ITP primaria: patogenesi immunitaria e difetto trombopoietico

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THE MEGAKARYOCYTES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA, A FORM OF HYPERSPLENISM

By WILLIAM DAMESHEK, M.D., AND CAPTAIN EDWARD B. MILLER, A.U.S.

1. The number of megakaryocytes per million nucleated blood cells ranged from 450 to 1565—far above the normal average of 183.
2. A third or less of the megakaryocytes showed evidence of platelet production.
3. Megakaryoblasts were increased.
4. Promegakaryocytes were much less plentiful than in the normal, but non-granular platelet production was frequently seen.
5. There was very little platelet production from adult megakaryocytes.
6. Degenerated forms were slightly increased over normal.

Dameshek & Miller, Blood 1946

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Conclusioni

la piastrinopenia in corso di ITP è probabilmente causata da una ridotta produzione megacariocitaria di piastrine

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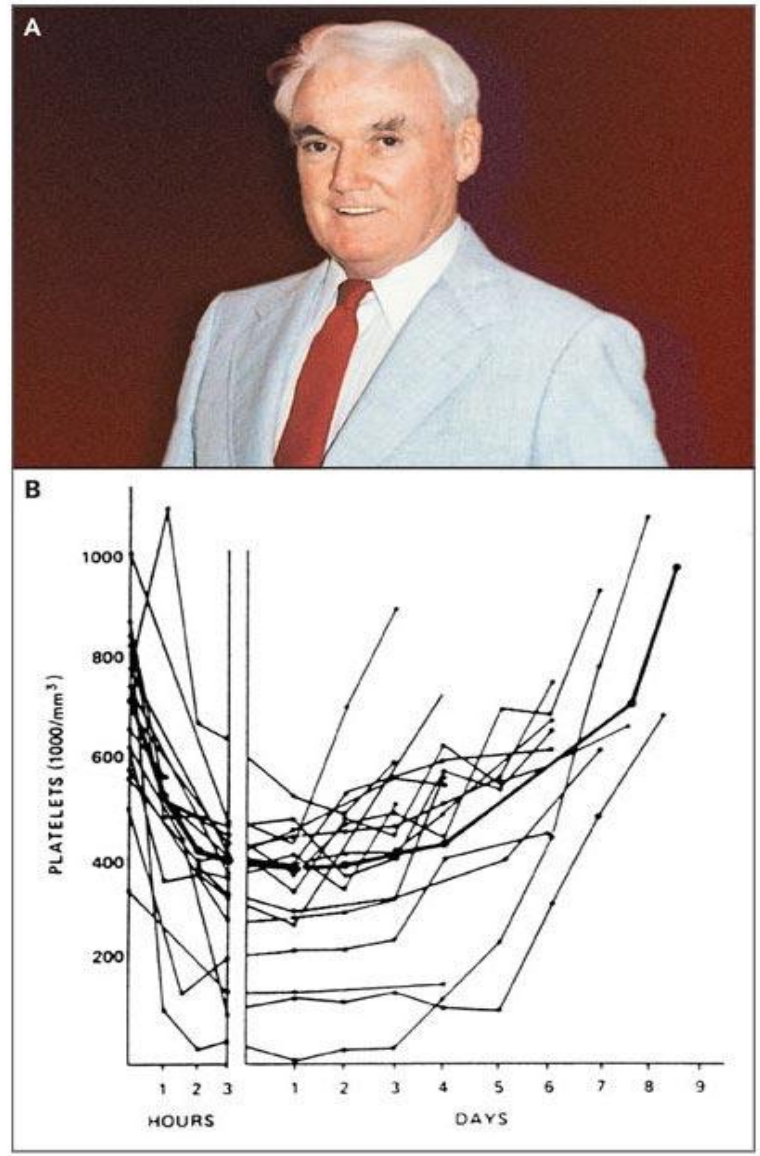
Dameshek & Miller, Blood 1946

The Harrington-Hollingsworth Experiment



Schwartz R. N Engl J Med 2007;357:2299-2301

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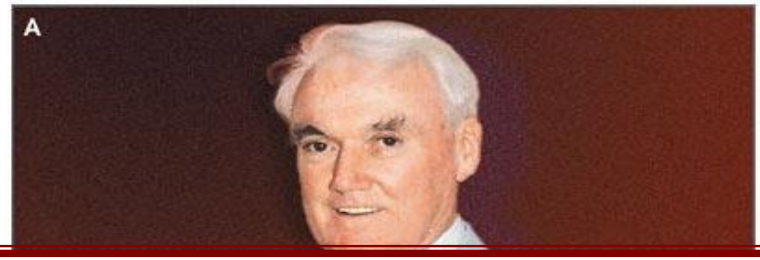
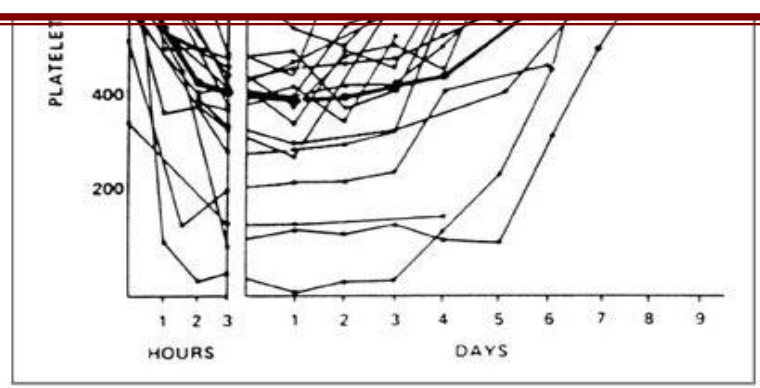


Conclusioni

la piastrinopenia in corso di ITP è associata a ridotta sopravvivenza piastrinica, attribuibile alla presenza di un fattore nel siero dei pazienti (in seguito identificato come anticorpo diretto contro glicoproteine di membrana)



