time.

Discussion if these anti-domain I antibodies should be included in the APS criteria.

According to Devreese, due to the reduced sensitivity of the assay, the anti-domain I assay cannot replace the anti-β2GPI assay.

IgG aβ2GP1-Dm1 and risk categories



Pengo V et al.

OTOMO SCORE

Relative risk of clinical manifestations of APS for each aPL test

Test	Cutoff	Sensitivity, %	Specificity, %	OR (95% CI)	aPL score
APTT mixing	>49 sec.	39.1	89.3	5.36 (2.53–11.4)	5
Confirmation test, ratio	>1.3	19.6	95.2	4.81 (1.79–12.9)	2
	>1.1	30.4	90.9	4.38 (1.96–9.76)	1
KCT mixing	>29 sec.	45.6	88.8	6.64 (3.17–13.9)	8
dRVVT mixing	>45 sec.	28.2	90.9	3.93 (1.74–8.88)	4
Confirmation test, ratio	>1.3	17.4	94.7	3.72 (1.38–10.1)	2
	>1.1	28.3	90.4	3.7 (1.65–8.27)	1
IgG aCL, GPL					
High titers	>30	15.2	98.4	11 (2.72–44.5)	20
Medium/low titers	>18.5	19.5	94.6	4.31 (1.63–11.3)	4
IgM aCL, MPL	>7	6.52	96.3	1.79 (0.45–7.22)	2
IgG anti-β_2GPI, units					
High titers	>15	23.9	98.4	19.3 (5.11–72.7)	20
Medium/low titers	>2.2	30.4	92.5	5.4 (2.35–12.4)	6
IgM anti-β_2GPI, units	>6	8.7	91.4	1.02 (0.32–3.20)	1
IgG anti-PS/PT, units					
High titers	>10	19.6	97.8	11.1 (3.25–38.1)	20
Medium/low titers	>2	28.3	95.7	8.81 (3.39–22.9)	13
IgM anti-PS/PT, units	>9.2	6.52	98.9	6.45 (1.05–39.8)	8

Otomo K, Artritis & Rheumat, 2012

Multivariate logistic regression analysis showed that only arterial hypertension, hyperlipidaemia, LA, aCL, anti-b2GPI and aPS/PT were independent risk factors for thrombosis and/or PL

	β-coefficient	GAPSS[a]
Hyperlipidaemia	1.73	3
Arterial hypertension	0.54	1
aCL IgG/IgM	2.63	5
Anti-β2GPI IgG/IgM	2.02	4
aPS/PT IgG/IgM	1.78	3
LA	2.35	4

nb. assignment of points to risk factors was based on a linear transformation of the corresponding b-regression coefficient

	AUC	Sensitivity	Specificity	NPV	PPV	*P*-value
GAPSS cut off = 10	0.736	0.709	0.793	0.7705	0.7045	0.000
GAPSS cut off = 12	0.697	0.578	0.817	0.7206	0.7027	0.001
GAPSS cut off = 15	0.664	0.378	0.950	0.6706	0.8500	0.004

aPL-S and GAPSS

	aPL-S (Otomo)	GAPSS (Sciascia)
	LA, aCL, abeta2GPI and aPS/PT included	LA, aCL, abeta2GPI and aPS/PT included
APL	Different LA tests titres or IgG/IgM represented as different scores	LA pos or neg Isotype not considered
Other risk factors	None	Hypertension, hyperlipidemia included
Function	Diagnosis of APS Prediction of thrombosis	Prediction of thrombosis

Summary

The GAPSS is a valid tool for a risk stratification for thrombosis both in SLE patients positive for aPL as well as in primary APS

Since in different patients population different cut-off values of GAPSS are able to predict thrombosis, a refinements of scoring is needed

aPL score may still have a role in patients with autoimmune disease

The combination of new sensitive lab markers (anti-D1, CAT?, others?) with GAPPS could furtherly improve to effort to identify the risk of events in the single subject

Clin Rheumatol
DOI 10.1007/s10067-015-3030-y

CASE BASED REVIEW

Thrombotic events in patients with antiphospholipid syndrome treated with rivaroxaban: a series of eight cases

Flavio Signorelli[1,2] · Felipe Nogueira[3] · Vinicius Domingues[4] · Henrique Ataide Mariz[5] · Roger A. Levy[3,6]

Comments

Until the results of trials will be available, VKAs remain the mainstay treatment in thrombotic APS

Ongoing trials on rivaroxaban in patients with APS

RAPS: Rivaroxaban in Antiphospholipid Syndrome
randomized controlled phase II/III clinical trial, including only APS patients with previous venous events
(Lupus; May 4, 2015. doi: 10.1177/0961203315581207)

TRAPS: Rivaroxaban in Thrombotic Antiphospholipid Syndrome
randomized controlled trial, including patients with all the clinical manifestations of APS, and with triple positivity
(https://clinicaltrials.gov/ct2/show/NCT02157272 Accessed May 13, 2015)

Rivaroxaban and LA assays

Arachchillage investigated if rivaroxaban influences LA assays and if anti-phospholipid antibodies affect the activity of rivaroxaban.
In vitro and ex vivo studies showed false positives with two commercial DRVVT assays at the peak concentration of rivaroxaban.
However, the Taipan venom time/Ecarin clotting (TVT/EC) time proved to be unaffected, allowing a reliable detection of LA.
Both by thrombin generation and rivaroxaban anti-Xa levels, she demonstrated that anti-phospholipid antibodies do not influence the activity of rivaroxaban.
In reply to a question she discourages the use of DRVVT tests in patients taking rivaroxaban but rather advises to incorporate the alternative TVT/EC test in each laboratory

RAPS Study

Cohen investigated if the superior results of rivaroxaban and other direct oral anticoagulants for the treatment of venous thrombosis can be extended to APS.

The **primary aim** of the prospective RAPS study was to demonstrate, in patients with APS and previous venous thromboembolism that the intensity of anticoagulation (CAT, F1+2, DD) achieved with rivaroxaban is not inferior to that of warfarin (percentage chang in ETP at 42 d).

Secondary aims were to compare rates of recurrent thrombosis, SAE and all bleeding and the quality of life in patients on rivaroxaban with those on warfarin (f-up 6 months).

Tissue factor-triggered thrombin generation was used to compare the inhibitory effects of the anticoagulants.

Cohen SSC-ISTH, 2015

RAPS Study results

116 pts randomized

P**rimary aim:** the intensity of anticoagulation achieved with rivaroxaban was inferior to that of warfarin in terms of ETP but not different considering all parameters of thrombogram and activation markers.

