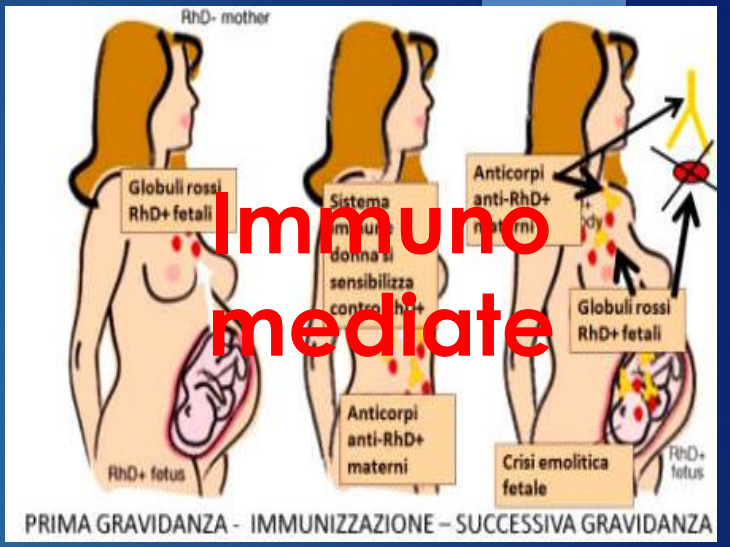
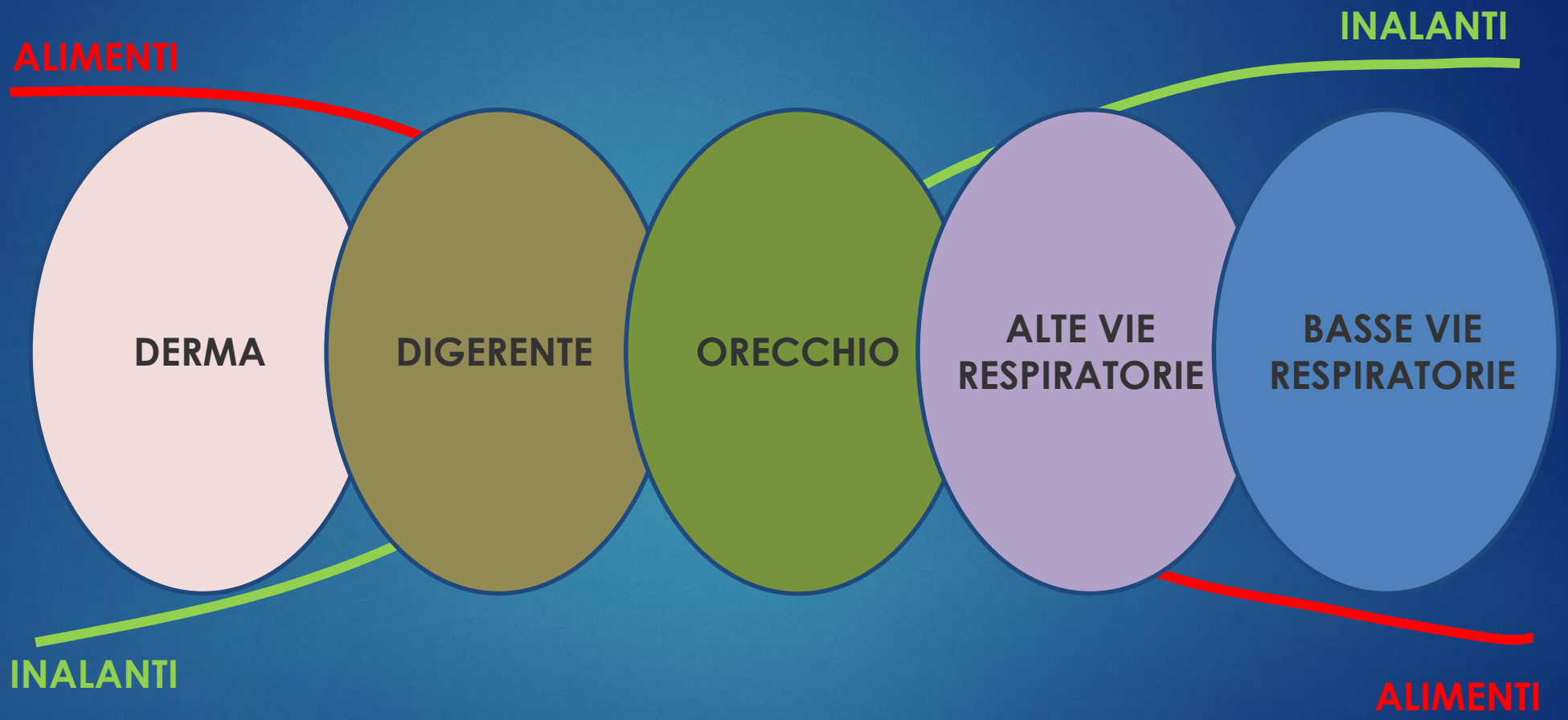
IL LABORATORIO NELLE ALLERGIE E INTOLLERANZE ALIMENTARI

