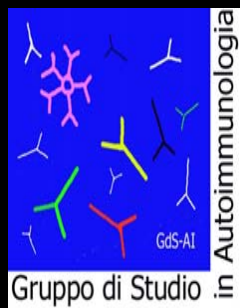


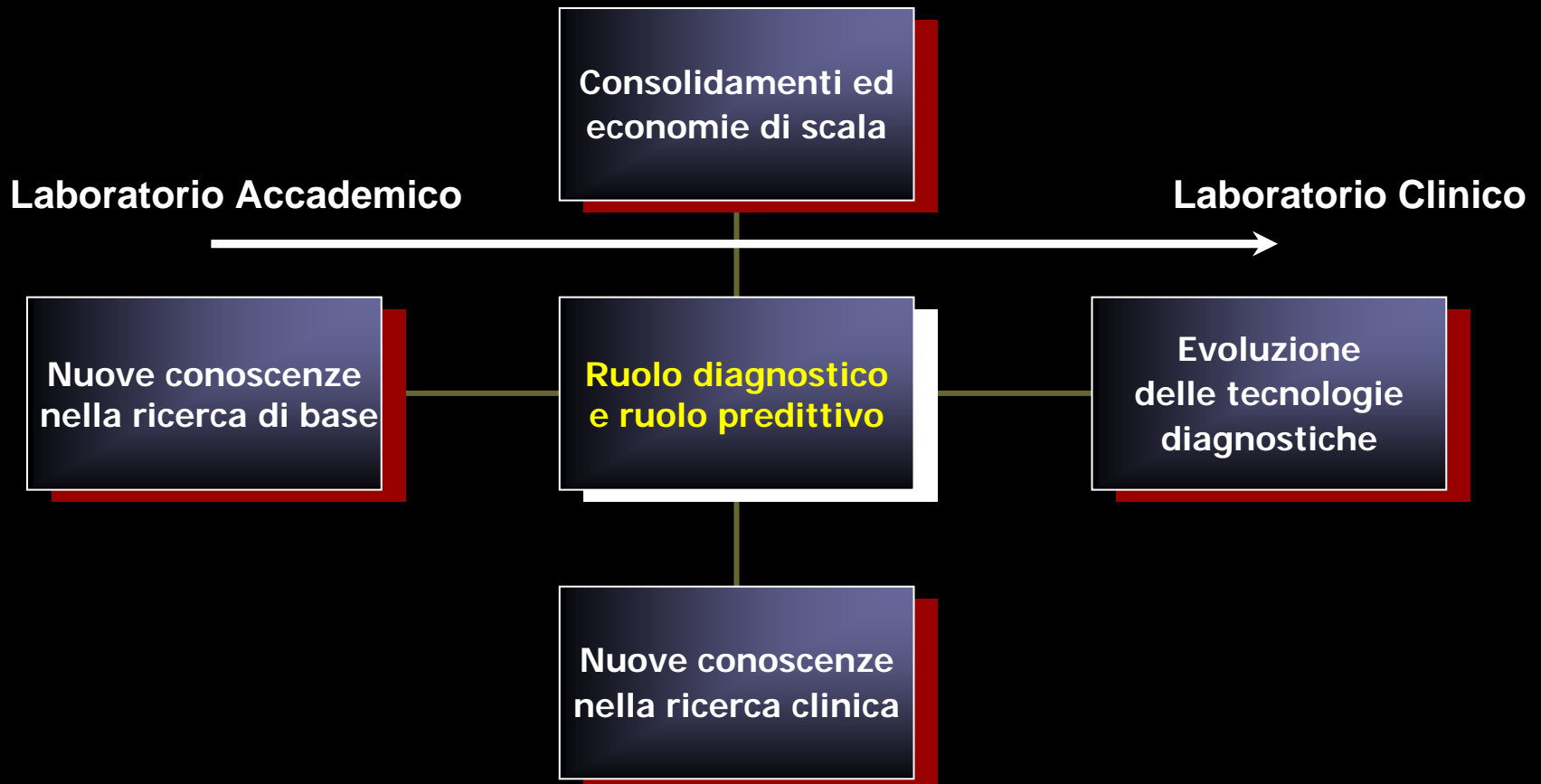


Ruolo della diagnostica autoanticorpale nel sistema neuro-endocrino e riproduttivo