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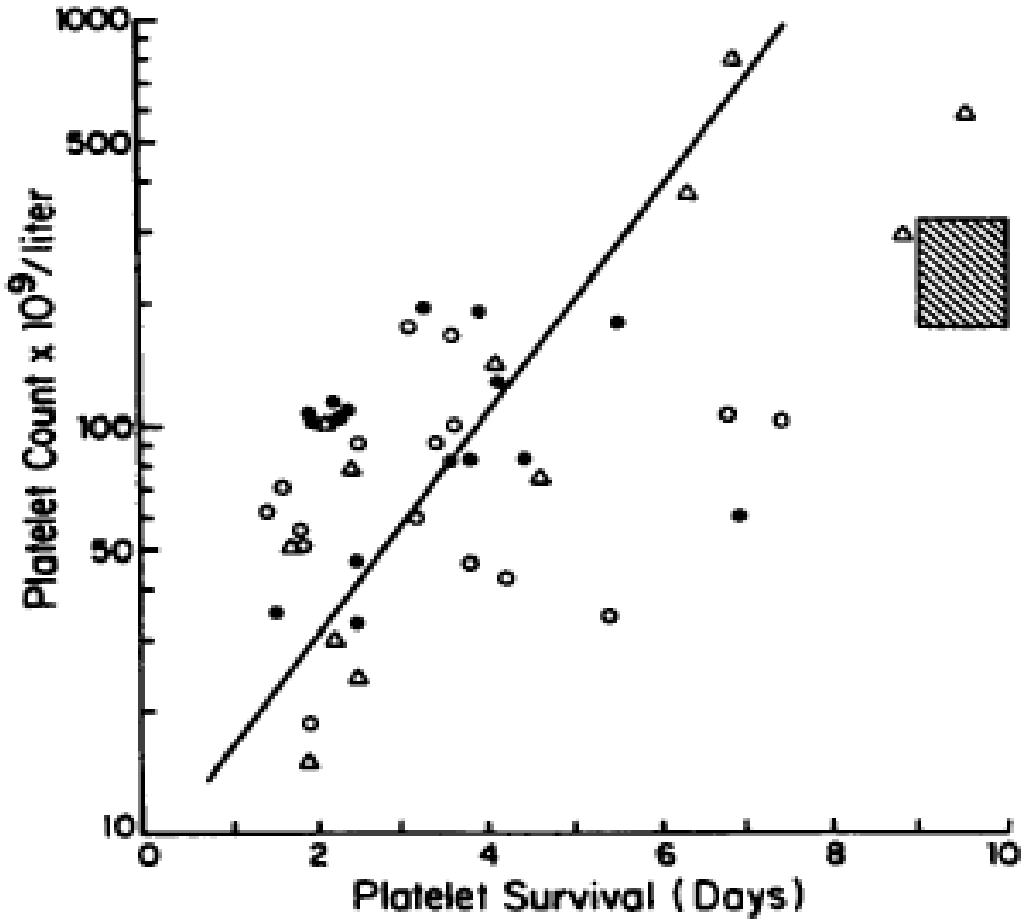
Mechanisms of Thrombocytopenia in Chronic Autoimmune Thrombocytopenic Purpura

Evidence of Both Impaired Platelet Production and Increased Platelet Clearance

Penny J. Ballem, Gerald M. Segal, John R. Stratton, Terry Gernsheimer, John W. Adamson, and Sherrill J. Slichter
Puget Sound Blood Center, Divisions of Hematology and Cardiology, University of Washington School of Medicine, Veterans Administration Medical Center, Seattle, Washington 98104

The Journal of Clinical Investigation, Inc.
Volume 80, July 1987, 33–40

Correlation between log platelet count and autologous platelet survival



R=0.64
P<0.001

Ballem PJ et al, JCI 1987

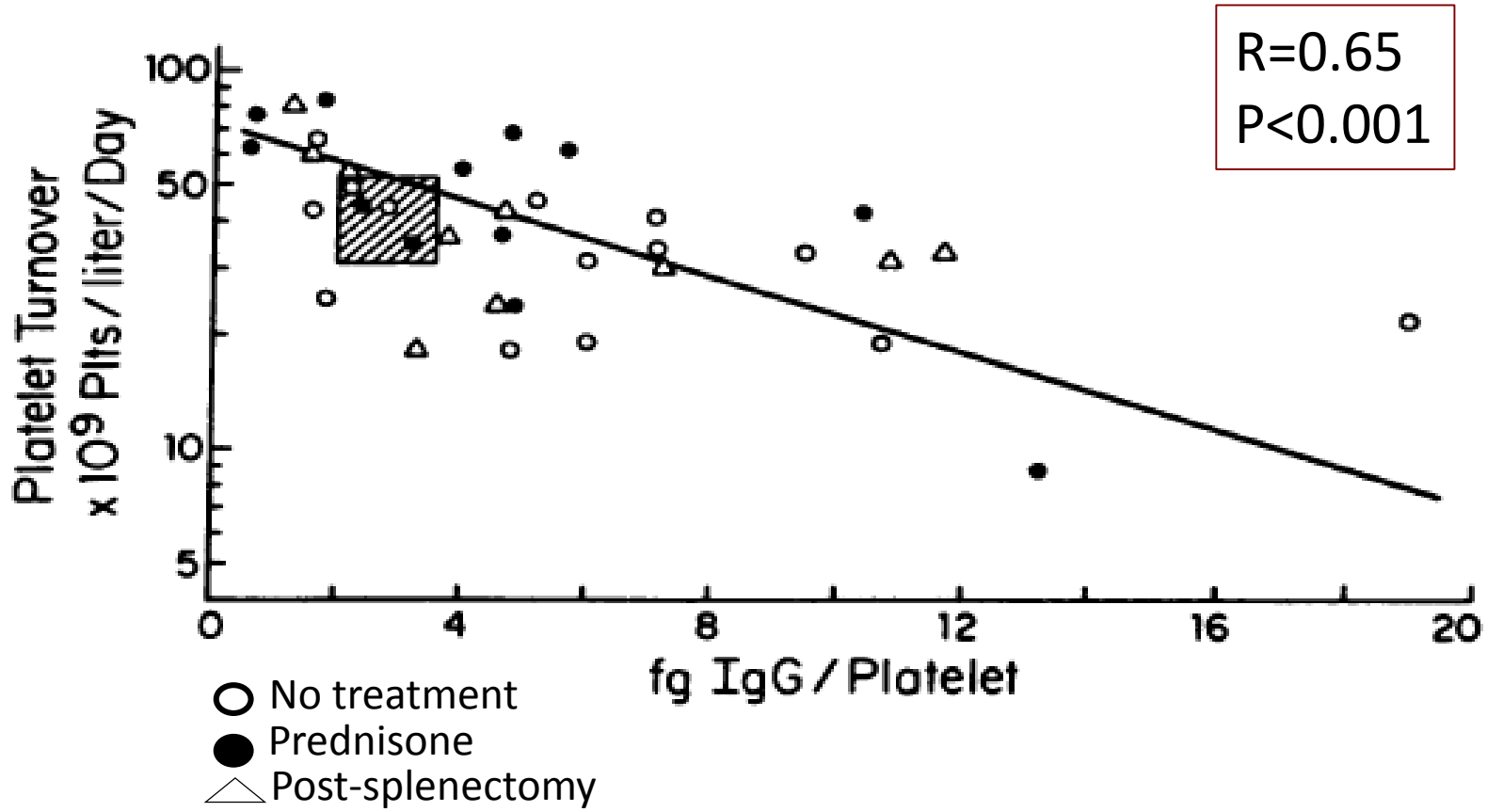
Platelet turnover in ITP patients, according to their treatment

	Turnover*	Untreated	Prednisone	Postsplenectomy	Total
	platelets/liter per d				
Decreased	$<31 \times 10^9$	7 (41%)‡	2 (13%)	4 (33%)	13 (30%)
Normal	$31–51 \times 10^9$	9 (53%)	6 (40%)	4 (33%)	19 (43%)
Increased	$>51 \times 10^9$	1 (6%)	7 (47%)	4 (33%)	12 (27%)
	Total	17	15	12	44