GIANLUCA MONTI,
CENTRO DIAGNOSTICO CLANIS
FORMIA 06/01/2017

REAZIONI AVVERSE



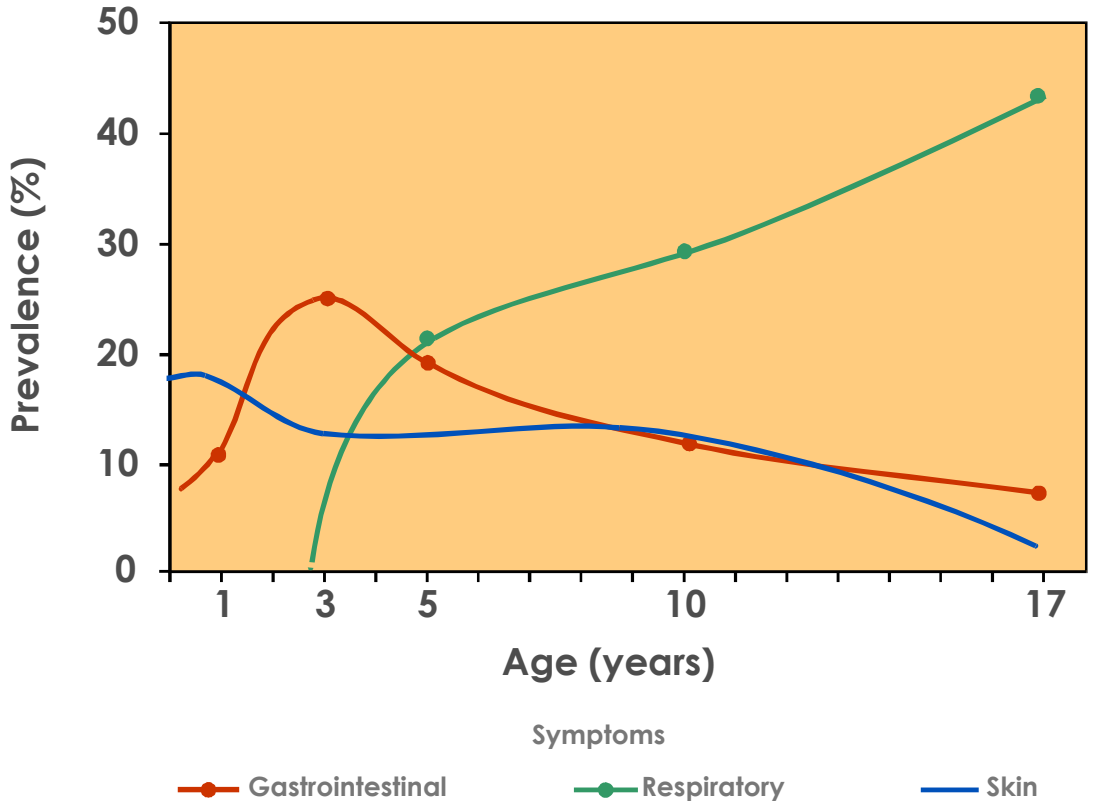
ATOPIA E MARCIA ALLERGICA



Selene K. Bantz J Clin Cell Immunol. 2014 Apr; 5(2): 202.

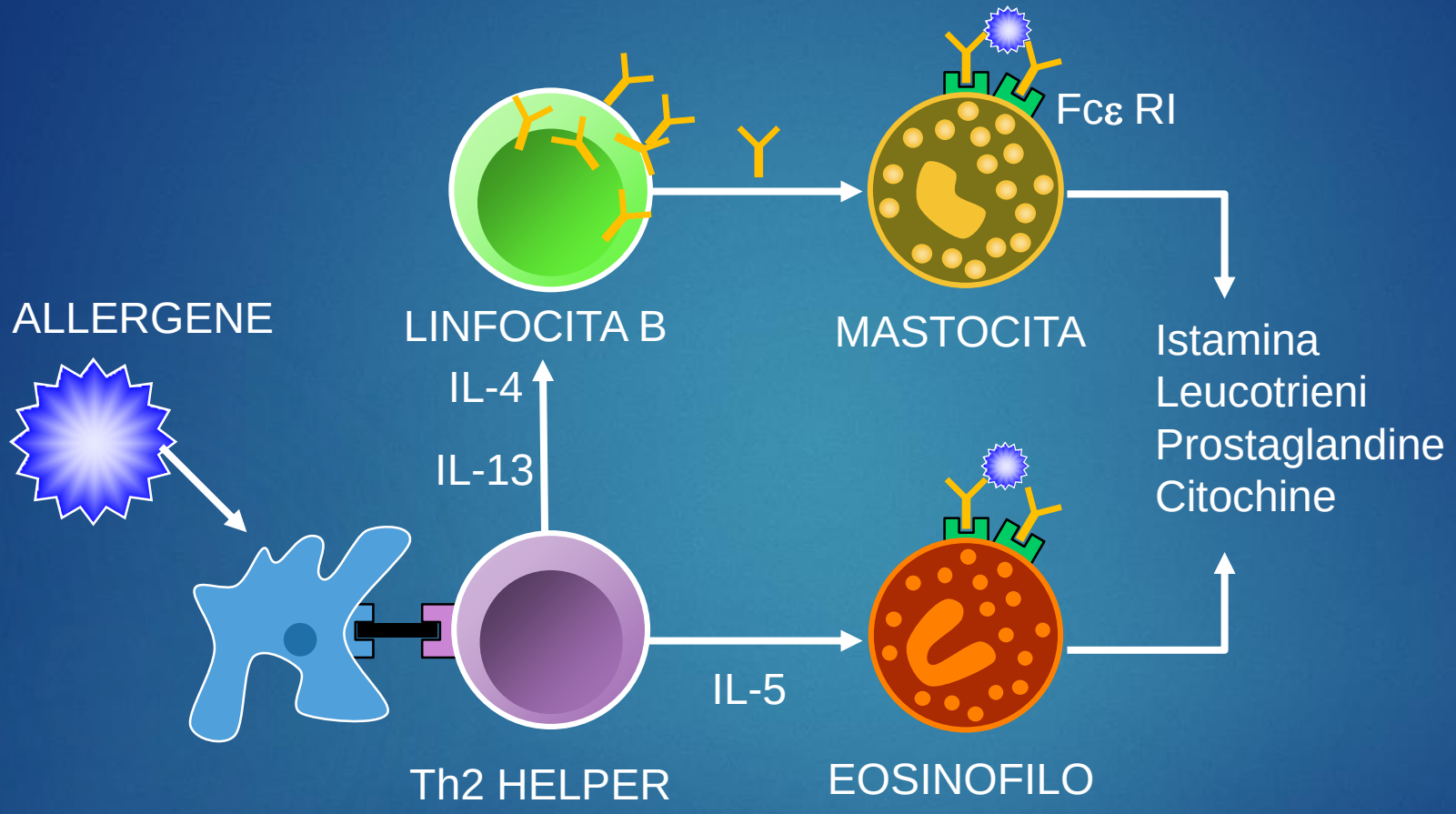
ALLERGIA

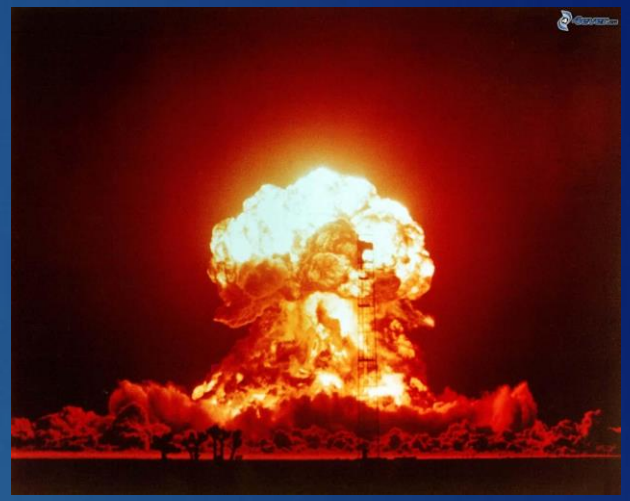
- 4° MALATTIA CRONICA (OMS)
- 20% DELLA POPOLAZIONE
- MALATTIA DEL MONDO OCCIDENTALE
- IPOTESI IGENISTA
- ESPOSIZIONE DOVUTA ALLO STILE DI VITA



Saarinen UM, Kajosaari M. Lancet. 1995;346:1065-1069.