Renato Tozzoli
Dipartimento dei Servizi Diagnostici
Ospedale di Latisana (Ud)
Firenze, 16 ottobre 2007

Il ruolo attuale del Laboratorio Clinico nelle malattie autoimmuni



Ruolo diagnostico e ruolo predittivo dei test autoanticorpali

Nuove conoscenze nella ricerca di base

Identificazione degli autoantigeni cardinali nel novero degli autoantigeni candidati

Evoluzione delle tecnologie biomediche

Standardizzazione delle procedure analitiche manuali (IIF)

Automazione dei test immunometrici convenzionali (EIA)

Miglioramento dell'accuratezza diagnostica

Introduzione dei metodi multiplex (array)

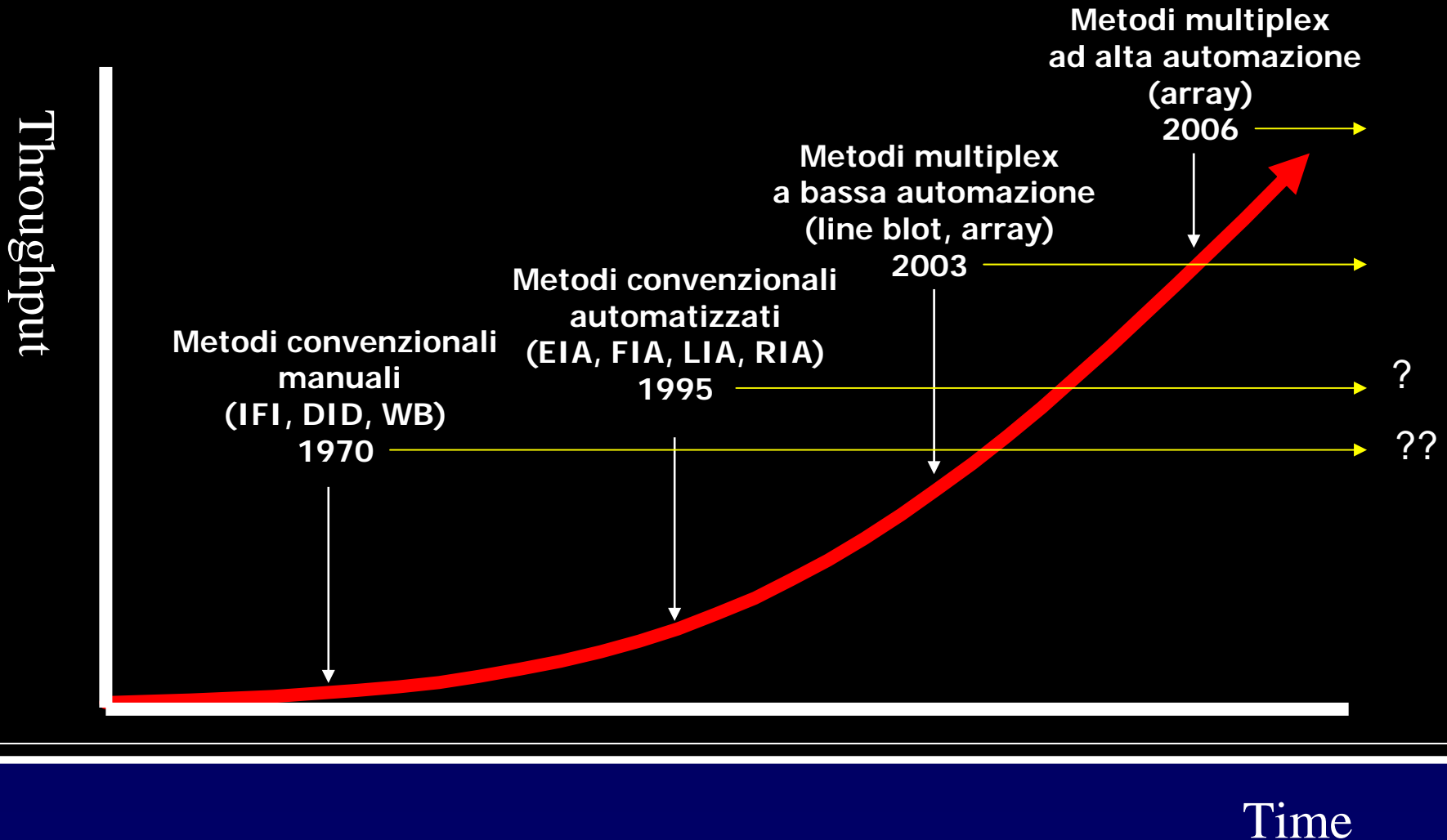
Nuove conoscenze nella ricerca clinica

Gli autoanticorpi come criteri diagnostici

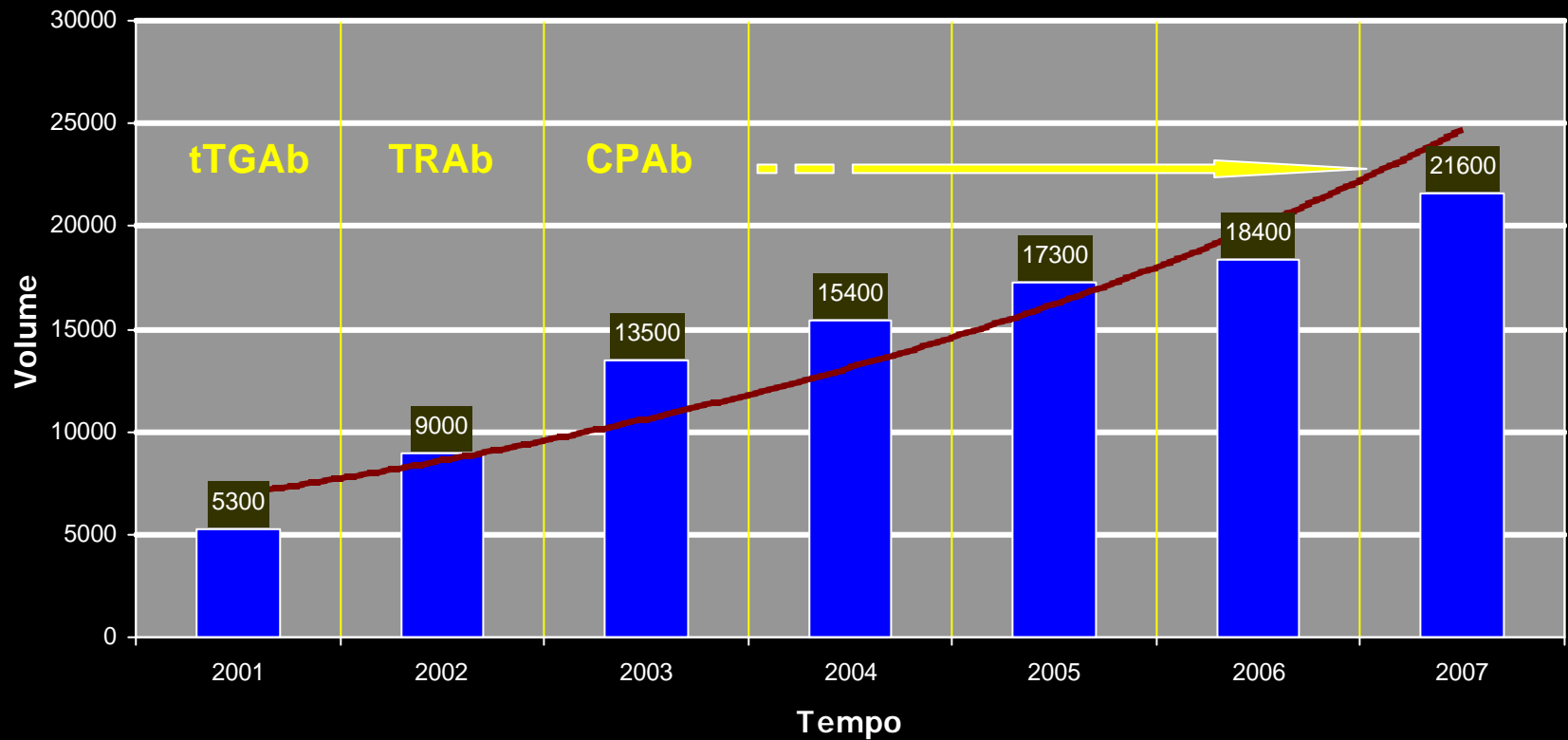
Gli autoanticorpi come marcatori predittivi