Normal subjects (n=15): 41±5x109 platelets/liter per day

Ballem PJ et al, JCI 1987

Correlation between log platelet turnover and IgG/platelet in ITP patients



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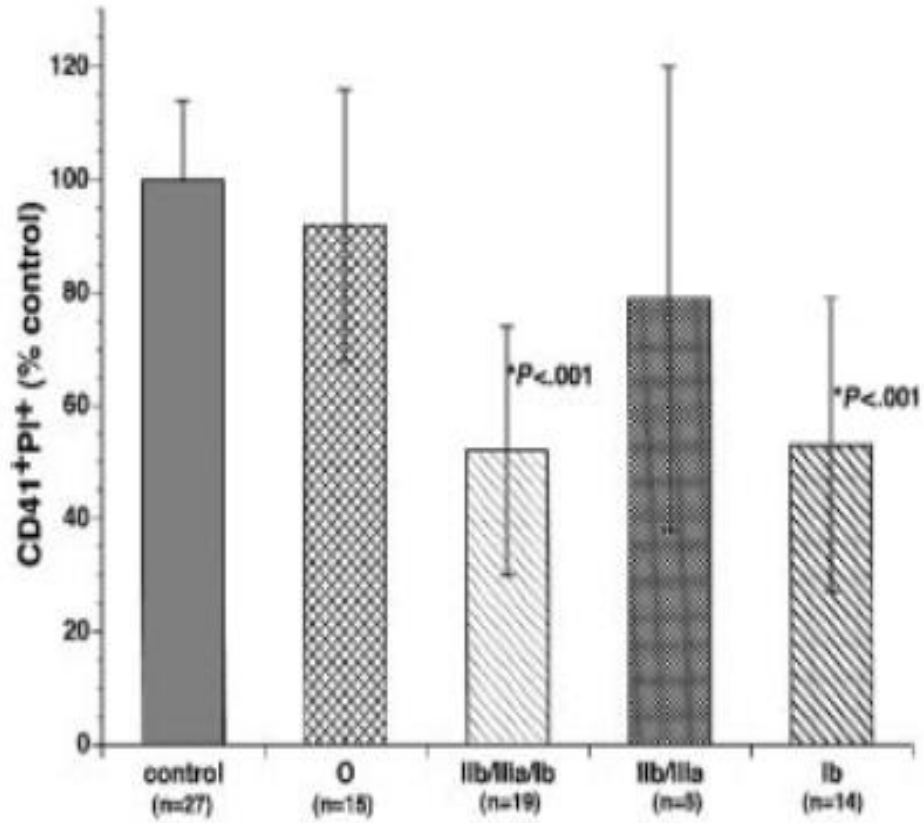
Conclusioni

sia una ridotta produzione piastrinica midollare, sia una aumentata eliminazione piastrinica contribuiscono alla patogenesi della piastrinopenia in corso di ITP

ITP plasma containing antibodies against GPIb inhibit megakaryocytopoiesis *in vitro,* in the presence of TPO



Chang M et al, Blood 2003

Reduction of plasma autoantibodies against GPIb/IX is associated with increased yield of $CD41^+$ PI^+ cells *in vitro*, in the presence of TPO

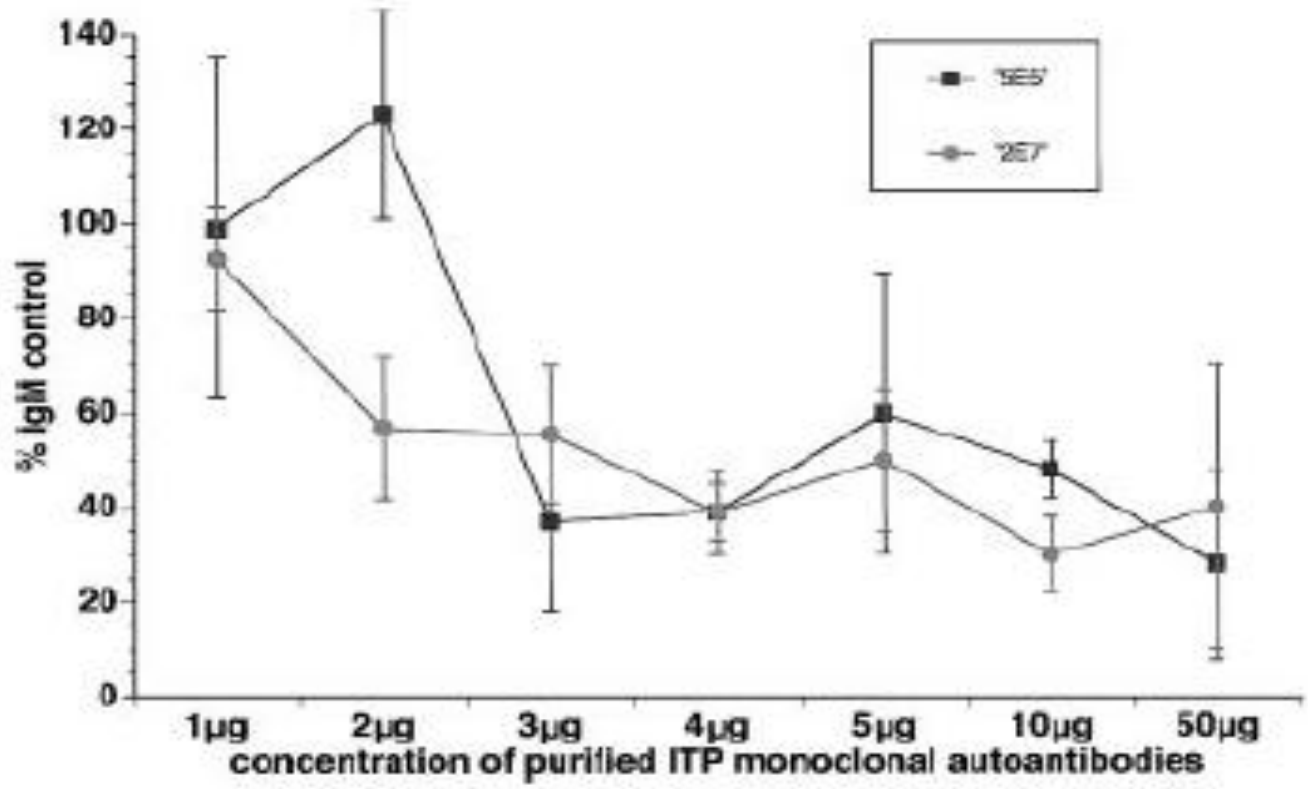
	Before absorption		
	Process 1*	Process 2†	After absorption
Acute ITP no. 1; Plt, $2 \times 10^3/\mu L$			
IgM titer	>160	>160	80
$CD41^+PI^+$, % of control	25	25	50
Acute ITP no. 2; Plt, $18 \times 10^3/\mu L$			
IgM titer	>20	>20	Undetectable
$CD41^+PI^+$, % of control	82	66	120
Healthy control no. 1; Plt, control			
IgM titer	Undetectable	Undetectable	Undetectable
$CD41^+PI^+$, % of control	100	94	100
Healthy control no. 2; Plt, control			
IgM titer	Undetectable	Undetectable	Undetectable
$CD41^+PI^+$, % of control	100	108	100

*Frozen plasma samples were defrosted in ice, and analyzed immediately in culture for their effect on in vitro megakaryocytopoiesis.