DOSAGGIO DELLE IGE SPECIFICHE





Abbas, Lichtman, Pillai, *Immunologia cellulare e molecolare*, ELSEVIER, 2012

Ishizaka K, Ishizaka T: Identification of IgE antibodies as a carrier of reaginic activity. J Immunol 99: 1187, 1967

STRUMENTI DIAGNOSTICI

"The diagnosis of human allergic disease begins and ends with the patient's clinical history and a physical examination."

Hamilton RG J Allergy Clin Immunol. 2010 Jul;126(1):33-8

Human IgE antibody serology: A primer for the practicing North American allergist/immunologist

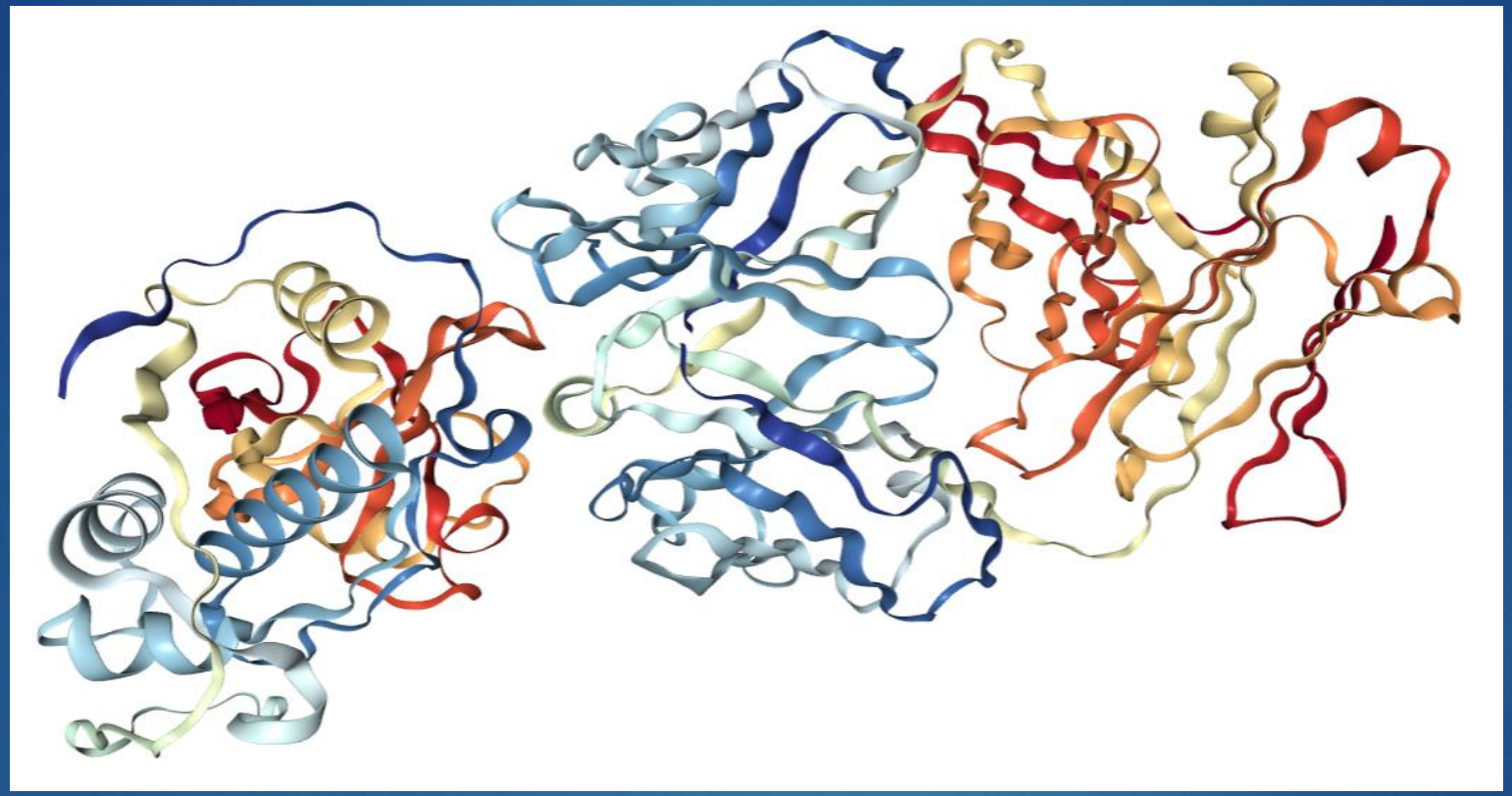
IgE SPECIFICHE

"When the clinical history identifies allergic symptoms in the patient that have a temporal relationship to a definable and relevant allergen exposure, IgE antibody sensitization is then confirmed using either in vivo skin test challenges (puncture/intradermal) or in vitro blood tests (allergen-specific IgE antibody serologic assays)."

Hamilton RG J Allergy Clin Immunol. 2010 Jul;126(1):33-8

Human IgE antibody serology: A primer for the practicing North American allergist/immunologist