Secondary aims : no thrombosis nor major bleeding after a 6-month period

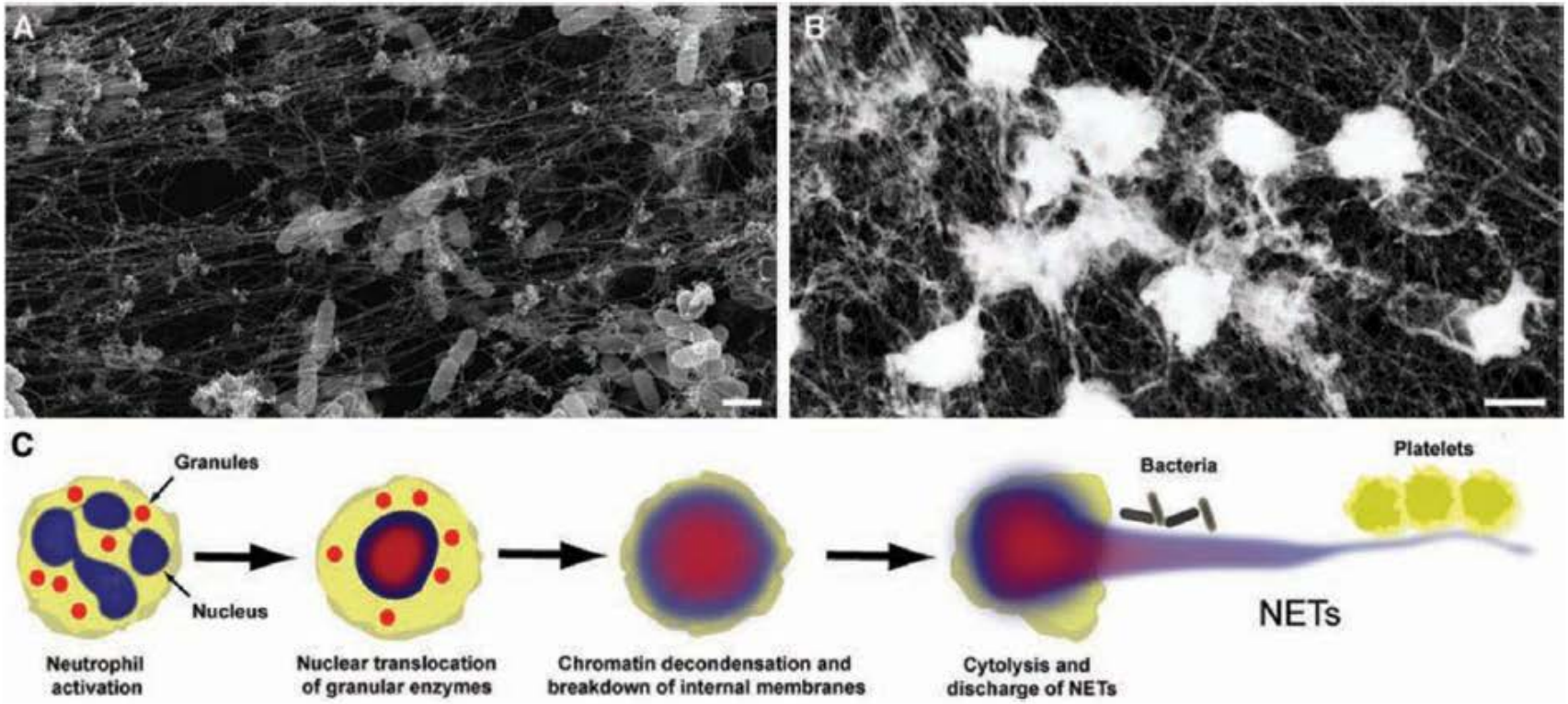
Cohen SSC-ISTH, 2015

Extracellular nucleic acids as potential biomarkers for venous thrombosis

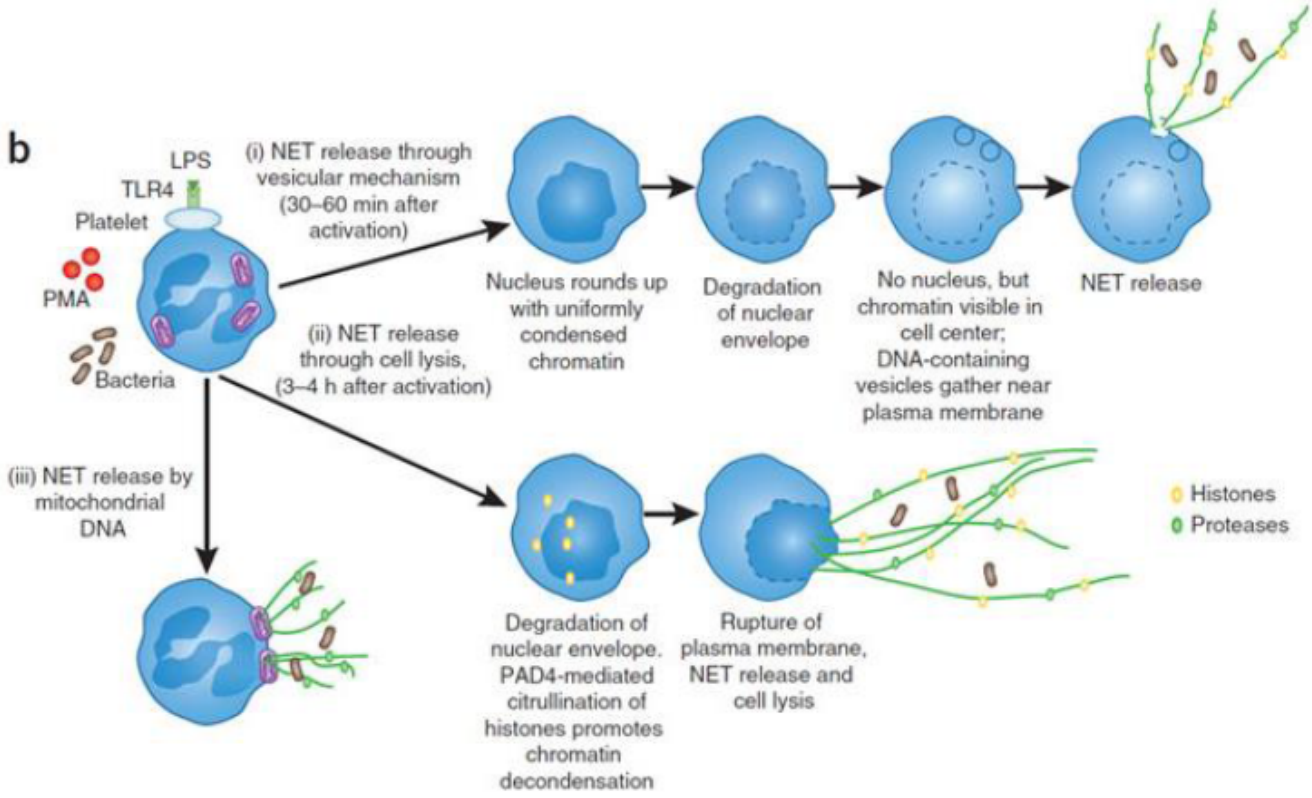
However, studies regarding its clinical applicability are still missing although there is considerable progress in understanding the interaction of extracellular DNA with the coagulation system

Neutrophil extracellular traps (NETs)

NETs are composed of a core DNA element to which histones, proteins (for example, lactoferrin and cathepsins) and enzymes (for example, MPO and neutrophil elastase) that are released from neutrophil granules, are attached. NETs immobilize pathogens, thus preventing them from spreading but also facilitating subsequent phagocytosis of trapped microorganisms. They are also thought to directly kill pathogens by means of antimicrobial histones and proteases.



NETs release mechanisms



(i) NETs can be released through a vesicular mechanism. Initially, the neutrophils become rounded with uniformly condensed chromatin and then undergo nuclear envelope breakdown. Within these cells, small vesicles containing DNA can be seen in the cytoplasm near the plasma membrane. The DNA-containing vesicles eventually fuse with the plasma membrane, and NETs are released to trap bacteria.

(ii) NETs can also be released through cell lysis, and this typically takes longer than the vesicular-mediated mechanism. The nuclear envelope is degraded, and chromatin decondensation occurs because of PAD4-mediated citrullination of histones.

(iii) NET release by mitochondria has also been observed.

NETs and thrombosis

PNAS PNAS PNAS

Extracellular DNA traps promote thrombosis

Tobias A. Fuchs[a,b,c], Alexander Brill[a,b,c], Daniel Duerschmied[a,b,c], Daphne Schatzberg[a,b], Marc Monestier[d], Daniel D. Myers, Jr.[e,f], Shirley K. Wrobleski[e], Thomas W. Wakefield[e], John H. Hartwig[g], and Denisa D. Wagner[a,b,c,1]

[a]Immune Disease Institute, Boston, MA 02115; [b]Program in Cellular and Molecular Medicine, Children's Hospital Boston, Boston, MA 02115; [c]Department of Pathology, Harvard Medical School, Boston, MA 02115; [d]Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140; [e]Section of Vascular Surgery and [f]Unit for Laboratory Animal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109; and [g]Translational Medicine Division, Brigham and Women's Hospital, Boston, MA 02115

Edited by Barry S. Coller, The Rockefeller University, New York, NY, and approved July 2, 2010 (received for review April 28, 2010)

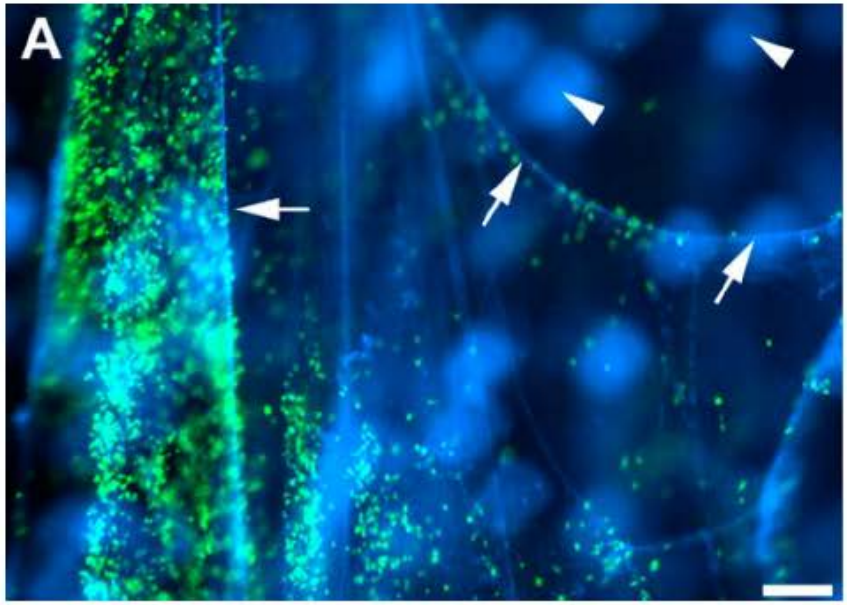
Neutrophil extracellular traps (NETs) are part of the innate immune response to infections. NETs are a meshwork of DNA fibers comprising histones and antimicrobial proteins. Microbes are immobilized in NETs and encounter a locally high and lethal concentration of effector proteins. Recent studies show that NETs are formed inside the vasculature in infections and noninfectious diseases. Here we report that NETs provide a heretofore unrecognized scaffold and stimulus for thrombus formation. NETs perfused with blood caused platelet adhesion, activation, and aggregation. DNase or the anticoagulant heparin dismantled the NET scaffold and prevented thrombus formation. Stimulation of platelets with purified histones was sufficient for aggregation. NETs recruited red blood cells, promoted fibrin deposition, and induced a red thrombus, such as that found in veins. Markers of extracellular DNA traps were detected in a thrombus and plasma of baboons subjected to deep vein thrombosis, an example of inflammation-enhanced thrombosis. Our observations indicate that NETs are a previously unrecognized link between inflammation and thrombosis and may further explain the epidemiological association of infection with thrombosis.

were activated (Fig. 1*C*). Perfusion of NETs with anticoagulated blood at high shear rates (900/s) (Fig. 1 *D*–*K* and Movie S1) or low, typically venous shear rates (200/s) (Movie S2) resulted in time-dependent platelet aggregation. Strings of NETs aligned (Fig. 1 *D* and *E*) in the direction of flow and, importantly, NETs were not a static surface but moved in three dimensions (Movie S1). Within 1 min from onset of perfusion, small platelet aggregates appeared on NETs (Fig. 1 *D* and *I*, arrows). Platelet adhesion and aggregation on NETs increased over the next 9 min (Fig. 1 *E* and *J*). DNase treatment simultaneously removed NETs and platelets, indicating that platelets were indeed attached to NETs (Fig. 1 *F* and *K*, and Movie S1). Quantification showed that areas covered by NETs were constant (Fig. 1*G*), whereas platelets adhered and aggregated in a time-dependent manner (Fig. 1*H*). Both platelet aggregates and NETs were removed by DNase (Fig. 1 *G* and *H*). When blood was supplemented with DNase at the beginning of the perfusion, NETs were degraded rapidly (Fig. 1*G*) and platelet aggregates did not form (Fig. 1*H*). Thus, NETs were the only prothrombotic substrate in these experiments.