†Frozen plasma samples were defrosted in ice, processed same as absorbed plasma samples except that no platelets were added to the plasma, and then analyzed in culture.

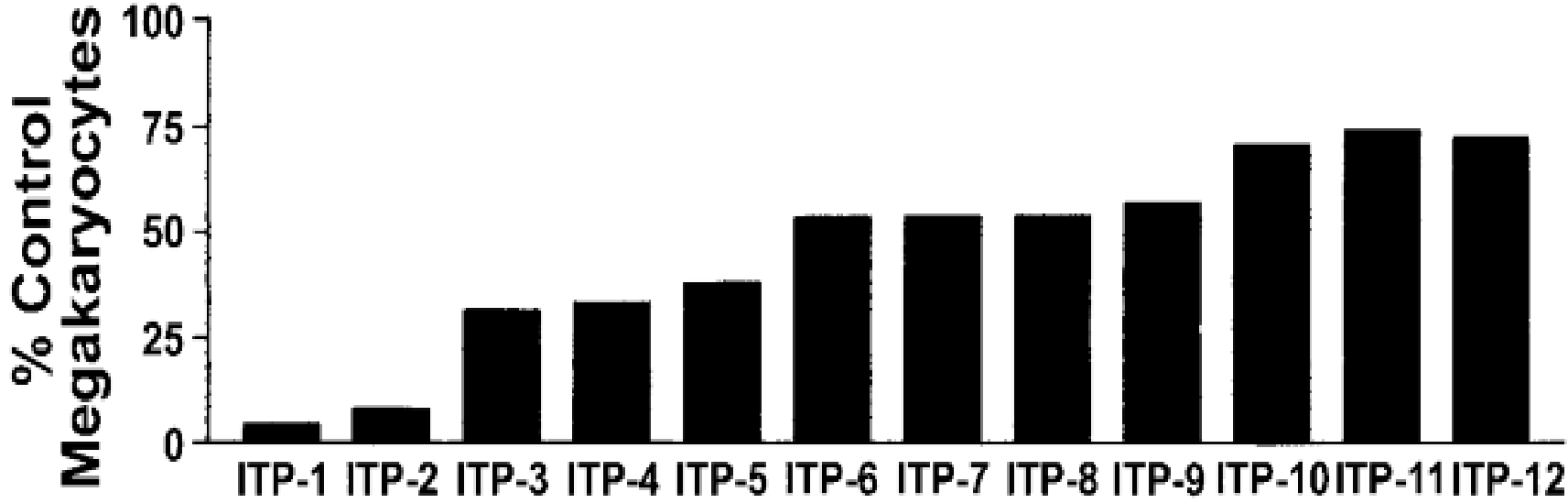
Chang M et al, Blood 2003

Dose response inhibition of $CD41^{+}PI^{+}$ growth *in vitro* by purified human antiplatelet GPIIb and GPIIIa monoclonal antibodies