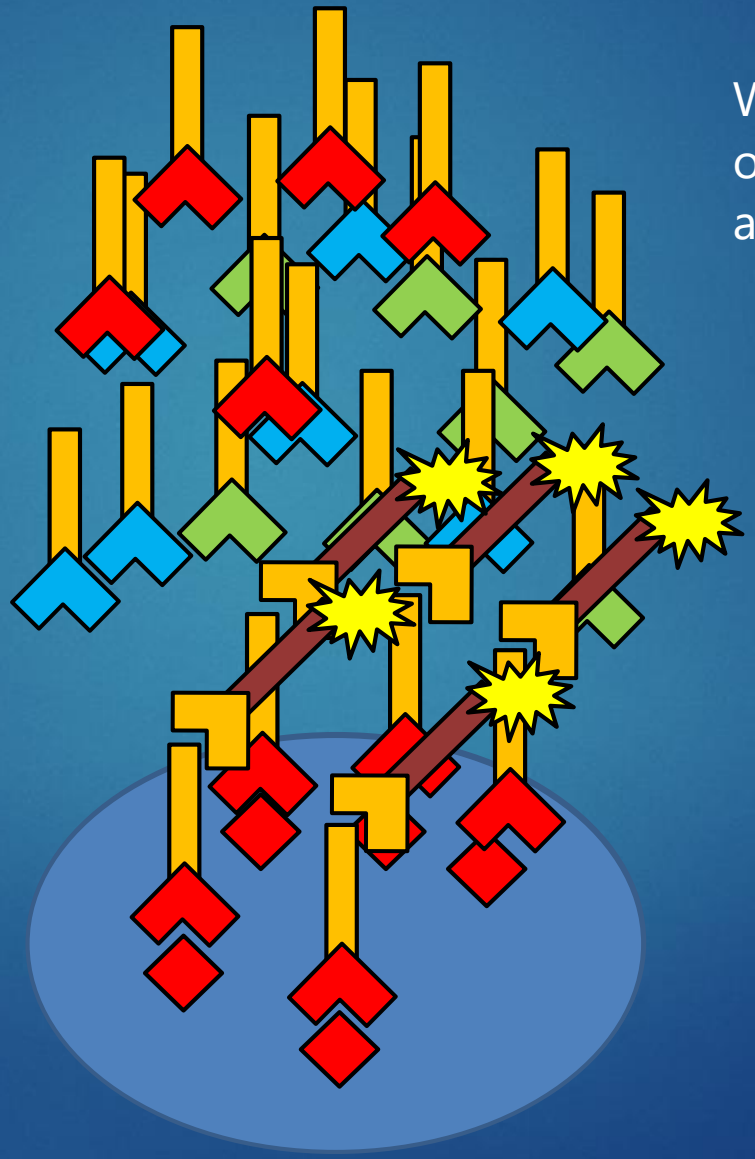
RICONOSCIMENTO ANTICORPALE



https://www.rcsb.org/pdb/ngl/ngl.do?pdbid=5VCO&bionumber=15VCO

THE CRYSTAL STRUCTURE OF DER P 1 ALLERGEN COMPLEXED WITH FAB FRAGMENT OF MAB 10B9 pdb 5VCO

DOSAGGIO IMMUNOLOGICO



Wide L, Bennich H, Johansson SGO. Diagno
of allergy by an in vitro test for allergen
antibodies. *Lancet* 1967;**2**:1105–1107.

UTILITA'

- IDENTIFICARE GLI ALLERGENI COINVOLTI
- PREVEDERE ALLERGIE NON ANCORA MANIFESTATE
- IMPOSTARE TERAPIE ADEGUATE
- RIDURRE O FERMARE LA PROGRESSIONE DELLA MARCIA

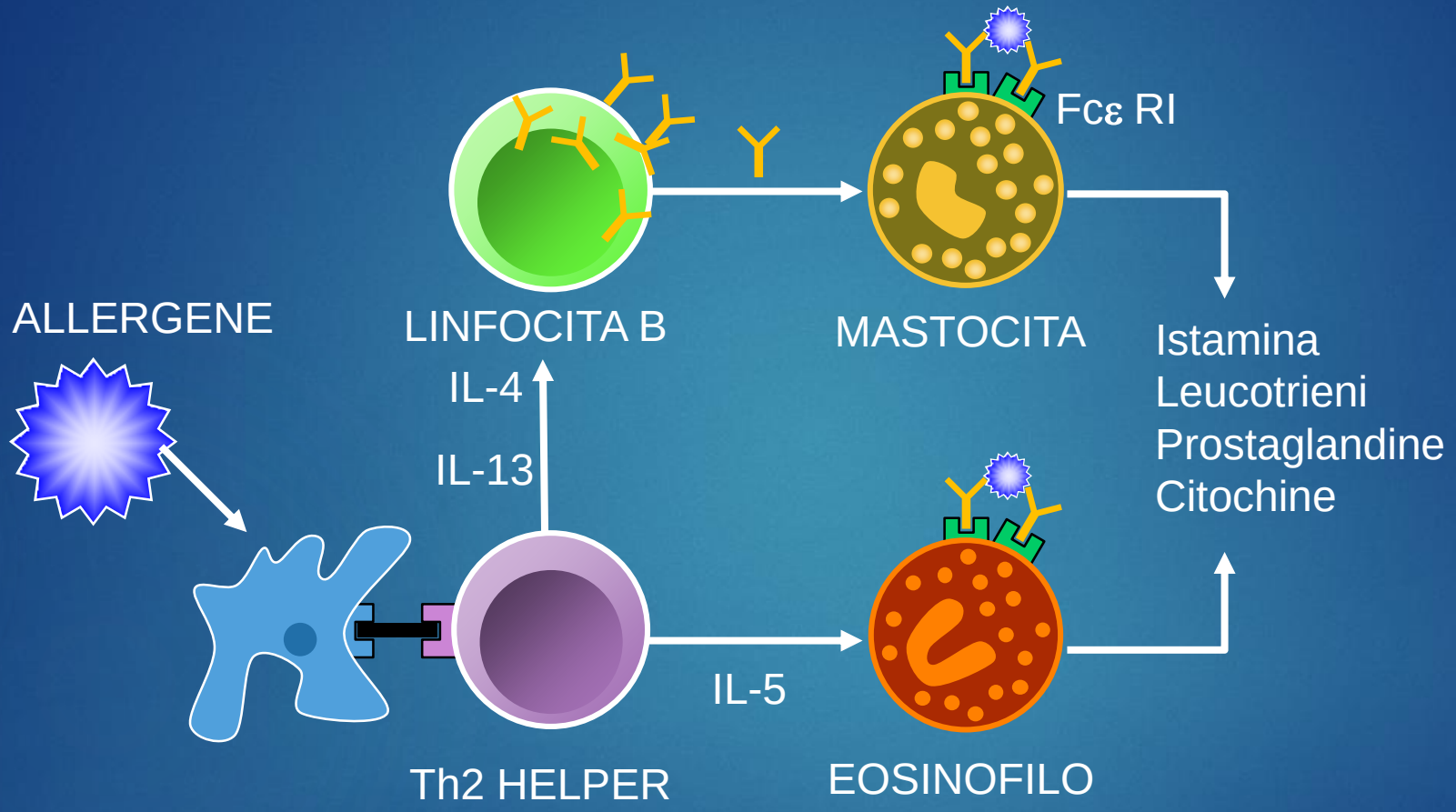
- INQUADRARE IL BABINO ATOPICO
- DISTINGUERE LE RINITI ALLERGICHE, LE NON ALLERGICHE, LE SINUSITI
- DISTINGUERE ASMA ALLERGICO, NON ALLERGICO, BRONCHITI