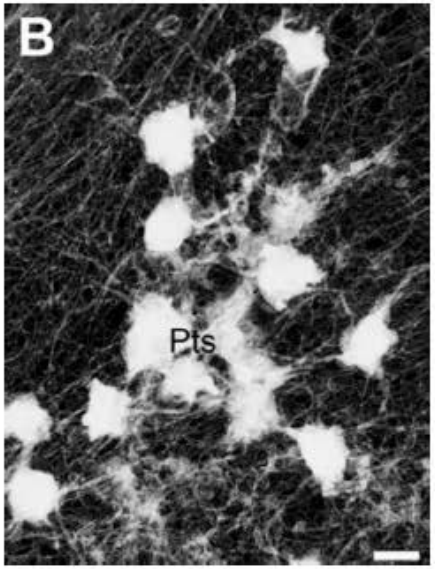
When we tested heparin, a common anticoagulant, on NET-

Proc Natl Acad Sci U S A. 2010 Sep 7;107(36):15880-5

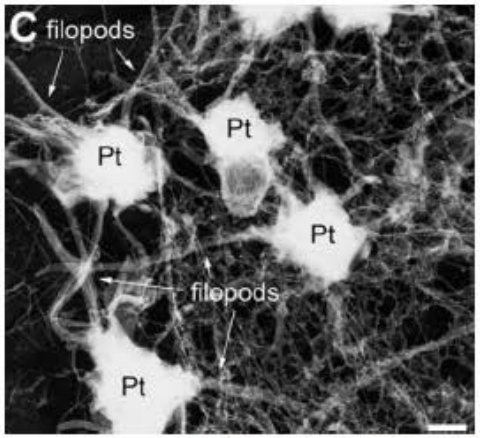
NETs provide a scaffold for platelet adhesion and aggregation



Platelets (green) bound to NETs (blue, arrows). Neutrophils (blue, arrowheads) were out of focus and did not bind platelets. (Scale bar, 20 μm.)

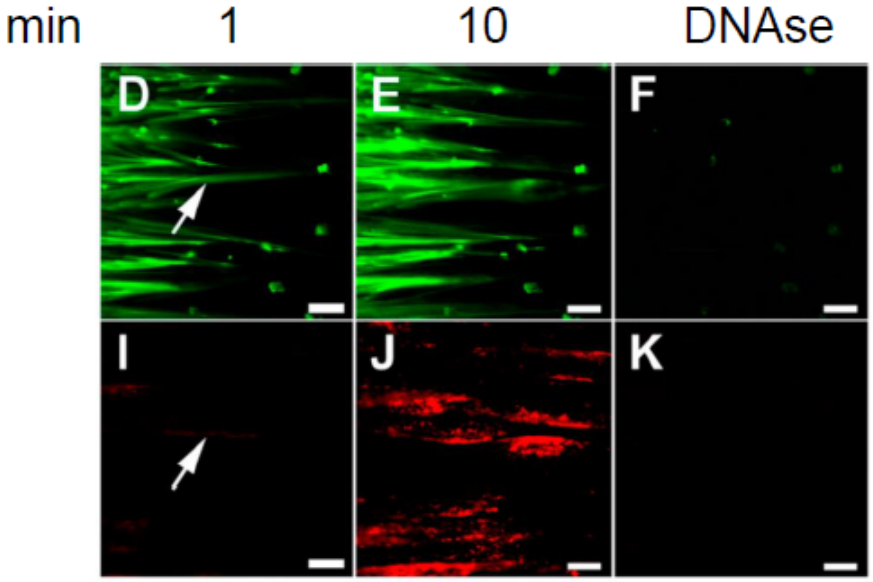


Electron micrograph of platelets (Pts) attached to a fibrous meshwork of NETs. (Scale bar,1 μm.)



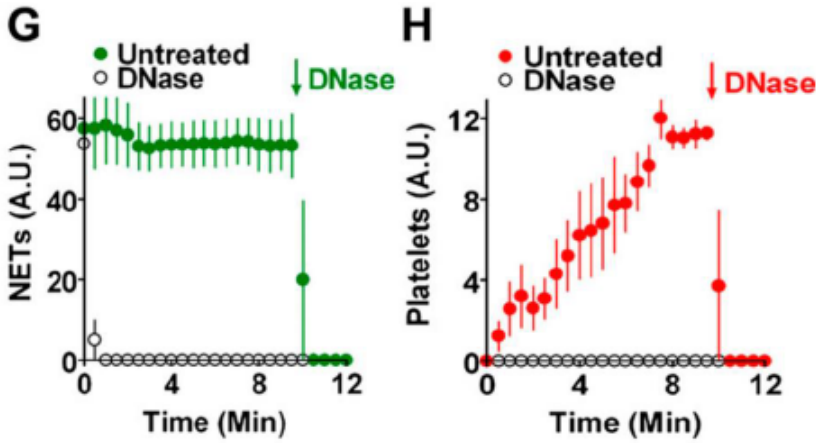
Numerous filopods indicated that platelets (Pt) on NETs were activated. (Scale bar, 0.5 μm.)

Platelets were attached to NETs



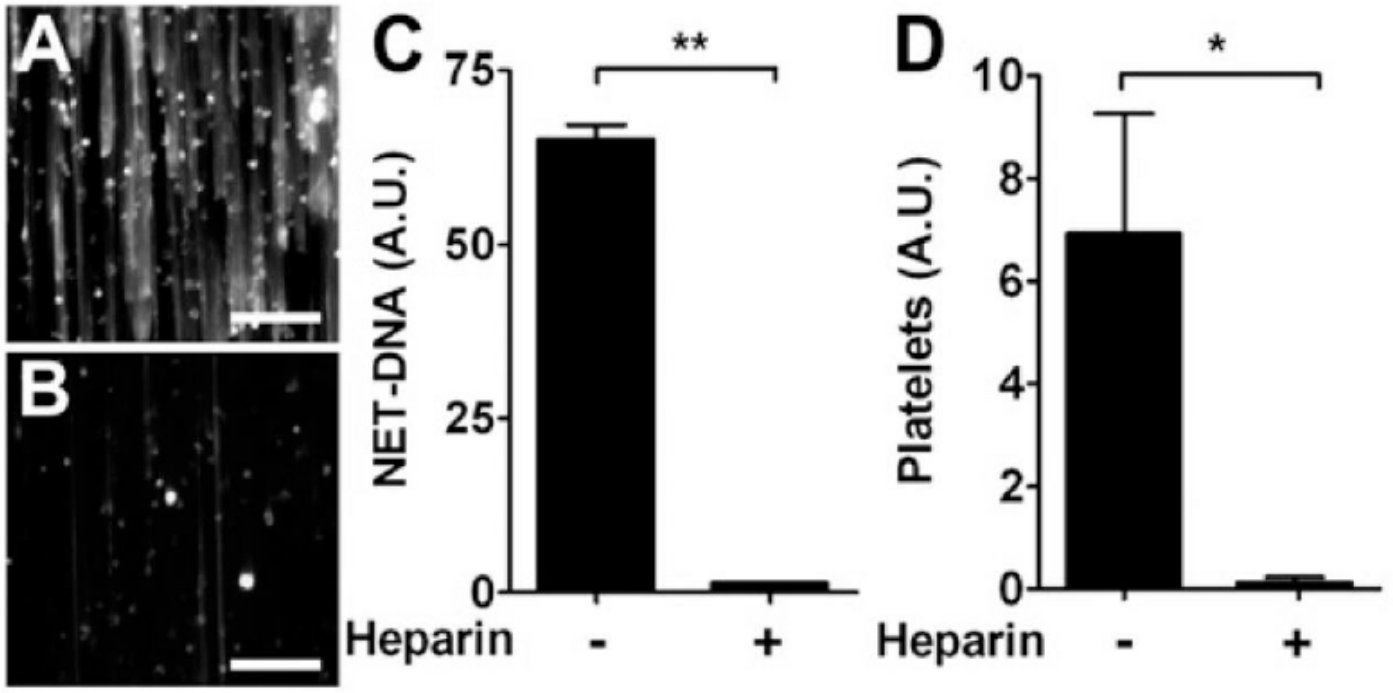
Time-course of platelet adhesion and aggregation on NETs.
NETs (D and E, green) were perfused with platelets (I and J, red) in whole blood. The flow direction was from left to right. Images showed NETs and platelets after 1 min (arrows in D and I) and 10 min (E, J) of perfusion. DNase added to blood after 10 min digested NETs (F) and removed platelets (K), indicating that platelets were attached to NETs.

DNase removes NETs and inhibits platelet aggregation



Quantification of NETs (G) or platelets (H) in the presence (open circles) or absence of DNase (closed circles). DNase was added to untreated samples after 10 min (arrow).