Variazioni dell'epidemiologia delle malattie autoimmuni

L'evoluzione delle tecnologie biomediche: il ruolo dell'automazione



L'evoluzione dell'attività del Laboratorio Clinico



I metodi multiplex: sfide ed opportunità per il laboratorio clinico

■ Motivi analitici

- Riduzione del volume dei campioni e dei reagenti

■ Motivi logistico-gestionali

- Associazione di analiti attualmente misurati con tecnologie differenti e contenimento dei costi

■ Motivi fisiopatologici e clinici

- Combinazione di marker in profili flessibili patologia-orientati od organo-orientati (profiling)

Planar and Nonplanar Microarrays

Tozzoli R, Bizzaro N. Autoantibodies 2006 (modified)

Support	Format	Detection system	AutoAbs tested	Estimated throughput	Commercial product	Reference
Nitrocellulose membranes	Line-blot	C	15	<50	Inno-LIA ? ?	Meheus, 1999
Microbeads	Wells	FL	9	1.000	FIDIS, AThENA, QuantaPlex	Rouquette, 2003
Nitrocellulose membranes	Microspots	CM	30	1.000	Biochip	Hentschel, 2004
Microbeads	Full automation	FL	15	2200	BioPlex 2200	Shovman, 2005
Glass slides	Microspots	FL	225	10.000	NA	Hueber, 2005
Barcoded particles	Wells	FL	9	10.000	UltraPlex	Pang, 2005
Glass-slides gold-coated	Microspots	SPR	24	10.000	NA	Segbers-Lokate, 2007
Polystyrene chips	Microspots	FL	20	10.000	Impact	Klause, 2007

I metodi a determinazione multipla: una nuova era per i test autoanticorpali

High-throughput protein-array techniques are already available in which **hundreds of autoantibodies specificities can be analyzed simultaneously.**


This technology might allow **prospective studies of large numbers of healthy individuals for the development of many autoimmune diseases.**

Scofield RH. Autoantibodies as predictors of disease. Lancet 2004

Autoantibody profiling: diagnostic purposes

Tozzoli R. *Autoimmun Rev*, 2007

Multiplex autoantibodies
detected in a single assay



New clinical correlations and paradigms,
prediction of autoimmune diseases
based on patients 'fingerprints' or 'signatures'



Clinical/analytical specificity of autoantibodies:
the management of unexpected results

Are We at a Stage to Predict Autoimmune Diseases?

Bizzaro N, Tozzoli R, Shoenfeld Y. *Arthritis Rheum*, 2007

.... In view of the low pretest probability of detecting autoimmune diseases at an early stage....., it is uncertain whether it will be useful or productive to submit the general population to autoantibody screening.....

.... It could be useful to test at-risk patients or group of patients:

- a) paucisymptomatic;
- b) with autoantibody positivity found by chance;
- c) with a systemic or organ-specific autoimmune disease;
- d) relatives of patients with autoimmune disease
- e) the female population from the age of puberty (?)

Ha oggi il laboratorio clinico di autoimmunità un ruolo nelle malattie autoimmuni del sistema neuroendocrino e riproduttivo ?

1. L'ipofisite autoimmune (AH/LYH) e le sindromi poliendocrine autoimmuni (APS)
2. L'insufficienza ovarica prematura (AI-POF)
3. L'infertilità secondaria: l'abortività e la poliabortività (RPL)

1. Epidemiology of LYH: a rare disease

Over the last 13 years the number of diagnosed cases has increased considerably, probably as a result of improved imaging criteria, and over 200 cases have been described so far.

Nevertheless, at the present time the LYH is still considered uncommon, and its true incidence and prevalence are unknown.

De Bellis A, Bizzarro A, Bellastella A.

BPR Clinical Endocrinology & Metabolism, 2005

Epidemiology of LYH: an underestimated disease?