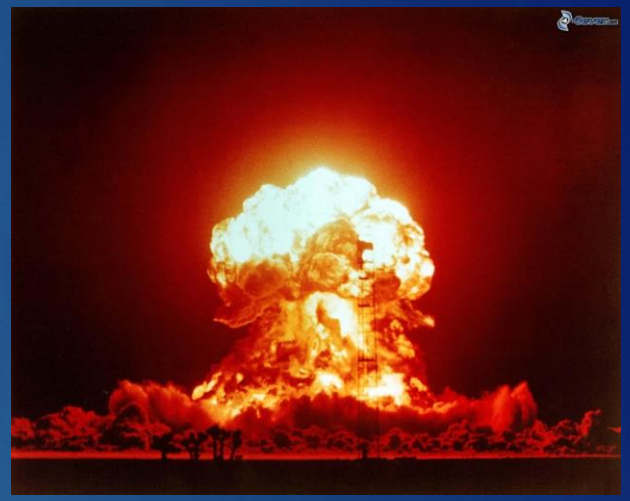
Sampson H. *Ann Allergy Asthma Immunol*. 2004;93:307-308
1Selner JC, et al. Ann Allergy Asthma Immunol. 1999;82:407-412
Guidelines for the Diagnosis and Management of Asthma.1997. NIH publication 97-4051

VANTAGGI

- NESSUN CONTATTO FRA PAZIENTE E ALLERGENE (REAZIONE IN VITRO)
- NON E' NECESSARIO SOSPENDERE LE TERAPIE IN ATTO
- POSSIBILITA' DI TESTARE > 200 ALLERGENI SU UN SINGOLO PRELIEVO
- CARATTERISTICHE DI SENSIBILITA' E SPECIFICITA' ANALOGHE A SPT
- TEST COMPELMENTARE A SPT (POSSIBILI INTEGRAZIONI)

DOSAGGIO DELLE IGE SPECIFICHE





Abbas, Lichtman, Pillai, *Immunologia cellulare e molecolare*, ELSEVIER, 2012

ALLERGENI

Allergens are proteins contained in allergenic sources; sensitization occurs when specific IgE are produced by atopic individuals and bind the trigger molecules. Allergy is an abnormal immunological reaction occurring in sensitized patients exposed to an allergen.

Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol. 2008;122

ALLERGENI ESTRATTIVI

t: tree	T4 CORYLUS AVELLANA (NOCCIOLO)
f: food	F17 NOCCIOLA
g: grass	G2 CYNODON DACTYLON (ERBA CANINA)
i: insect	I1 APIS MELLIFERA (APE)
w : wheat	G15 TRITICUM AESTIVUM (GRANO)
d: dust	D1 DERMATOPHAGOIDES PTERONYSSINUS
e: epithelia	E1 EPITELIO DI GATTO
p: parasite	P4 ANISAKIS
k: work	K82 LATTICE
m: mix	GM1 GRAMINACEE 3,4,5,6,8

ALLERGENICITA'

- EFFETTIVA DISPONIBILITA' IN VIVO DEL DETERMINANTE ANTIGENICO
  - CLASSE 1: RESISTENTI A CALORE E DIGESTIONE, SENSIBILIZZAZIONE GI, SOLUBILI IN ACQUA, BASSO MW, SENSIBILITA' PRIMARIA
  - CLASSE 2: TERMOLABILI E/O DIGERIBILI, DERIVANTI DALLE PIANTE, DIFFICILI DA ISOLARE, INSTABILI, CROSS REATTIVI
- CONFORMAZIONE SPAZIALE ALL'ESPOSIZIONE AL LEUCOCITA DEPUTATO
- AVIDITà DELL'ANTICORPO IGE
- CROSS-REATTIVITA'