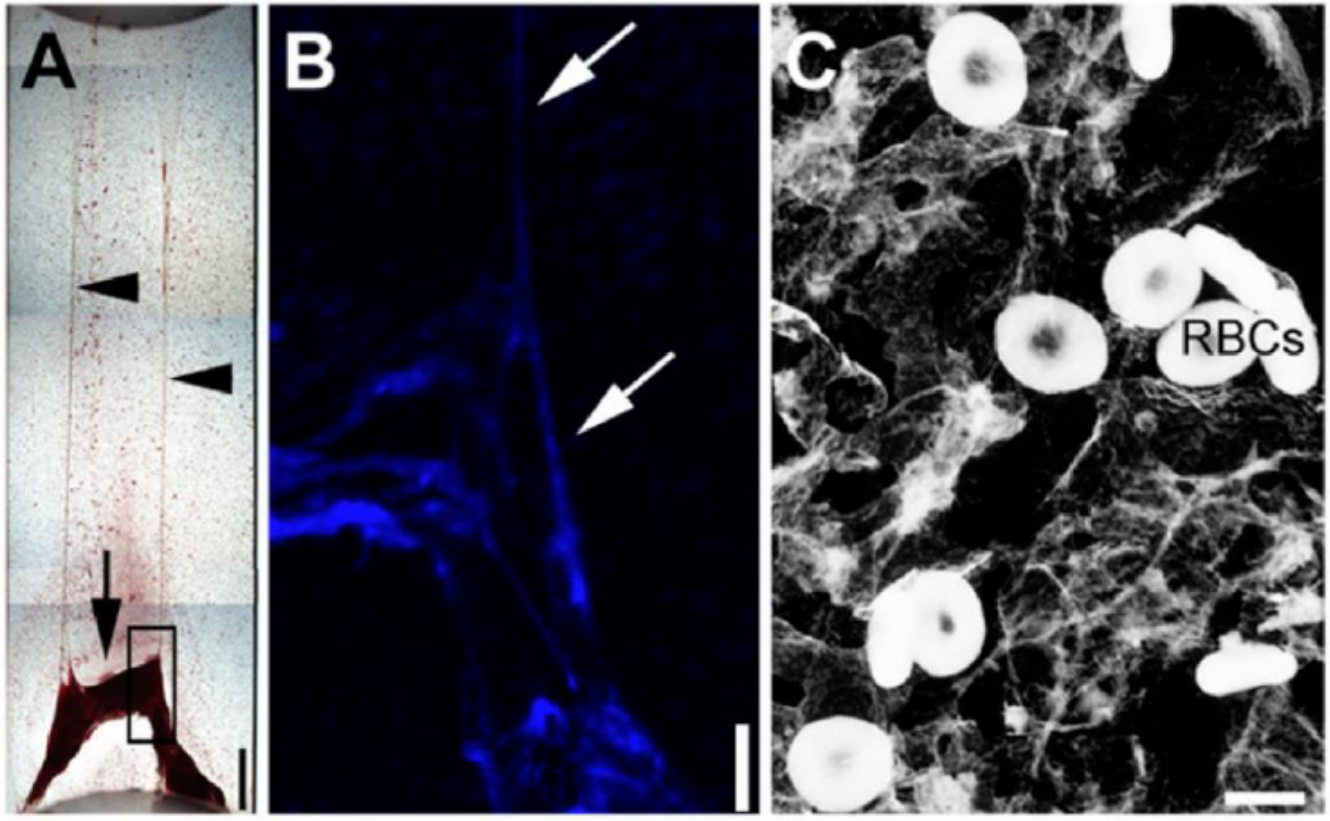
Heparin dismantles NETs and prevents histone induced platelet aggregation



SytoxGreen staining of NETs perfused for 10 min with blood in the absence (A) or presence (B) of heparin.
(C) Quantification of NETs after 10 min perfusion with normal (-) or heparinized (+) blood.
(D) Quantification of platelets on NETs perfused for 10 min with blood before (-) and after (+) treatment with heparin.

Heparin has the highest negative charge density of any known biological molecules

NETs provide a scaffold for RBC-rich thrombi



(A)Flow chamber coated with NETs after perfusion with blood. Light microscopy of a red thrombus (arrow) anchored on two strings (arrowheads). Figure is a composite of multiple photographs of the flow chamber.

(B)DNA staining of thrombus (rectangle in A). Strings of DNA are seen in the thrombus (arrows).

(C)Electron microscopy shows individual RBCs attached to NETs.

NETs

1-NETs provide a heretofore unrecognized scaffold and stimulus for thrombus formation

2-NETs perfused with blood caused platelet adhesion, activation and aggregation

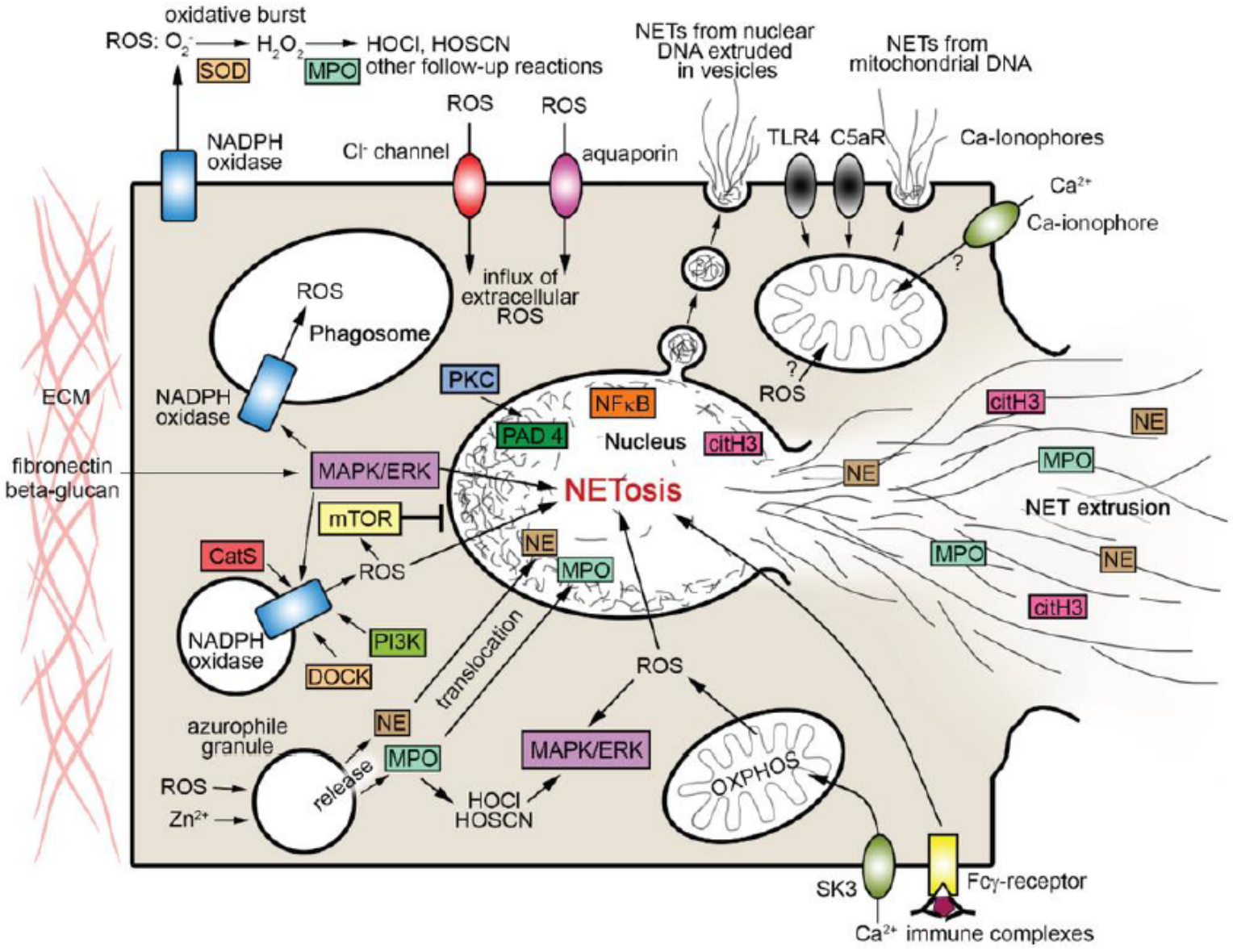
3-DNase or the anticoagulant heparin dismantled the NET scaffold and prevented thrombus formation

4-Stimulation of platelets with purified histones was sufficient for aggregation

5-NETs recruited red blood cells, promoted fibrin deposition and induced a red thrombus

6-Markers of NETs were detected in a thrombus and plasma of baboons subjected to deep vein thrombosis, an example of inflammation-enhanced thrombosis

Pathways of interaction between ROS and NET formation



OR193

DNA in plasma indicates disease extent and predicts mortality in patients with venous thromboembolism

Fuchs T[1,2], Jiménez-Alcázar M[2], Limacher A[3], Méan M[4], Renné T[1,2], Aujesky D[4] and Lämmle B[5]

[1]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [2]Clinical Chemistry and Laboratory Medicine, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]Department of Clinical Research and Institute of Social and Preventive Medicine, University of Bern; [4]Universitätsspital Bern, Bern, Switzerland; [5]Centrum für Thrombose und Hämostase, Universitätsmedizin Mainz, Mainz, Germany

Aims:
We and others have previously identified elevated levels of DNA in plasma from patients or animals with deep vein thrombosis. The diagnostic and prognostic value of plasma DNA in VTE is not known. We hypothesized that levels of plasma DNA or DNase1, the predominant DNA-degrading enzyme in plasma, correlate with the extent of VTE at diagnosis and are indicative of the clinical outcome

Results
-Plasma DNA and DNase1 were positively ($P < 0.001$) and negatively ($P < 0.022$) correlated with the extent of VTE at enrollment, respectively.
-Neither DNA nor DNase1 were associated with the clinical outcomes VTE recurrence or major bleeding,
-High DNA levels (top quartile) were predictive for mortality within 12 months post VTE diagnosis (adjusted HR 2.40, 95% CI 1.53–3.75, $P < 0.001$).

Conclusion:
Increased DNA and decreased DNase1 in plasma are associated with the extent of VTE at time of diagnosis. Quantification of DNA in plasma may help identifying patients at risk of dying within months after acute VTE.

JTH, 2015

PO534-MON

Increased circulating dna, calprotectin and mieloperoxidase, as neutrophil extracellular trap markers, are risk factors for deep vein thrombosis

Martos L, Navarro S, Ramón LA, Ferrando F, Cid AR, Bonanad S, España F and Medina P

Haemostasis, Thrombosis, Atherosclerosis and Vascular Biology Research Group, La Fe Sanitary Research Institute, Valencia, Spain

Aims:

To measure the levels of these 3 circulating NET components in plasma from 193 DVT patients and 198 healthy controls in order to assess the role of NETs on the risk of DVT in vivo

Results

Patients with DVT had significantly higher levels of circulating DNA, CP and MPO compared with controls ((P < 0.001). There was a significant correlation between the levels of DNA and CP both in cases (r = 0.245;P = 0.003) and controls (0.271, P = 0.001) and between CP and MPO in patients (r = 0.194, P = 0.024). Individuals with levels in the 4th quartile (Q) of controls had a significant increase in the risk of VTE compared with those in the 1st Q. Multivariate analysis including the 4th Q of DNA, CP and MPO showed a significantly increased OR only for DNA (15.6; 1.2–203.0).