Addison's disease

Central diabetes insipidus

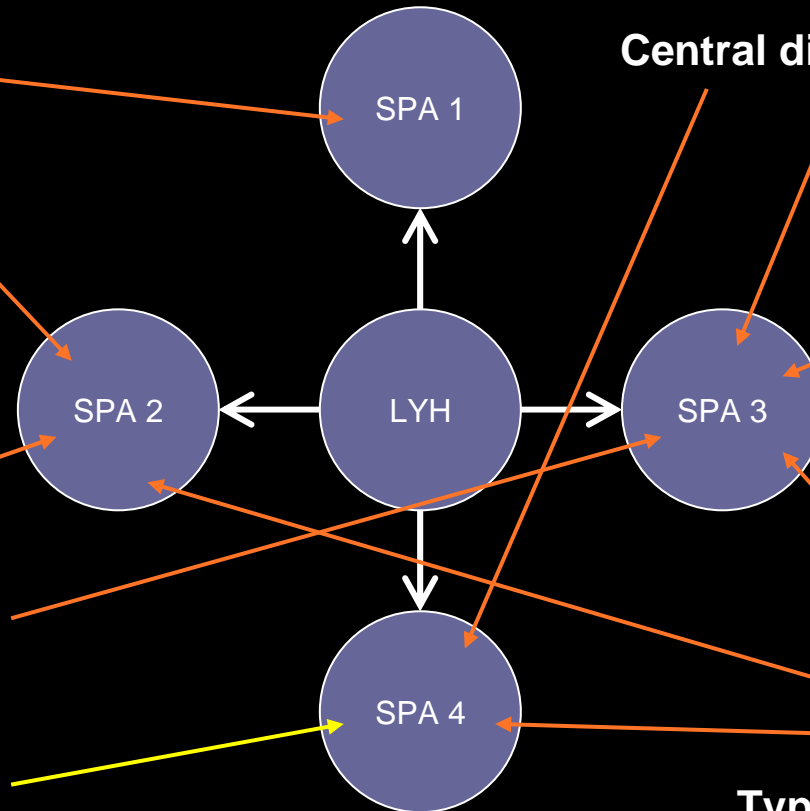
Chronic atrophic gastritis

Autoimmune hepatitis

Autoimmune thyroid diseases

Type 1 diabetes mellitus

**Systemic lupus
Pernicious anemia
Primary biliary cyrrhosis**



I metodi di rilevazione degli anticorpi anti-ipofisi (APA)

Fissazione del complemento (FC) (Engelbert, 1965)

Immunofluorescenza indiretta (IFI) (Bottazzo, 1975)

Immunoblot (IB) (Crock, 1990)

Radioimmunoprecipitazione (RIP) (Tanaka, 2002)

Detection of APAs by IIF: analytical variability and lack of standardization?

Gluck M, Scherbaum WA. Substrate specificity for the detection of autoantibodies to anterior pituitary cells in human sera.

Horm Metab Res 1990; 22:541-5

Human foetal pituitary tissue

De Bellis A, Bizzarro A, Conte M, et al. Antipituitary antibodies in adults with apparently idiopathic growth hormone deficiency and in adults with autoimmune endocrine diseases.