Sampson HA. Adverse Reactions to foods. Allergy Principles and Practice. 2003

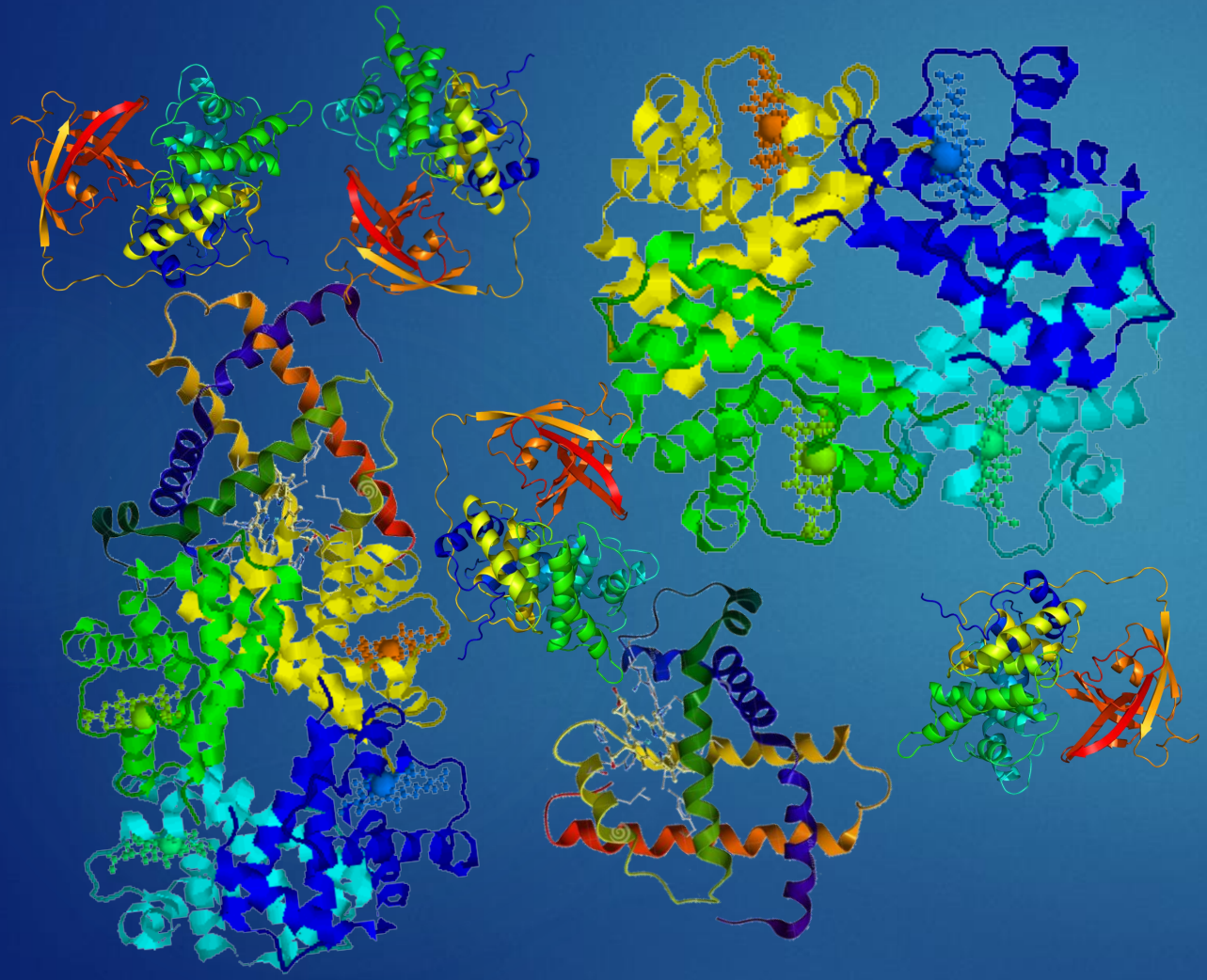
ALLERGENI 

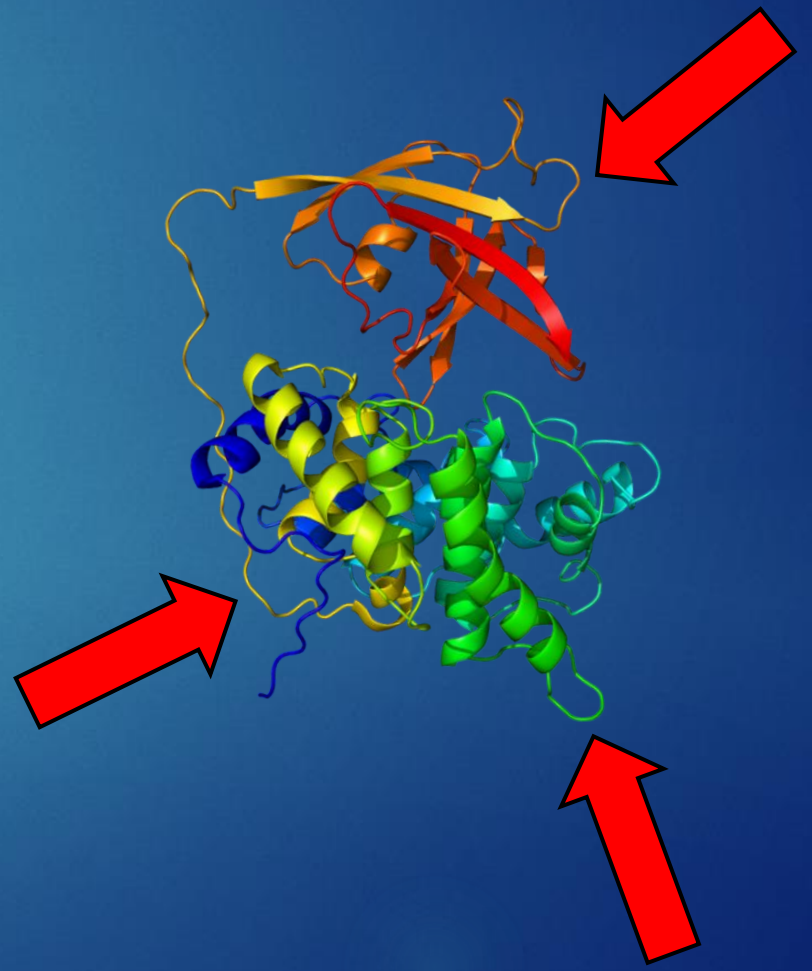
- IN GENERE PROTEINE (O APTENI CONIGATI A CARRIER)
- PESO MOLECOLARE 5 – 150 kDa
- OGNI ALLERGENE PUO' AVERE PiU' EPITOPI
- EPITOPI CONFORMAZIONALI E LINEARI
- ESISTONO MIGLIAIA ALLERGENI CON PROBABILE RILEVANZA CLINICA

F2 LATTE

- SECRETO
  - 87% ACQUA
  - 4% GRASSI
  - 5% ZUCCHERI
  - 3% PROTEINE
    - CASEINA
    - ALFA-LATTOALBUMINA
    - BETA-LATTOGLOBULINA
    - LATTOFERRINA
    - BSA
    - ENZIMI, ANTICORPI ….

PROTEOMICA > ALLERGOMICA





ALLERGENI IN VITRO

- ESTRATTI ALLERGENICI
  - VARIABILITA' FONTE NATURALE
  - PROCESSO DI ESTRAZIONE
- MISCELE DI ESTRATTI
- ALLERGENI MOLECOLARI
- ESTRATTI ARRICHHITI

Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 1967;**2**:1105–1107.

ESTRATTI COMMERCIALI

Gli estratti di allergeni commercialmente disponibili nell'UE sono standardizzati in termini di attività allergica totale nei confronti delle IgE, ma non per il contenuto proteico specifico.

Ciò rende impossibile confrontare e scambiare estratti prodotti da diverse aziende dalla stessa fonte allergizzanti.

Zimmer J, Vieths S, Kaul S. Standardization and regulation of allergen products in the European Union. Curr Allergy Asthma Rep. 2016;16(3):21. doi: 10.1007/s11882-016-0599-4