Conclusion:

Increased levels of DNA, CP and MPO were associated with a significant increase in the risk of DVT.

The multivariate analysis suggests that the increased VTE risk is via the increase in DNA levels.

These results support the potential use of DNase as a new therapeutic tool for DVT prevention or thrombolysis.

JTH, 2015

INVITED REVIEW

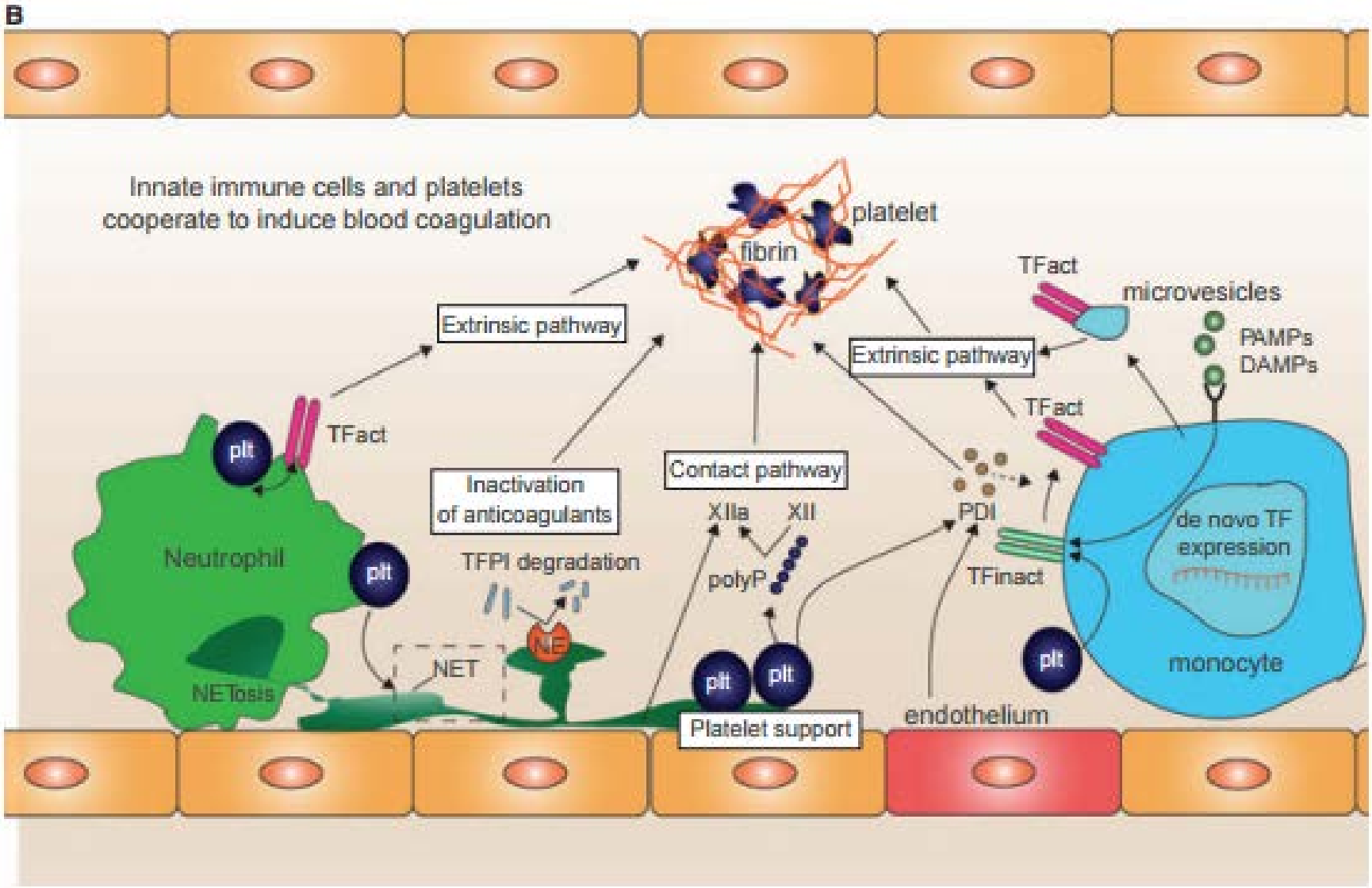
Crossroads of coagulation and innate immunity: the case of deep vein thrombosis

C. SCHULZ,*† B. ENGELMANN‡ and S. MASSBERG§¶

*Cardiovascular Division, King's College London British Heart Foundation Centre of Excellence; †CMCBI, Division of Immunology, Infection and Inflammatory Diseases, Guy's Campus, King's College, London, UK; ‡Institut für Laboratoriumsmedizin, Klinikum der Universität, Ludwig-Maximilians-Universität; §Medizinische Klinik und Poliklinik I, Klinikum der Universität, Ludwig-Maximilians-Universität; and ¶DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

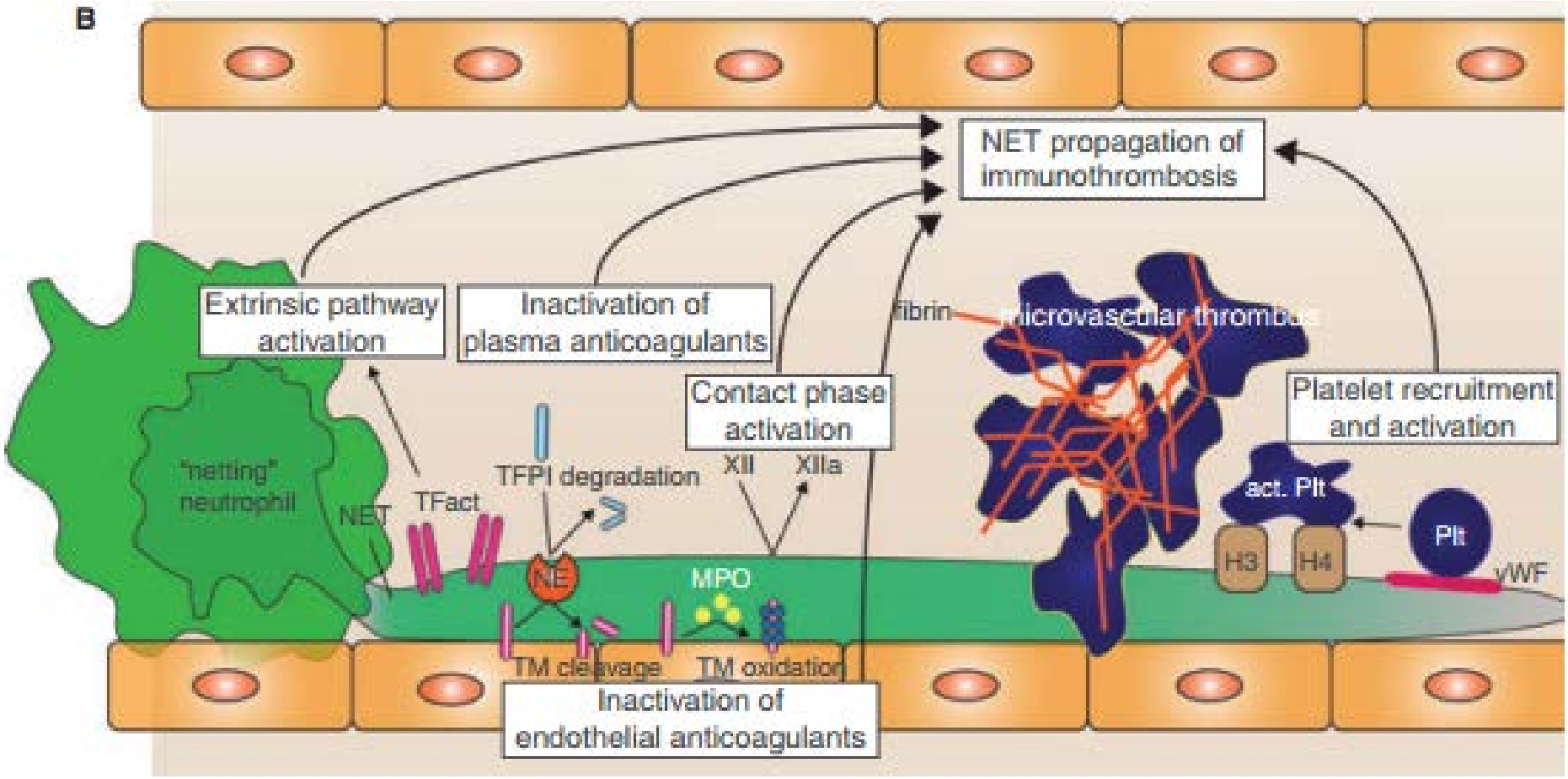
Schultz C, JTH 2013

Activation of blood coagulation in immunothrombosis and DVT



Schultz C, JTH 2013

NET formation is a feature of immunothrombosis and DVT



Schultz C, JTH 2013

Journal of Thrombosis and Haemostasis, **13** (Suppl. 1): S82–S91 DOI: 10.1111/jth.12977