Young baboon pituitary tissue

APAs by IIF in AITD

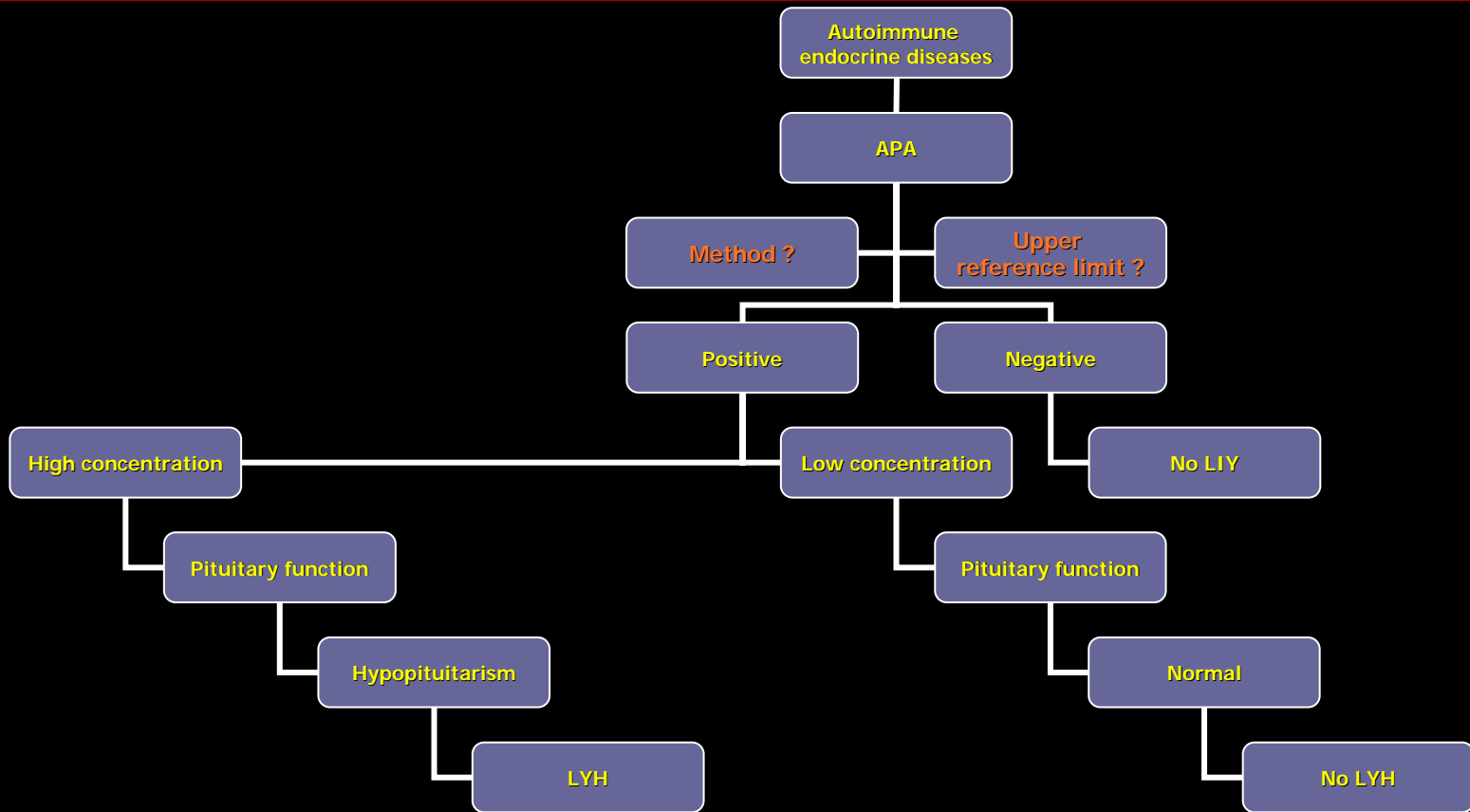
Disease	N/T	%	N/T	%
HT	92/707	13.0	13/86	15.1
GD	18/254	7.1	2/23	8.7
Non-AITD	3/329	0.9	-	-
HS	0/135	0	-	-

Manetti et al, JCEM 2007

Our preliminary study

Diagnostic flowchart of LYH

De Bellis A, Bizzarro A, Bellastella A. BPRCEM 2005 (modified)



Candidate autoantigens in pituitary autoimmunity: Pituitary Proteome ?

AA	PM (kDa)	Author
NSE α	49	O'Dwyer, 2002
NSE γ	53	Tanaka, 2003
hGH	22	Tanaka, 2002
PGSF 1 α	35	Tanaka, 2002
PGSF 2	42	Tanaka, 2002
TDRD 6	36	Bensing, 2005
ITD type 2	86	Nakahara, 2005

2. Insufficienza ovarica primitiva autoimmune (AI-POF)

Associata ad altre malattie autoimmuni

TAI

Morbo di Addison

RA

Vitiligine

Diabete autoimmune

LES

Miastenia

Morbo di Crohn

SPA 1,2,3,4

Isolata

Prevalenza

1.0% delle donne fertili

Eziologia

Alterazioni genetiche

Difetti enzimatici

Insulti fisici

Alterazioni (post)-recettoriali

Patologia diencefalo/ipofisi

0.8%

Autoimmune

0.2%

Autoantibodies and AI-POF

Perhaps it would be reasonable to view POF as a complex disease with multiple underlying etiopathogenetic mechanisms that include a cohort of patients with underlying ovarian autoimmunity and subcohorts with global dysregulation and polyglandular involvement, as well as patients with autoimmunity confined to the ovaries involving autoreactivity targeted to any of several potential ovarian-specific antigens.