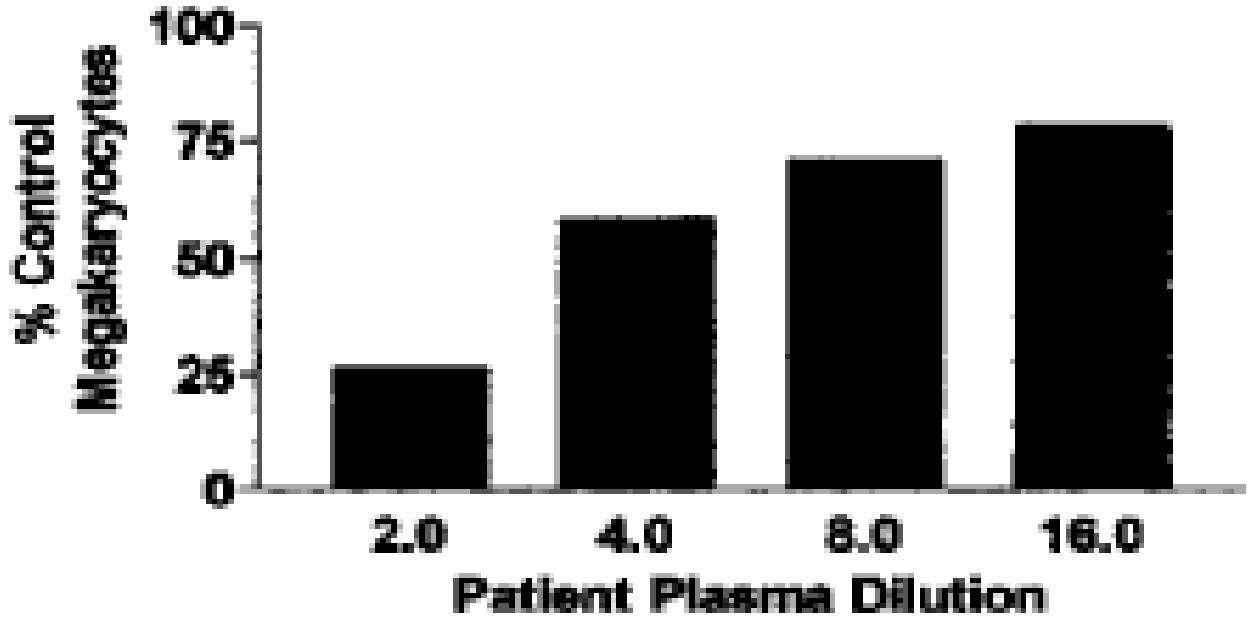
Chang M et al, Blood 2003

Suppression of megakarycytopoiesis *in vitro* by ITP plasma



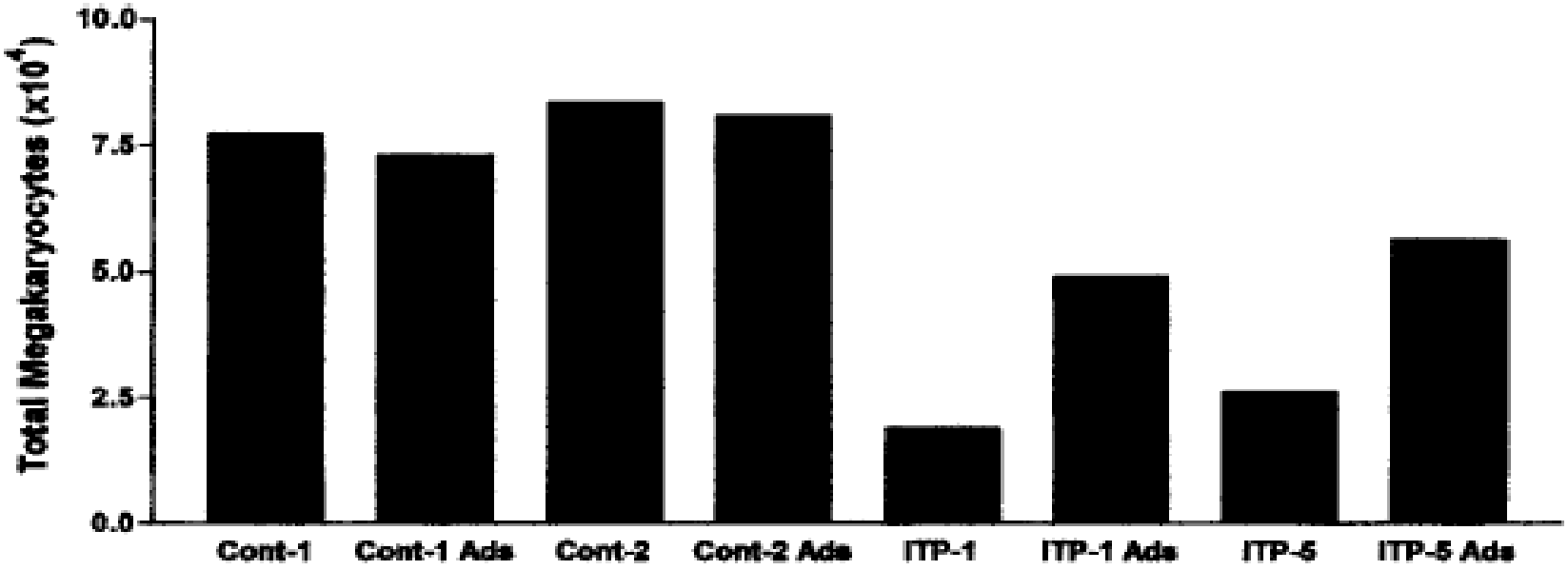
Mc Millan R et al, Blood 2004

Suppression of megakaryocyte production by ITP plasma dilutions



Mc Millan R et al, Blood 2004

Effect of autoantibody absorption with immobilized GPIIbIIIa on megakaryocyte production *in vitro* in the presence of control or ITP plasma



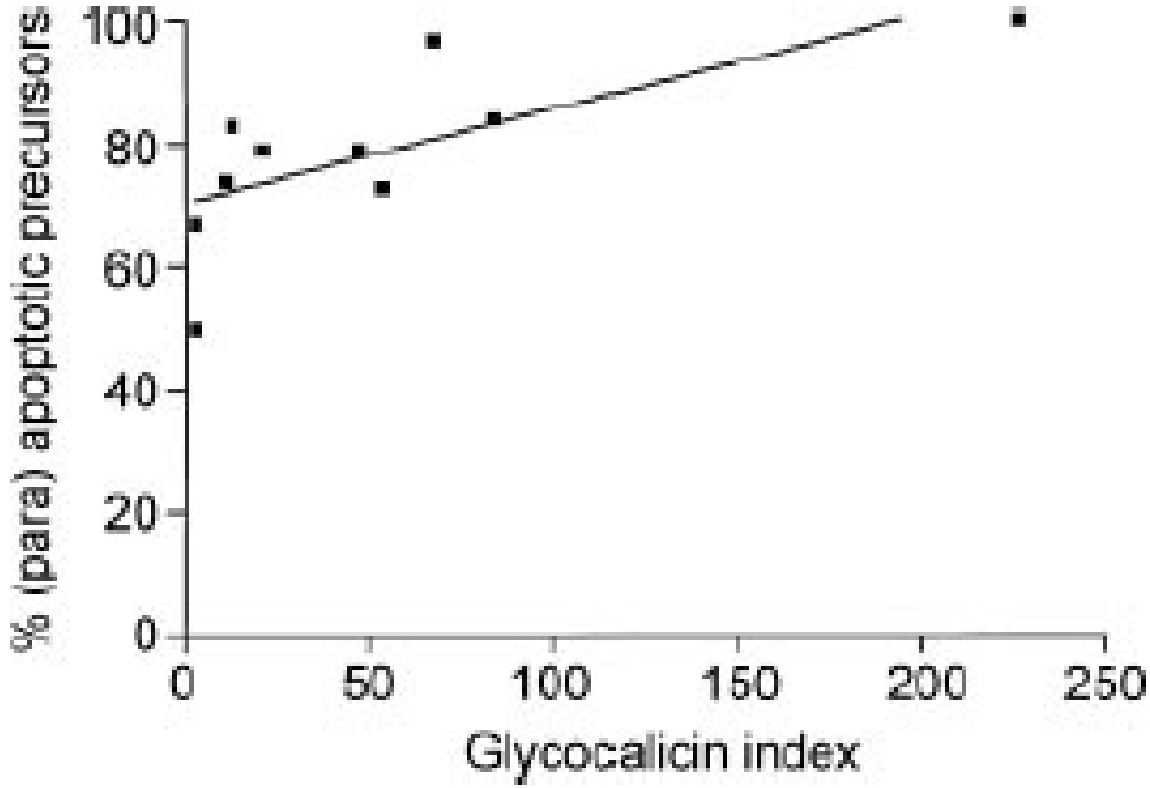
Mc Millan R et al, Blood 2004

Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura

Ewout J. Houwerzijl, Nel R. Blom, Johannes J. L. van der Want, Mariet T. Esselink, Jan J. Koornstra, Jan W. Smit, Henk Louwes, Edo Vellenga, and Joost Th. M. de Wolf