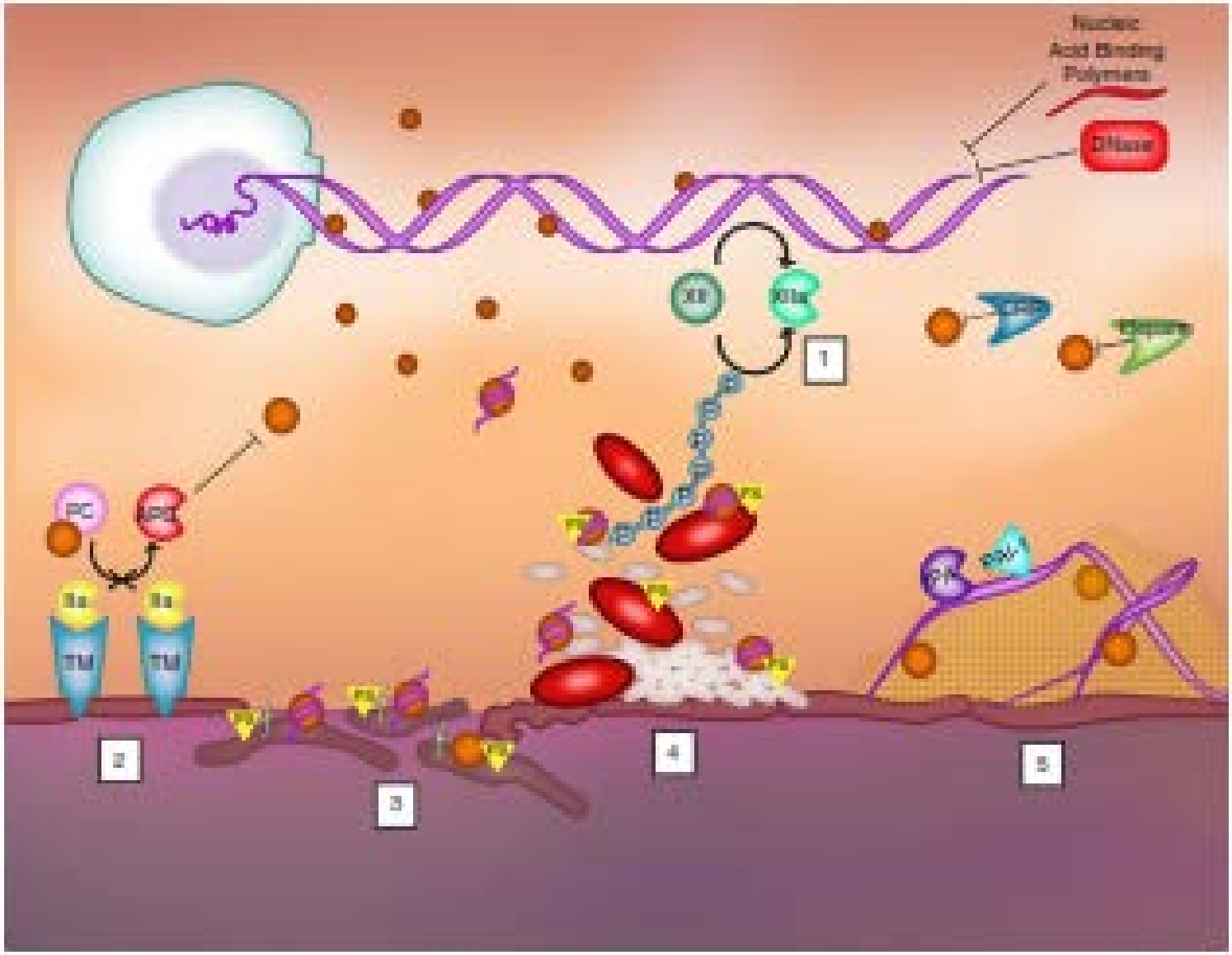
INVITED REVIEW

Extracellular DNA and histones: double-edged swords in immunothrombosis

T. J. GOULD,*† Z. LYSOV*† and P. C. LIAW†‡
*Department of Medical Sciences; †Thrombosis and Atherosclerosis Research Institute (TaARI); and ‡Department of Medicine, McMaster University, Hamilton, ON, Canada

Summary. The existence of extracellular DNA in human plasma, also known as cell-free DNA (cfDNA), was first described in the 1940s. In recent years, there has been a resurgence of interest in the functional significance of cfDNA, particularly in the context of neutrophil extracellular traps (NETs). cfDNA and histones are key components of NETs that aid in the host response to infection and inflammation. However, cfDNA and histones may also exert harmful effects by triggering coagulation, inflammation, and cell death and by impairing fibrinolysis. In this article, we will review the pathologic nature of cfDNA and histones in macrovascular and microvascular thrombosis, including venous thromboembolism, cancer, sepsis, and trauma. We will also discuss the prognostic value of cfDNA and histones in these disease states. Understanding the molecular and cellular pathways regulated by cfDNA and histones may provide novel insights to prevent pathological thrombus formation and vascular occlusion.

Modulation of coagulation, inflammation, and fibrinolysis by cell-free DNA and histones



Gould TJ, JTH 2015

Functional properties of cell free DNA and histones

	Biological processes modulated by cfDNA	Biological processes modulated by histones
Coagulation and fibrinolysis	Activates the intrinsic pathway of coagulation [32,34–37] Enhances neutrophil elastase-mediated degradation of TFPI [39] Binds to fibrin degradation products and holds fibrin network together [55] Potentiates fibrinolysis by stimulating fibrin-independent plasminogen activation [40] Inhibits fibrinolysis by accelerating tPA inactivation by PAI-1 [40]	Promote prothrombin autoactivation [51] Impair TM-mediated activation of protein C [54] Bind to anionic phospholipids and prolongs clotting times [98]
Endothelial and epithelial cells	Unknown	Activation of inflammation and cell death pathways via TLR-2 and TLR-4 [41–43,45,49] Induction of Ca^{2+} influx by direct binding of histones to membranes [49]
Neutrophils	Unknown	Induce formation of NETS and stimulate cytokine and MPO release [41,42,49]
Platelets	Unknown	Induce platelet aggregation; and induce P-selectin, PS, FV/FVa expression, and prothrombinase activity [53] Induce Ca^{2+} influx and aggregation [50] Mediate platelet-dependent thrombin generation [35]
Dendritic cells, macrophages, Kupffer cells	Unknown	Activate TLR-2 and TLR-4 which contributes to acute kidney injury [43] Activate NLRP3 inflammasome [47,48]
Hepatic non-parenchymal cells	Unknown	Enhance DNA-mediated inflammation through TLR-9 [46]
Red blood cells	Unknown	Induce phosphatidylserine exposure [52]

Gould TJ, JTH 2015

Conclusion

-cfDNA and histones exert both protective and harmful effects to the host.

-Elevated levels of these molecules appear to have prognostic value in predicting poor outcome.

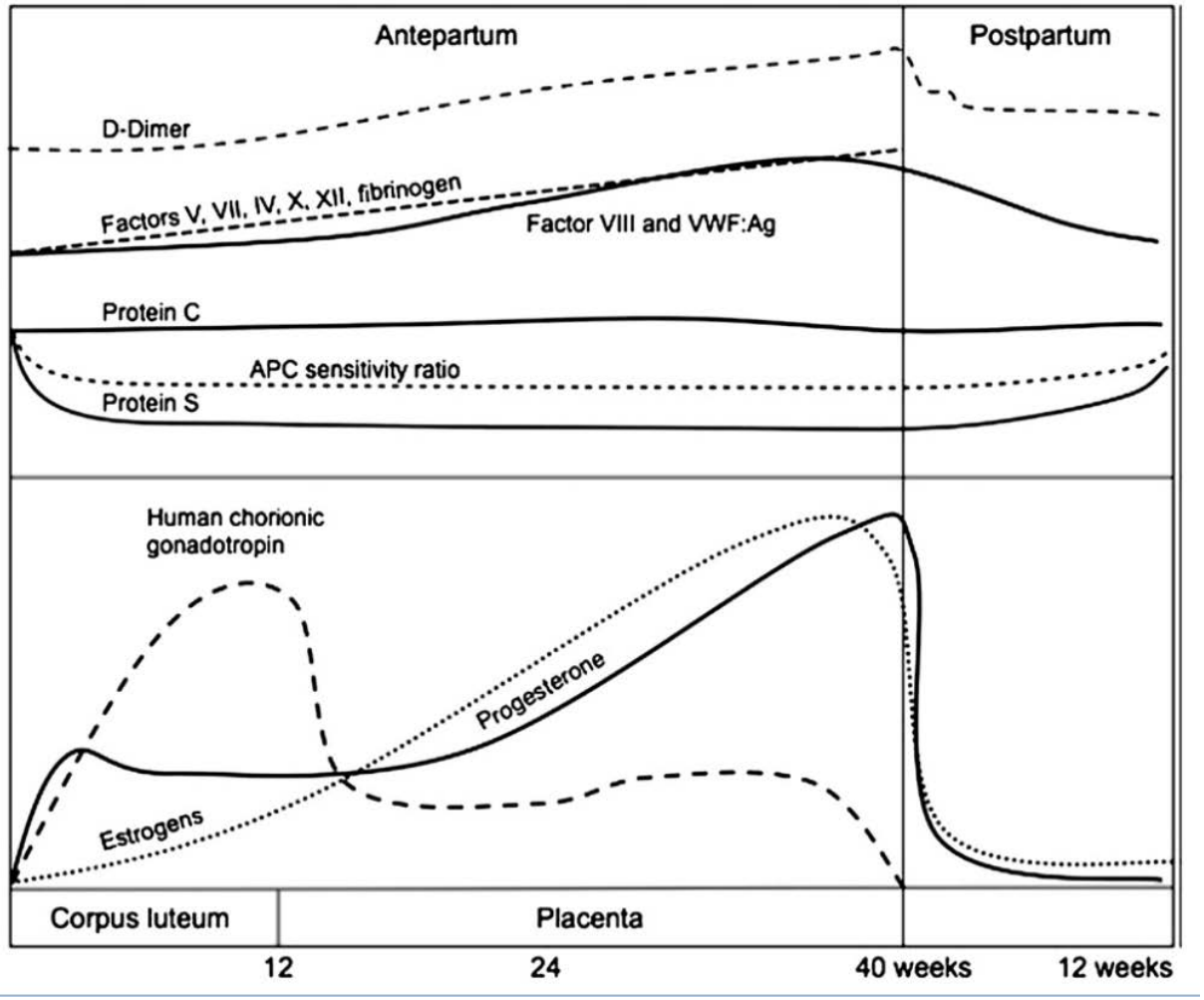
-In pathologic conditions characterized by excessive activation of coagulation and inflammation, pharmacological strategies that inhibit NETosis or that neutralize the toxic effects of cfDNA and histones are of great clinical interest.