Tuohy VK, Altuntas CZ. Curr Opin Obstet Gynecol, 2007;19:366-9

Frequency of autoantibodies in POF

Autoab	Method	Disease	%	Author
StCA	IIF	POF/APS1	60	Forges, 2004
	IIF	POF/APS2	20-40	Hoek, 1997
	IIF	POF/AD	73-100	Dal Pra, 2003
	IIF	POF/NAD	0-10	Betterle, 1993
	IIF	Isolated POF	0-10	Forges, 2004
	IIF	Healthy	0	Forges, 2005

Frequency of autoantibodies in POF

Autoab	Method	Disease	%	Author
AOA	IIF	Isolated POF	2-50	Forges, 2005
	IIF	Healthy	31-38	Novosad, 2003
	ELISA	Isolated POF	30-67	Forges, 2005
	ELISA	Healthy	0-5	Forges, 2005
	WB	Isolated POF	30	Pires, 2007
	WB	Healthy	0-5	Pires, 2007

Detection of AOA: analytical variability and lack of standardization

Indirect Immunofluorescence assay

Ovarian antibodies as detected by indirect immunofluorescence are unreliable in the diagnosis of autoimmune premature ovarian failure.

Novosad JA et al. BMC Women's Health, 2003

Enzyme-linked immunosorbent assay

...ELISA testing often utilizes undefined ovarian homogenates, inherently predisposing to an inability to define targeted self antigen.

Forges T, et al. Human reproduction update, 2004

... the most reliable and convincing way to demonstrate the presence of AOA in POF is to use highly purified recombinant or extracted target antigens in rigorously applied assays....

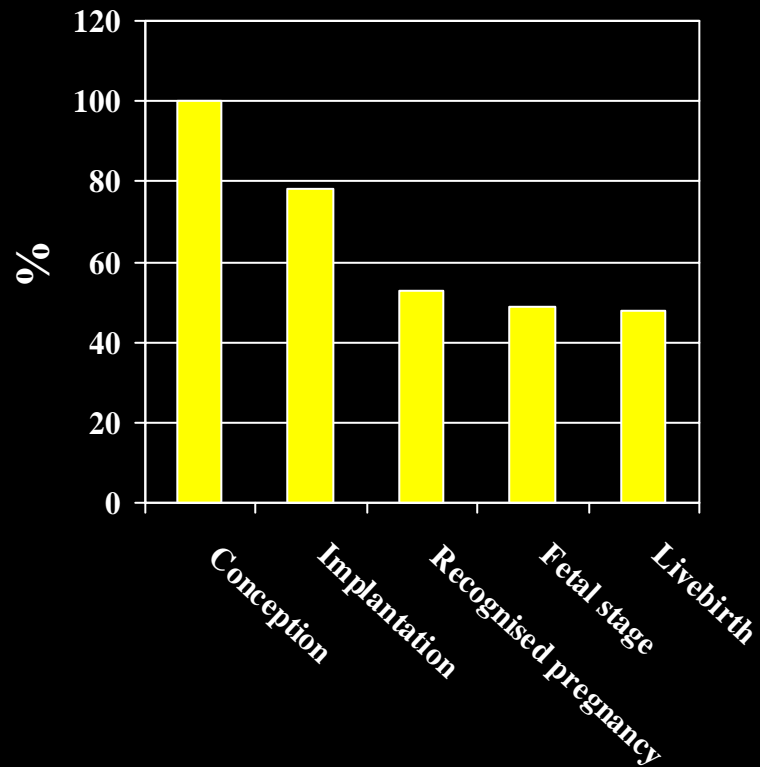
Tuohy VK, et al. Curr Opin Obstet Gynecol, 2007

Candidate autoantigens in ovarian autoimmunity: ovarian proteome ?