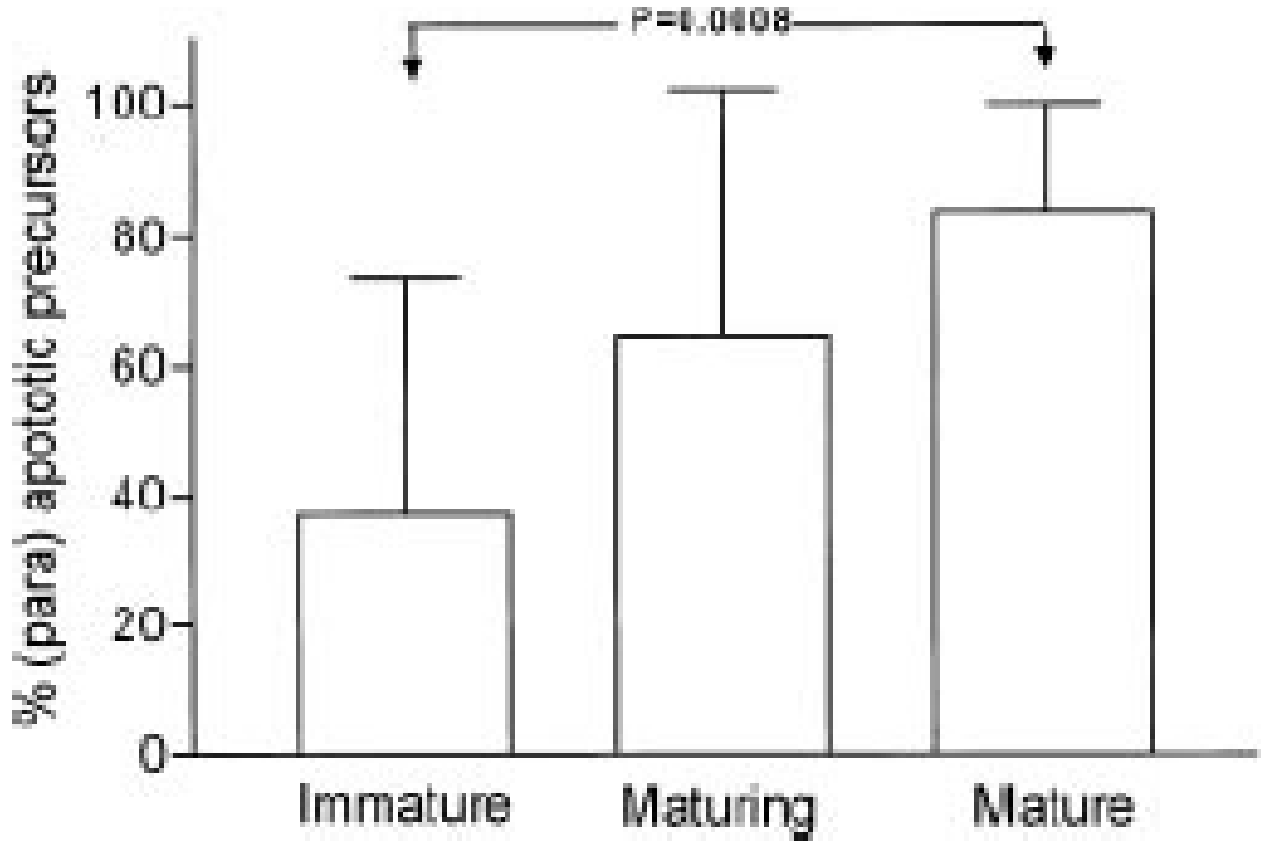
To investigate whether altered megakaryocyte morphology contributes to reduced platelet production in idiopathic thrombocytopenic purpura (ITP), ultrastructural analysis of megakaryocytes was performed in 11 ITP patients. Ultrastructural abnormalities compatible with (para-)apoptosis were present in 78% ± 14% of ITP megakaryocytes, which could be reversed by in vivo treatment with prednisone and intravenous immunoglobulin. Immunohistochemistry of bone marrow biopsies of ITP patients with extensive apoptosis showed an increased number of megakaryocytes with activated caspase-3 compared with normal (28% ± 4% versus 0%). No difference, however, was observed in the number of bone marrow megakaryocyte colony-forming units (ITP, 118 ± 93/10^5 bone marrow cells; versus controls, 128 ± 101/10^5 bone marrow cells; $P = .7$). To demonstrate that circulating antibodies might affect megakaryocytes, suspension cultures of $CD34^+$ cells were performed with ITP or normal plasma. Morphology compatible with (para-)apoptosis could be induced in cultured megakaryocytes with ITP plasma (2 of 10 samples positive for antiplatelet autoantibodies). Finally, the plasma glycocalicin index, a parameter of platelet and megakaryocyte destruction, was increased in ITP (57 ± 70 versus 0.7 ± 0.2; $P = .009$) and correlated with the proportion of megakaryocytes showing (para-) apoptotic ultrastructure ($P = .02$; $r = 0.7$). In conclusion, most ITP megakaryocytes show ultrastructural features of (para-) apoptosis, probably due to action of factors present in ITP plasma. (Blood. 2004; 103:500-506)

Correlation between percentage of damaged megakaryocytes and glycocalicin index



Houverzijl et al, Blood 2004

Percentage of damaged ITP megakaryocytes in different stages of differentiation



Houverzijl et al, Blood 2004

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Conclusioni

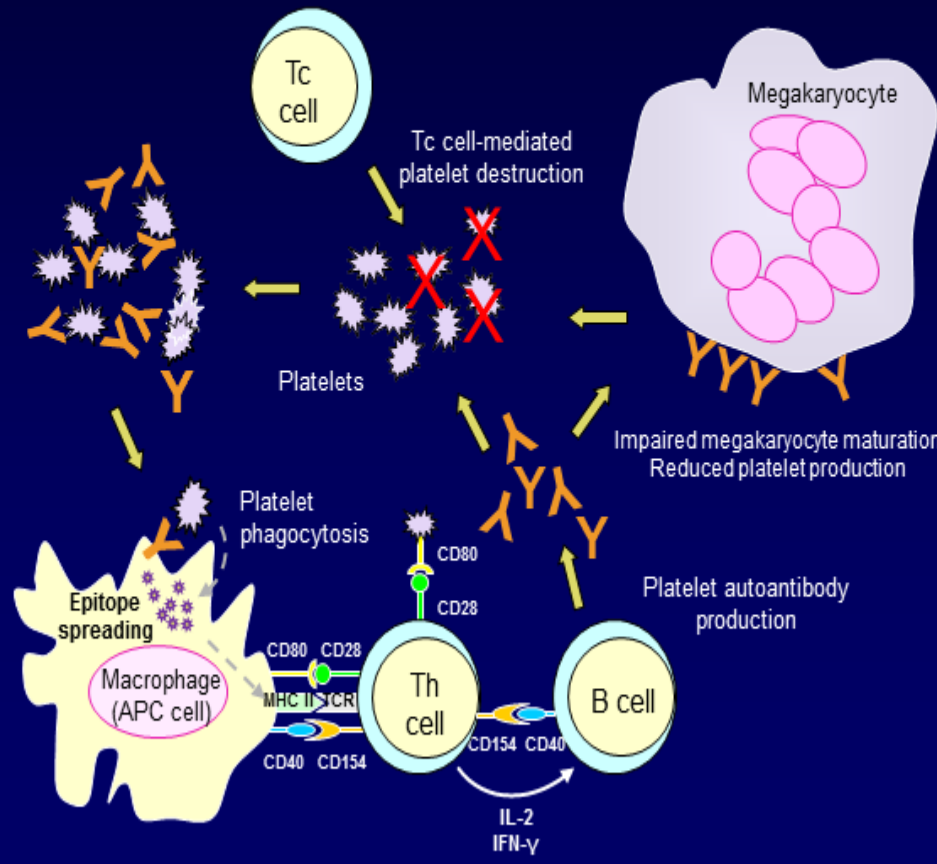
Molti megacarioci di pazienti ITP mostrano segni ultrastrutturalei di (para)apoptosi, probabilmente causata da fattori plasmatici

megakaryocytes, which could be reversed by in vivo treatment with prednisone and intravenous immunoglobulin. Immunohistochemistry of bone marrow biopsies of ITP patients with extensive apoptosis showed an increased number of megakaryocytes with activated caspase-3 com-

sion cultures of CD34+ cells were performed with ITP or normal plasma. Morphology compatible with (para-)apoptosis could be induced in cultured megakaryocytes with ITP plasma (2 of 10 samples positive for antiplatelet autoantibodies). Finally, the plasma glycocalicin

apoptosis, probably due to action of factors present in ITP plasma. (Blood. 2004; 103:500-506)