-It is possible that combination therapies that reduce coagulation and inflammation, as well as promote thrombolysis, would be the most beneficial.

Gould TJ, JTH 2015

Women’s health and thrombophilia

Qualitative levels and hemostatic direction of hormone changes during normal pregnancy



Blecker S, Blood Review 2014

Risk of pregnancy-related VTE in thrombophilic women stratified by family history for VTE

Thrombophilic defect	Prevalence in population, % [96,142–145]	Estimated RR OR (95% CI)	Absolute risk of VTE[a], % of pregnancies (95% CI)	
			Family studies	Non-family studies
Factor V Leiden, heterozygous	2.0–7.0	8.3 (5.4–12.7) (133)	3.1 (2.1–4.6) (106,107)	1.2 (0.8–1.8)
Factor V Leiden, homozygous	0.2–0.5	34.4 (9.9–120) (133)	14.0 (6.3–25.8) (129,130)	4.8 (1.4–16.8)
Prothrombin heterozygous	2.0	6.8 (2.5–18.8) (133)	2.6 (0.9–5.6) (105,108)	1.0 (0.3–2.6)
Prothrombin homozygous	Very rare	26.4 (1.2–559) (133)	–	3.7 (0.2–78.3)
Antithrombin deficiency	<0.1–0.6	4.7 (1.3–17.0) (133)	3.0 (0.08–15.8) (135)	0.7 (0.2–2.4)
Protein C deficiency	0.2–0.3	4.8 (2.2–10.6) (133)	1.7 (0.4–8.9) (135)	0.7 (0.3–1.5)
Protein S deficiency	<0.1–0.1	3.2 (1.5–6.9) (133)	6.6 (2.2–14.7) (135)	0.5 (0.2–1.0)

Blecker S, Blood Review 2014

Current Commentary

Inherited Thrombophilia and Pregnancy Complications Revisited

Marc A. Rodger, MD, FRCPC, MSc, Michael Paidas, MD, McLintock Claire, MBChB, FRACP, FRCPA, Saskia Middeldorp, MD, Susan Kahn, MD, Ida Martinelli, MD, William Hague, MD, Karen Rosene Montella, MD, and Ian Greer, MD

"Inherited thrombophilias are not yet established as a cause of placenta-mediated pregnacy complication, such as fetal growth restriction, preeclampsia, abruption, and pregnancy loss.

An inherited thrombophilia is only one of many factors that lead to development of these diseases and is unlikely to be the unique factor that should drive management in subsequent pregnancies."

Obstetrics & Gynecology, 2008

OPEN ACCESS Freely available online PLoS MEDICINE

The Association of Factor V Leiden and Prothrombin Gene Mutation and Placenta-Mediated Pregnancy Complications: A Systematic Review and Meta-analysis of Prospective Cohort Studies

Marc A. Rodger[1,2*], Marisol T. Betancourt[1,2], Peter Clark[3], Pelle G. Lindqvist[4], Donna Dizon-Townson[5], Joanne Said[6,7], Uri Seligsohn[8], Marc Carrier[1,2], Ophira Salomon[8], Ian A. Greer[9]

1 Th ttawa, Otta ent of Tran dinge, Swe dicine, The 8 The Ama gdom

"Further research is required to determine if FVL and PGM are associated with placental abruption and whether PGM is associated with important increases in pregnancy loss".

CONCLUSIONS: Women with FVL appear to be at a small absolute increased risk of late pregnancy loss. Women with FVL and PGM appear not to be at increased risk of pre-eclampsia or birth of SGA infants.

2010 PLoS MEDICINE

Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study

M. A. RODGER,*†‡ M. C. WALKER,*§¶ G. N. SMITH,**†† P. S. WELLS,*†‡ T. RAMSAY,*‡‡ N. J. LANGLOIS,* N. CARSON,§§¶¶ M. CARRIER,*†‡ R. RENNICKS WHITE,* S. SHACHKINA*** and S. W. WEN*§¶

Introduction

Evidence from systematic reviews and meta-analyses of case control studies indicates that women with thrombophilia are not only at increased risk of VTE during pregnancy [1], but may also be at increased risk of common placenta-mediated pregnancy complications, including preeclampsia [2], small for gestational age (SGA) infants [3], placental abruption [2], miscarriage and stillbirth [4]. It has been postulated that placental vascular thrombosis and abnormal placentation are at least partly responsible for these pregnancy complications [5]. Large cohort studies have failed to demonstrate an association between factor V Leiden (FVL) or prothrombin gene mutation (G20210A) (PGM) and placenta-mediated pregnancy complications.

Discussion

Our meta-analysis had sufficient power to conclude that an association between thrombophilia (FVL and/or PGM) and any preeclampsia, SGA < 10th percentile and placental abruption does not exist and additional research is not warranted.

Journal of Thrombosis and Haemostasis, **12**: 469–478

Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study

M. A. RODGER,*†‡ M. C. WALKER,*§¶ G. N. SMITH,**†† P. S. WELLS,*†‡ T. RAMSAY,*‡‡ N. J. LANGLOIS,* N. CARSON,§§¶¶ M. CARRIER,*†‡ R. RENNICKS WHITE,* S. SHACHKINA*** and S. W. WEN*§¶

Introduction

Evidence from systematic reviews and meta-analyses of case control studies indicates that women with thrombo-

In conclusion, women who are carriers of FVL or PGM are not at increased risk of preeclampsia, placental abruption or having an SGA infant. In our updated meta-analysis FVL confers a small absolute increased risk of pregnancy loss that is not observed in women with PGM.

factor V Leiden (FVL) or prothrombin gene mutation (G20210A) (PGM) and placenta-mediated pregnancy complications.

Discussion

Our meta-analysis had sufficient power to conclude that an association between thrombophilia (FVL and/or PGM) and any preeclampsia, SGA < 10th percentile and placental abruption does not exist and additional research is not warranted.

Journal of Thrombosis and Haemostasis, **12**: 469–478

Antepartum dalteparin versus no antepartum dalteparin for the prevention of pregnancy complications in pregnant women with thrombophilia (TIPPS): a multinational open-label randomised trial

www.thelancet.com Vol 384 November 8, 2014

Background Thrombophilias are common disorders that increase the risk of pregnancy-associated venous thromboembolism and pregnancy loss and can also increase the risk of placenta-mediated pregnancy complications (severe pre-eclampsia, small-for-gestational-age infants, and placental abruption). We postulated that antepartum dalteparin would reduce these complications in pregnant women with thrombophilia.

Methods In this open-label randomised trial undertaken in 36 tertiary care centres in five countries, we enrolled consenting pregnant women with thrombophilia at increased risk of venous thromboembolism or with previous placenta-mediated pregnancy complications. Eligible participants were randomly allocated in a 1:1 ratio to either antepartum prophylactic dose dalteparin (5000 international units once daily up to 20 weeks' gestation, and twice daily thereafter until at least 37 weeks' gestation) or to no antepartum dalteparin (control group).

The primary composite outcome was independently adjudicated severe or early-onset pre-eclampsia, small-for-gestational-age infant (birthweight <10th percentile), pregnancy loss, or venous thromboembolism. We did intention-to-treat and on-treatment analyses. This trial is registered with ClinicalTrials.gov, number NCT00967382, and with Current Controlled Trials, number ISRCTN87441504.

Findings Between Feb 28, 2000, and Sept 14, 2012, 292 women consented to participate and were randomly assigned to the two groups.

Interpretation Antepartum prophylactic dalteparin does not reduce the occurrence of venous thromboembolism, pregnancy loss, or placenta-mediated pregnancy complications in pregnant women with thrombophilia at high risk of these complications and is associated with an increased risk of minor bleeding.

Marc A Rodger, William M Hague, John Kingdom, Susan R Kahn, Alan Karovitch, Mathew Sermer, Anne Marie Clement, Suzette Coat, Wee Shian Chan, Joanne Said, Evelyne Rey, Sue Robinson, Rshmi Khurana, Christine Demers, Michael J Kovacs, Susan Solymoss, Kim Hinshaw, James Dwyer, Graeme Smith, Sarah McDonald, Jill Newstead-Angel, Anne McLeod, Meena Khandelwal, Robert M Silver, Gregoire Le Gal, Ian A Greer, Erin Keely, Karen Rosene-Montella, Mark Walker, Philip S Wells, for the TIPPS Investigators

PO474-TUE

New thrombophilic risk factors in patients with vascular placental complications (VPC) and pregnancy or hormonal therapy – related thrombosis and management in the clinical practice. Results from the international team project

Santamaria A[1,2], on behalf of Team Project, Medina C[3], on behalf of TEAM Project, Oliver A[4], on behalf of TEAM Project, Marti E[5],

Aims:

From 2009 almost 60 centres have participated.: 1. Women with hormonal therapy or pregnancy related thrombosis disease, 2. Women with history of VPC, 3. Thromboprophylaxis in women with VPC, 4. Thromboprophylaxis in women with thrombophilia.