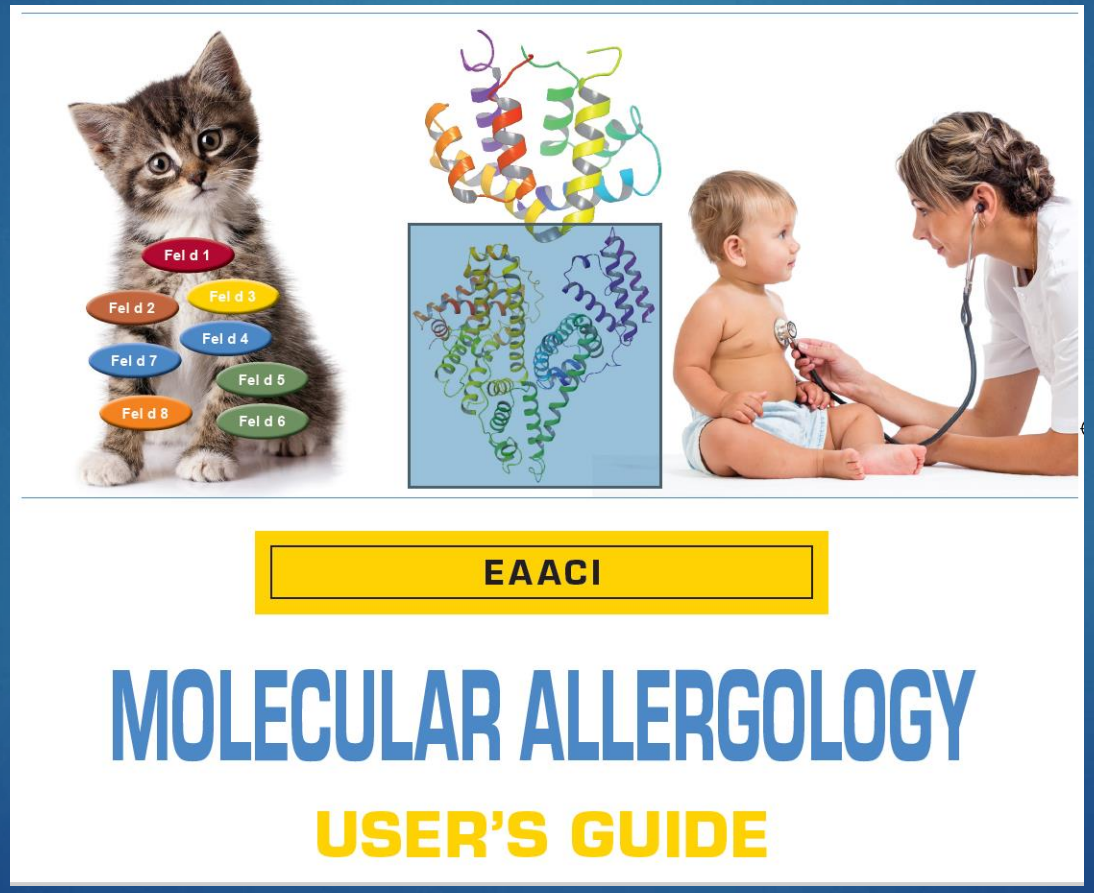
PRINCIPALI ALLERGENI CLASSE 1

- LATTE VACCINO
  - CASEINA, A-LATTALBUMINA, B-LATTOGLOBULINA, ALBUMINA
- UOVO
  - OVOMUCOIDE, OVALBUMINA, OVOTRASFERRINA
- ARACHIDI
  - VICILLIN, CONGLUTIN, GLYCININ
- SOYA
  - GLYCININ, PROFILIN, INIBITORI TRIPSINA
- GAMBERI
  - TROPOMIOSINA
- LTPS VEGETALI
  - MELA, PESCA, ALBICOCCA, MAIS

PRINCIPALI ALLERGENI CLASSE 2

- Glucanase (Gruppo 2)
  - LATTICE, AVOCADO, BANANA
- Pathogen-related protein (Gruppo 3, chitinase):
  - Latex (Hev b6), avocado
- Pathogen-related protein 5 (thaumatin-like):
  - Cherry, apple, kiwi
- Birch Bet 1 homologues (pathogen-related proteins 10):
  - Apple, cherry, apricot, peach, pear, carrot, celery, parsley, hazelnut
- Birch Bet 2 homologues (celery-mugwort-spice syndrome) profilin:
  - Latex, celery, potato, pear, peanut, soybean

DA UNO A MOLTI



ALLERGENI MOLECOLARI

Arachis hypogaea

Ara h 1
Ara h 2
Ara h 3

http://www.allergen.org/index.php

Species	Allergen	Biochemical name	MW(SDS-PAGE)	Food Allergen	Entry Date	Modified Date
Arachis hypogaea (Peanut, groundnut)						
	Ara h 1	Cupin (Vicillin-type, 7S globulin)	64	Yes	2003-06-24	2010-04-29
	Ara h 2	Conglutin (2S albumin)	17	Yes	2003-10-27	2010-04-29
	Ara h 3	Cupin (Legumin-type, 11S globulin, Glycinin)	60, 37 (fragment)	Yes	2003-06-24	2012-05-04
	Ara h 4	renamed to Ara h 3.02, number not available for future submissions		Yes	2003-06-24	2012-05-04
	Ara h 5	Profilin	15	Yes	2003-06-24	2010-04-29
	Ara h 6	Conglutin (2S albumin)	15	Yes	2003-06-24	2010-04-29
	Ara h 7	Conglutin (2S albumin)	15	Yes	2003-06-24	2010-04-29
	Ara h 8	Pathogenesis-related protein, PR-10, Bet v 1 family member	17	Yes	2004-04-25	2013-06-04
	Ara h 9	Nonspecific lipid-transfer protein type 1	9.8	Yes	2007-10-30	2015-02-05
	Ara h 10	16 kDa oleosin	16 kDa	Yes	2008-07-11	2016-01-05
	Ara h 11	14 kDa oleosin	14 kDa	Yes	2008-07-11	2010-04-29
	Ara h 12	Defensin	8 kDa (reducing), 12 kDa (non-reducing), 5.184 kDa (mass)	Yes	2015-12-23	2016-10-11
	Ara h 13	Defensin	8 kDa (reducing), 11 kDa (non-reducing), 5.472 kDa (mass)	Yes	2015-12-23	2016-10-11
	Ara h 14	Oleosin	17.5 kDa	Yes	2015-05-09	2016-10-11
	Ara h 15	Oleosin	17 kDa	Yes	2015-05-02	2016-10-11
	Ara h 16	non-specific Lipid Transfer Protein 2	8.5 by SDS PAGE reducing	Yes	2015-05-22	2016-10-11
	Ara h 17	non-specific Lipid Transfer Protein 1	11 kDa by SDS-PAGE reducing	Yes	2015-05-22	2016-10-11