Understanding ITP pathophysiology will help us to find new therapeutic strategies



Steps

- Loss of tolerance
- Cleaving of platelet Ab by APC cell and express on APC cell
- APC–Th cell–B cell interactions
- B cell differentiation and Ab production
- Ab opsonize platelet: pathological loop

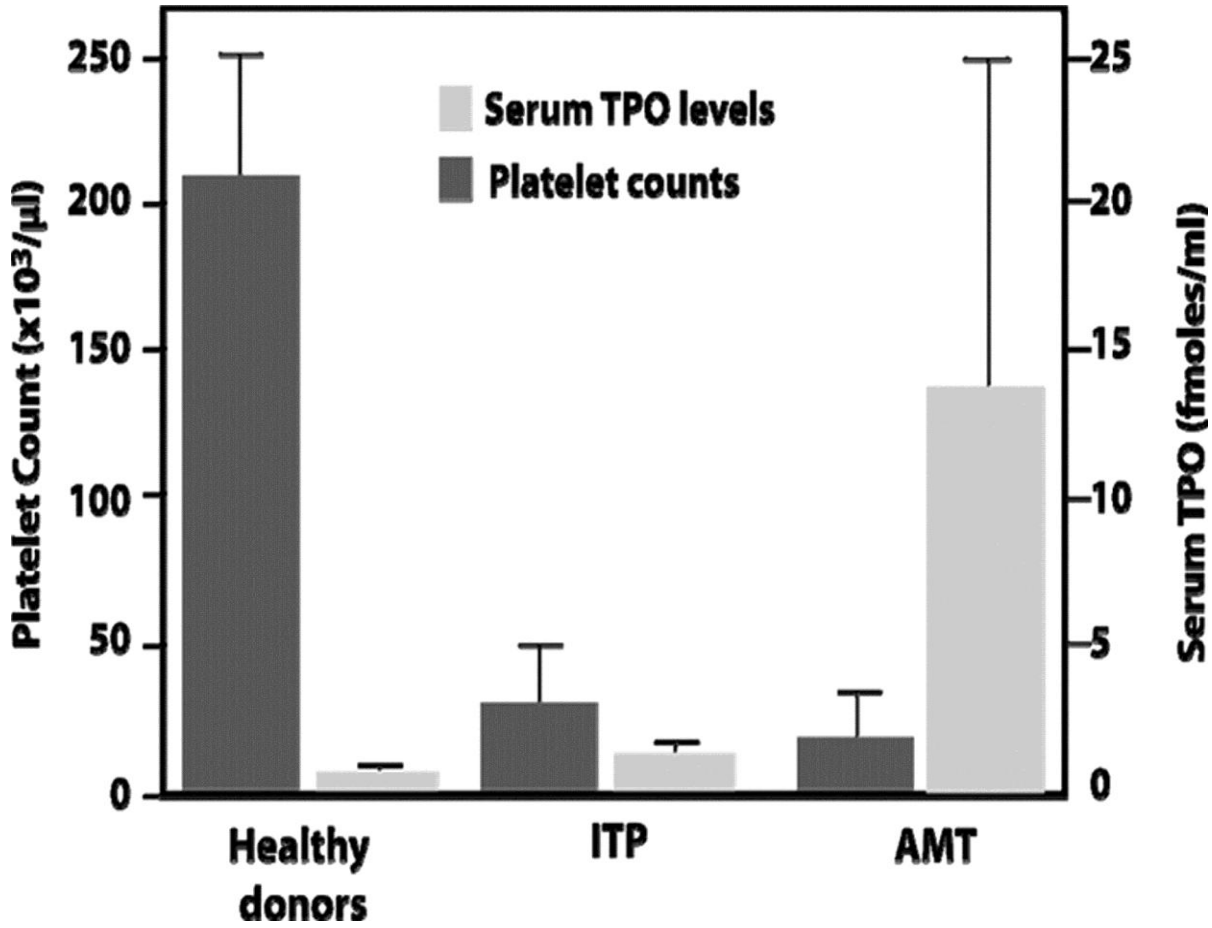
Additional steps

- T-cell-mediated platelet destruction
- Impaired megakaryocyte production

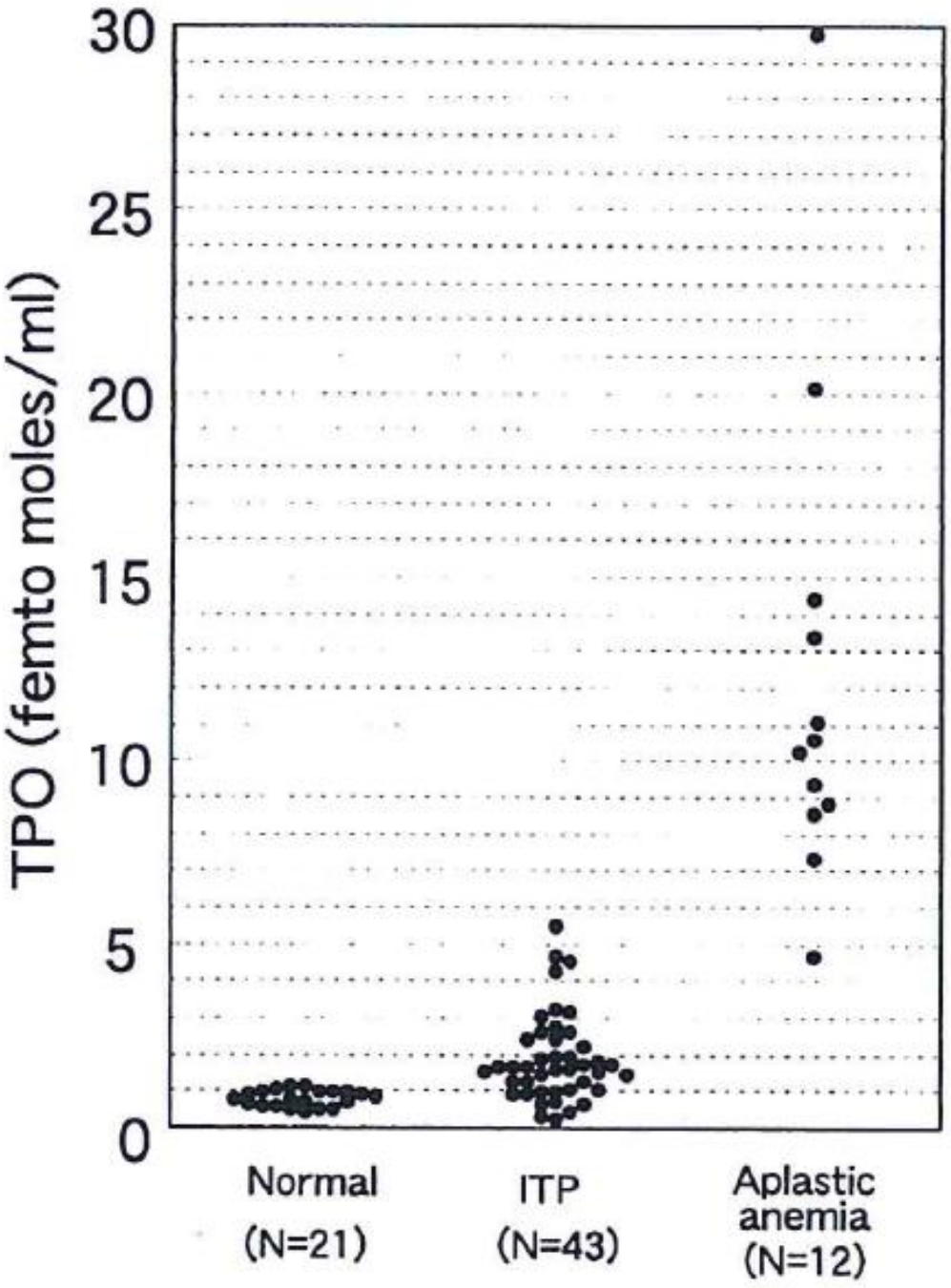
Ab=antibody, APC=antigen-presenting cell