Cells	Autoantigen	Disease	Frequency	Author
Steroid producing	17-0H	POF/APS	50	Falorni, 2002
	SCC	POF/APS	71	
	3-HSD	POF/APS	3.7	Dal Pra, 2003
	170H/SCC	POF/APS	93	
Granulosa	FSH-R	POF	?	Chiauzzi, 1982
	β -FSH	POF	90	Forges, 2004
	Inhibin- α	EAO	?	Altuntas, 2006
Oocyte	MATER	EAO/POF	?	Tuohy, 2007
Corpus luteum	67 kDa	EAO	?	Pasoto, 1999
Zona pellucida	ZP3	EAO	?	Dean, 1992
Various	α -enolase	POF/APS	20	Sundblad, 2006

3. Miscarriage or pregnancy loss

Rai R, Legan L. Lancet 2006;368:601-11



% of infertile couples

Sporadic (SPL): 50%

Recurrent (RPL):

3 or more losses 1%

2 or more losses 5%

Association between autoantibodies and recurrent pregnancy loss (RPL) in women without autoimmune disease: meta-analysis of retro- and prospective studies

Autoantibody	Authors	Patients	OR	P
LAC	8 studies (1986-1997)	2026	15.4	0.0001
aCL IgG	7 studies (1988-2006)	2724	4.68	0.0001
aCl IgM	4 studies (1991-2006)	1700	5.61	0.02
Prothrombin	Shoenfeld, 2006	77	5.4	0.05
Tg and/or TPO	7 studies (1993-2005)	250		0.05
β 2GPI IgG	4 studies (1990-2006)	1266		n.s.
Annexin V	4 studies (1994-2005)	1193		n.s.
ANA	Bizzaro, 2005	116		n.s.
Gliadin-tTG	Bustos, 2006	128		0.04
Anti-tTG	Bustos, 2006	128		0.04
ASCA	Shoenfeld, 2006	77	3.9	0.05
Anti-laminin 1	Inagaki, 2001	207		0.004
Anti-elastin	Konova, 2007	10		0.005

Detection of autoantibody profile in early pregnancy: universal screening or targeted high-risk case finding?

- Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of **therapeutic trials**. Empson M. et al. Obstet Gynecol 2002
- Immunomodulatory effects of **intravenous immunoglobulins** as a treatment for autoimmune diseases, cancer, and recurrent pregnancy loss. Sapir T. et al. ANYAS 2005
- **Levothyronine** treatment in euthyroid pregnant women with AITD: effects on obstetrical complications. Negro R. et al. JCEM 2007
- The influence of **selenium** supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. Negro R. et al. JCEM 2007

Screening for autoantibodies in pregnancy: the role of microarrays

- aCL IgG
- aCL IgM
- Anti-prothrombin (?)
- TPOAb
- TgAb (?)
- Anti-laminin (?)
- Anti-elastin (?)

L'autoimmunità organo-specifica nella Medicina Clinica e di Laboratorio

**L'AUTOIMMUNITÀ TIROIDEA
NELLA MEDICINA
CLINICA E DI LABORATORIO**

Manifestazione effettuata con il patrocinio della
REGIONE VENETO

e
delle Società Scientifiche
AIPaC - AIRA - SIBioC - SIIC

*Chioggia (VE)
18 e 19 Maggio 2001
Centro Congressi Kursaal*

**INCONTRO DI
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ORGANO - SPECIFICA
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E DI LABORATORIO**

NAPOLI - CASTEL DELL'OVO
26 + 28 GIUGNO 2003

Con il Patrocinio delle Società Scientifiche:
S.I.E. - A.I.M.N. - A.I.S.F. - S.I.G.E.
S.I.D. - A.I.Pa.C. - S.I.Me.L. - S.I.Bio.C.

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ALLA PRATICA CLINICA**

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S.I.M.I. - S.I.N. - S.I.G.O. - S.I.P.

Sala 500
Centro Congressi del Lingotto - TORINO
28 - 29 giugno 2005

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MEDICINA CLINICA E
DI LABORATORIO**

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A.M.E. - ELAS Italia - S.I.Bio.C. - S.I.E. - S.I.G.E. - S.I.G.G. -
S.I.L.C.A. - S.I.Me.L. - S.I.M.I. - S.I.N. - S.I.P. -
Società Italiana di Diabetologia

Palazzo dei Congressi - FIRENZE
15 e 16 Ottobre 2007

Un ringraziamento

F. Aloisi

M. Amato

V. Barnaba

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C. Betterle

S. Bombardieri

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