Results

The most frequent thrombophilic factors were FII G20210A
factor V Leiden polymorphisms and new risk factors such as high factor VIIIc levels.The most frequent factors in the VPC profile were the presence of antiphospholipid antibodies , high factor VIIIc levels, deficiency of protein S (10 women), and also 12 women were carriers of the allele A1 of the genotype ABO and homozygous polymorphism F12 46C/T

Conclusion:

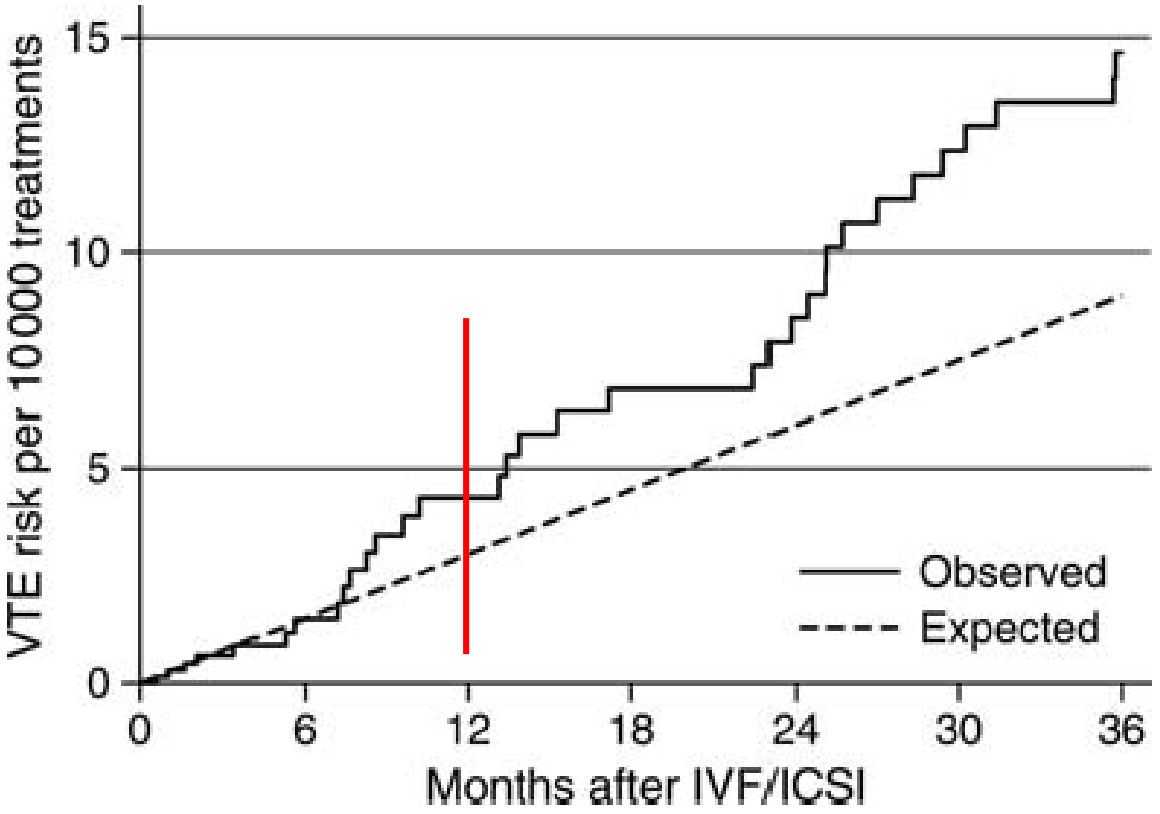
New thrombophilia factors seem to be important in this scenario such as Factor VIIIc and homozygous polymorphism F12 46C/T and the allele A1 (genotype ABO)

JTH, 2015

No evidence that assisted reproduction increases the risk of thrombosis: a Danish National cohort study

30,884 Danish women undergoing 75,141 treatment (IVF/ICSI): National Patient and IVF Registry

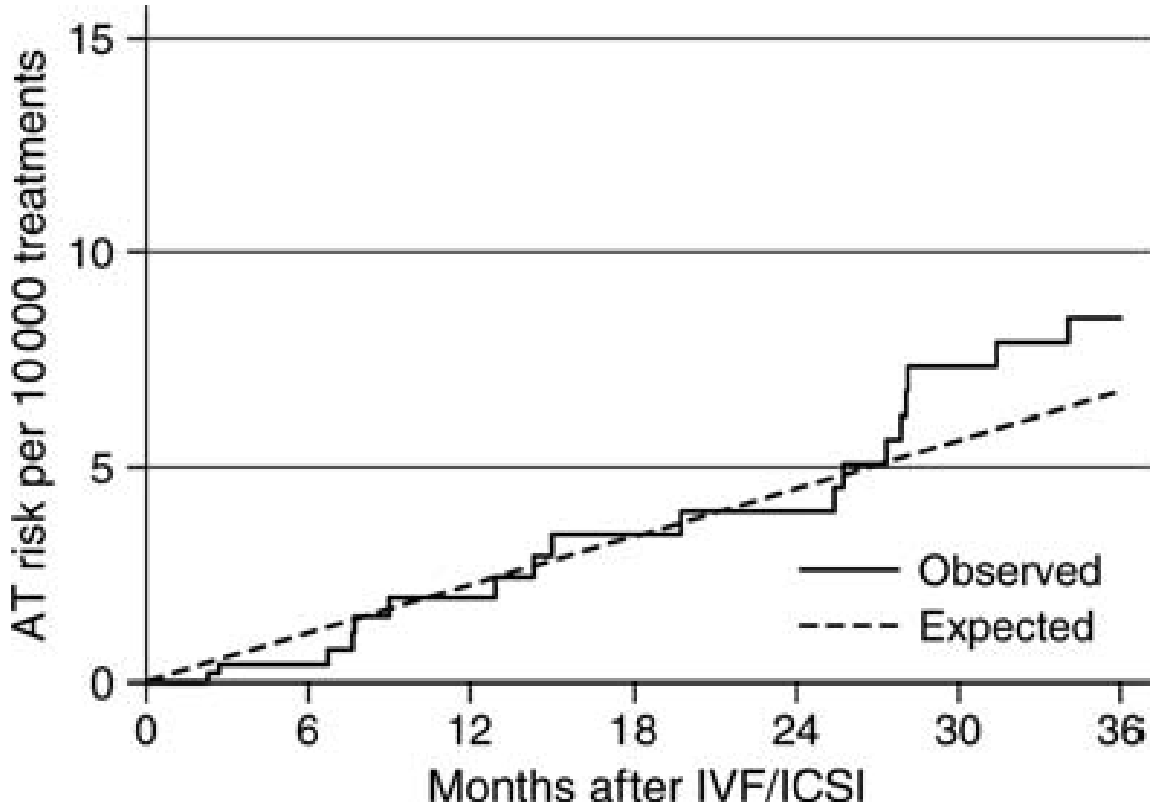
Cumulative incidence of venous thrombosis (VTE) for the first three years after IVF/ICSI, compared with the incidence in the reference population



Hansen AT, Human Reproduction, 2012

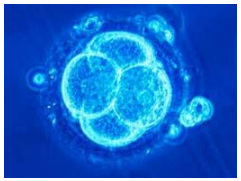
No evidence that assisted reproduction increases the risk of thrombosis: a Danish National cohort study

Cumulative incidence of arterial thrombosis (AT) for the first three years after IVF/ICSI, compared with the incidence in the reference population

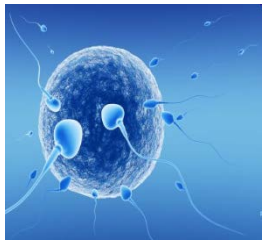


Hansen AT, Human Reproduction, 2012

PMA

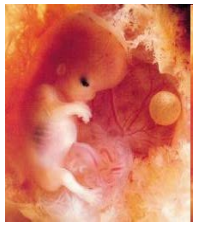


Incremento del rischio trombotico in caso di OHSS

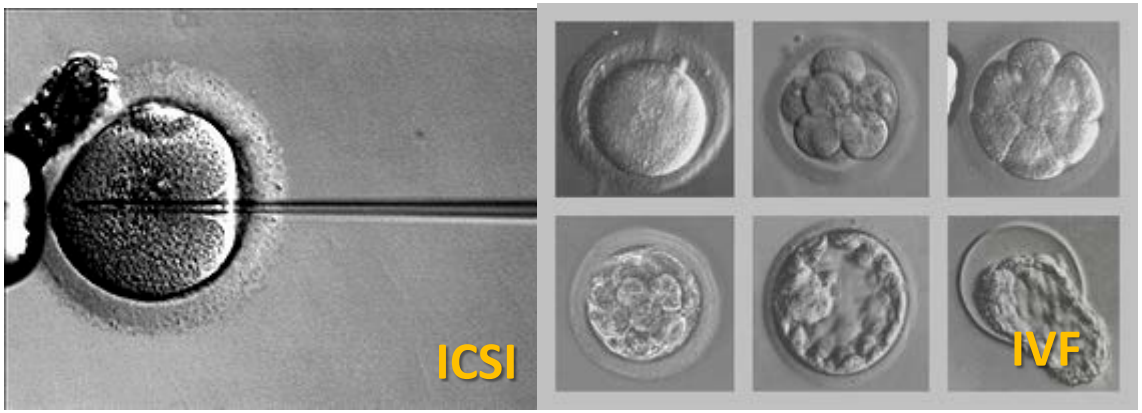


Incremento del rischio trombotico prevalentemente venoso nel corso di tutta la gravidanza ed in particolare nel primo trimestre e nelle prime settimane del post partum

Più alta incidenza di ipertensione e stroke ma non malattia coronarica



Non incremento del rischio trombotico arterioso a lungo termine



Pro-coagulant changes:

Increased coagulation factors

Decreased natural anticoagulant activity

Reduced fibrinoltytic activity

similar to changes seen during pregnancy

FIRsT and OTTILIA Registry

Thrombophilia screening after repeated failures in assisted reproductive techniques - FIRsT registry introduced.
A prospective registry aiming to collect data on whether there is benefit in giving LMWH to improve ART outcomes.
Plan to **collect and evaluate** data on the 1st cycle **after 2 or more ART failures**. In addition aim to collect and evaluate **thrombophilia screening** data if available.
OTTILIA registry update presented.
OTTILIA is an observational study on **antithrombotic prevention** in thrombophilia and pregnancy loss. To date 114 centers involved with 122 women recruited (143 pregnancies).

Grandone E, SSC-ISTH, 2015