Stasi R, et al. Thromb Haemost 2008;99:4–13

Differences in TPO plasma levels between patients with ITP or amegakaryocytic thrombocytopenia



Mukai et al, Thromb Haemost 1996



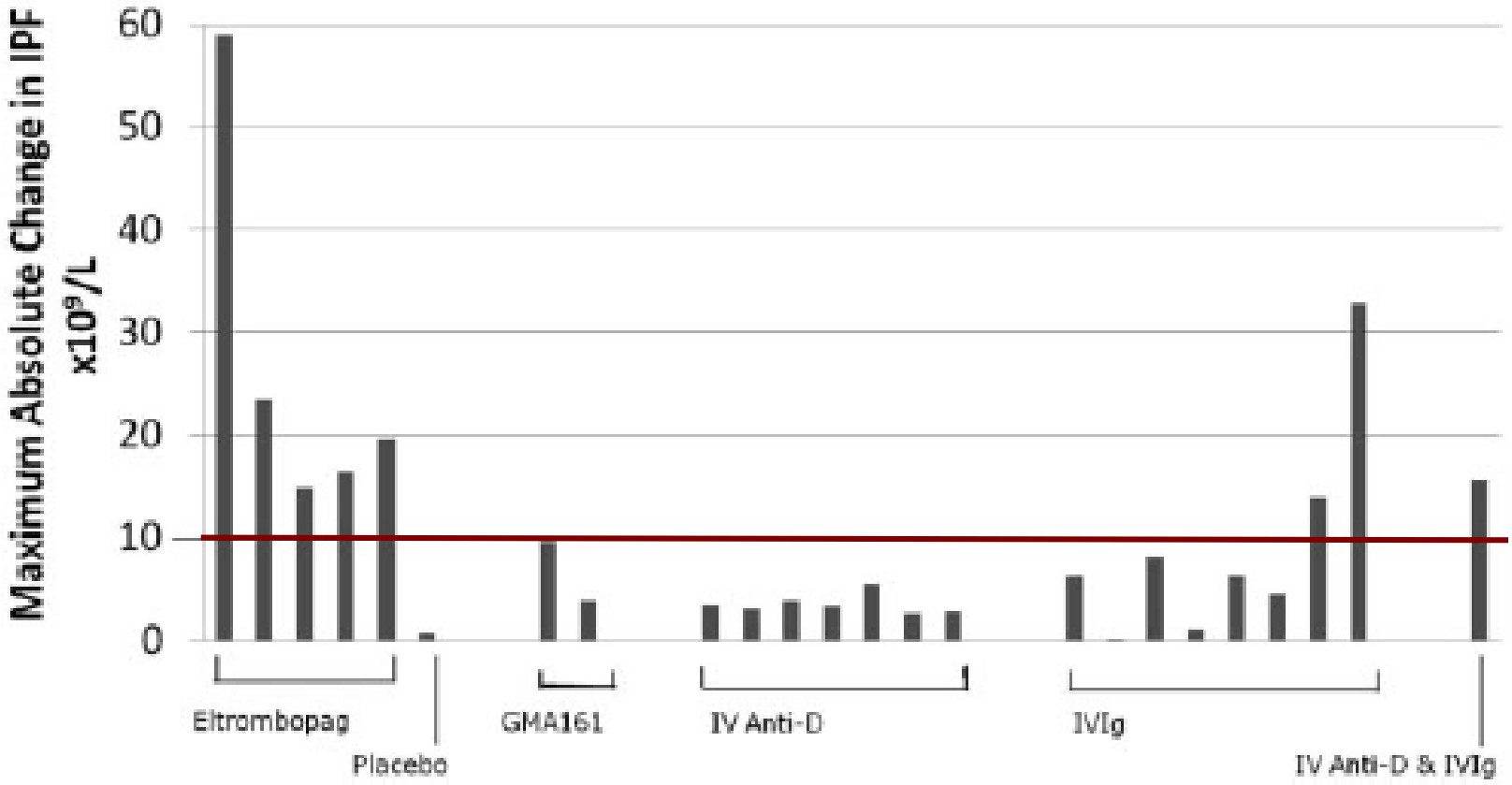
TPO plasma levels in normal subjects and patients with ITP or aplastic anemia

Kosugi A et al, BJH 1996

ITP - Strategie terapeutiche

- Farmaci immunosoppressori/immunomodulanti
- Agonisti del recettore per la trombopoietina

Maximum observed change in Absolute Immature Platelet Fraction (A-IPF) within 10 days of successful treatment in ITP patients (n=24)



GMA161: anti-FcγRIII MoAb *(inhibits destruction of Ab-coated platelets)*

Barsam SJ et al, Blood 2011

Conclusioni

- La ITP primaria riconosce due meccanismi patogenetici
  - Aumentata distruzione piastrinica periferica
  - Ridotta megacariocitopoiesi e piastrinopoiesi midollare
- Terapie efficaci:
  - Riduzione della distruzione piastrinica immuno-mediata
  - Incremento della magacariocitopoiesi e piastrinopoiesi midollare