ALLERGENI MOLECOLARI

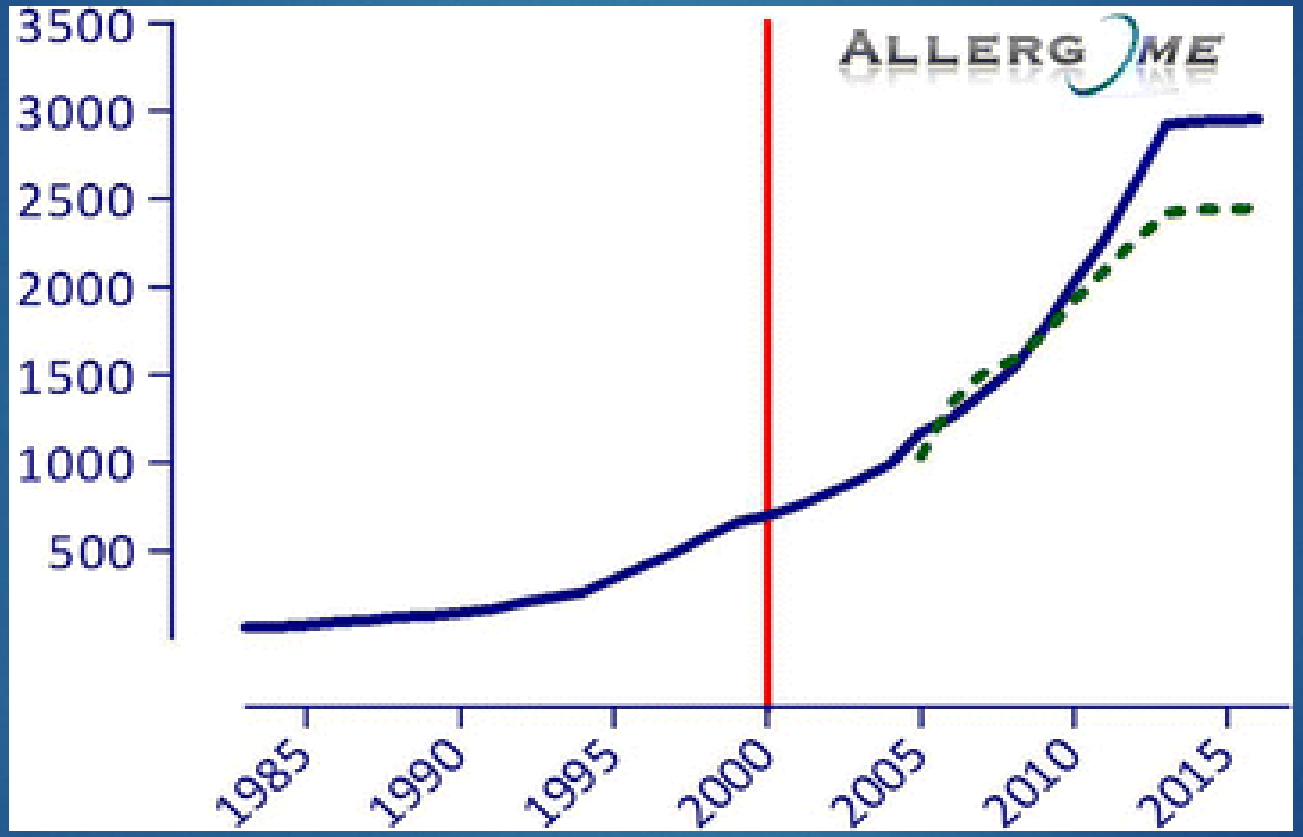
Dermatophagoides farinae

Der f 1
Der f 2
Der f 3

http://www.allergen.org/index.php

Species	Allergen	Biochemical name	MW(SDS-PAGE)	Food Allergen	Entry Date	Modified Date
Dermatophagoides *farinae* (American house dust mite)						
	Der f 1	Cysteine protease	27	No	2003-04-29	2010-04-29
	Der f 2	NPC2 family	15	No	2003-04-30	2010-04-29
	Der f 3	Trypsin	29	No	2003-04-30	2014-11-19
	Der f 4	alpha-amylase	57.9 kDa	No	2016-04-02	2016-10-11
	Der f 6	Chymotrypsin	25	No	2003-05-06	2010-04-29
	Der f 7		30-31	No	2003-05-06	2010-04-29
	Der f 8	Glutathione S-transferase	32 kD	No	2014-10-28	2016-10-11
	Der f 10	Tropomyosin	37	No	2003-04-29	2010-04-29
	Der f 11	Paramyosin	98	No	2003-04-29	2010-04-29
	Der f 13	Fatty acid binding protein		No	2007-02-08	2014-11-19
	Der f 14	Apolipophorin	177	No	2003-04-30	2010-04-29
	Der f 15	Chitinase	98/109	No	2003-04-30	2010-04-29
	Der f 16	Gelsolin/villin	53	No	2003-04-30	2010-04-29
	Der f 17	Calcium binding protein	53	No	2003-04-30	2010-05-20
	Der f 18	Chitin-binding protein	60	No	2003-05-06	2013-01-31
	Der f 20	Arginine kinase	40 kD	No	2014-11-03	2016-10-11
	Der f 21		14 kDa	No	2014-03-01	2016-10-11
	Der f 22			No	2007-02-08	2010-04-29
	Der f 24	Ubiquinol-cytochrome c reductase binding protein homologue	13	No	2013-04-10	2016-10-11
	Der f 25	Triosephosphate isomerase	34 kDa	No	2014-11-03	2016-10-11
	Der f 26	Myosin alkali light chain	18 kDa	No	2014-11-05	2016-10-11
	Der f 27	Serpin	48 kD	No	2014-11-04	2016-10-11

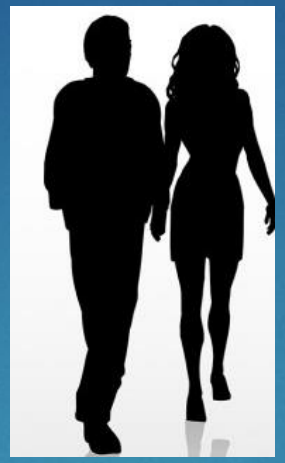
MOLECOLE ALLERGENICHE IDENTIFICATE



ALESSANDRI C. Clin Transl Allergy. 2017; 7: 21.

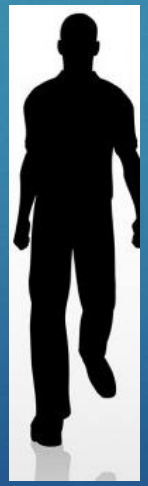
http://www.allergome.org/

F49 MELA



Mal d 1

Mal d 2



Mal d 3

Mal d 4

La concentrazione di nsLTPs (Mal d 3) e PR-10 (Mal d 1) varia sensibilmente considerando il cultivar.

La concentrazione di nsLTPs è maggiore nella mela bio e nelle varietà Golden e Granny Smith.

Non ci sono differenze nella concentrazione di Mal d 1 tra una mela biologica ed una a coltura convenzionale.

Le varietà Elise e Santana sono le meno allergizzanti mentre le Pink Lady e le Golden Delicious le più allergizzanti 7. Bisognerebbe pertanto eseguire SPT con diverse varietà di mele.

La concentrazione di nsLTPs e PR-10 aumenta con il tempo di conservazione e la maturazione del frutto 8.

Diaferio L Rivista di Immunologia e Allergologia Pediatrica 2017 I-IV

La conservazione standard a freddo (2°C in aria ambiente) incrementa del 15% l'allergenicità versus la conservazione controllata (3° C, con O2 al 2,5% e CO2 all'1%).

Per eseguire un prick by prick alla mela è consigliabile prelevare buccia e polpa da un'area prossima al gambo in quanto ricca di Mal d 1.

La reattività del prick test è inferiore se si utilizza il frutto fresco (ottobre-novembre) rispetto a quello conservato da sei mesi in quanto i prick sarebbero eseguiti in concomitanza del periodo di impollinazione della betulla.

Per l'esecuzione del OFC la scelta rimane la Golden bio (la più allergizzante e la più ricca di LTP), senza la buccia

Choice of relevant allergens for allergen-specific immunotherapy products.

The use of molecular diagnosis techniques may allow physicians to better identify whether children with allergic respiratory symptoms are sensitized to major allergens or to crossreactive molecules. This is of particular interest in patients who are sensitized to several pollens, to prescribe AIT only for major allergens.

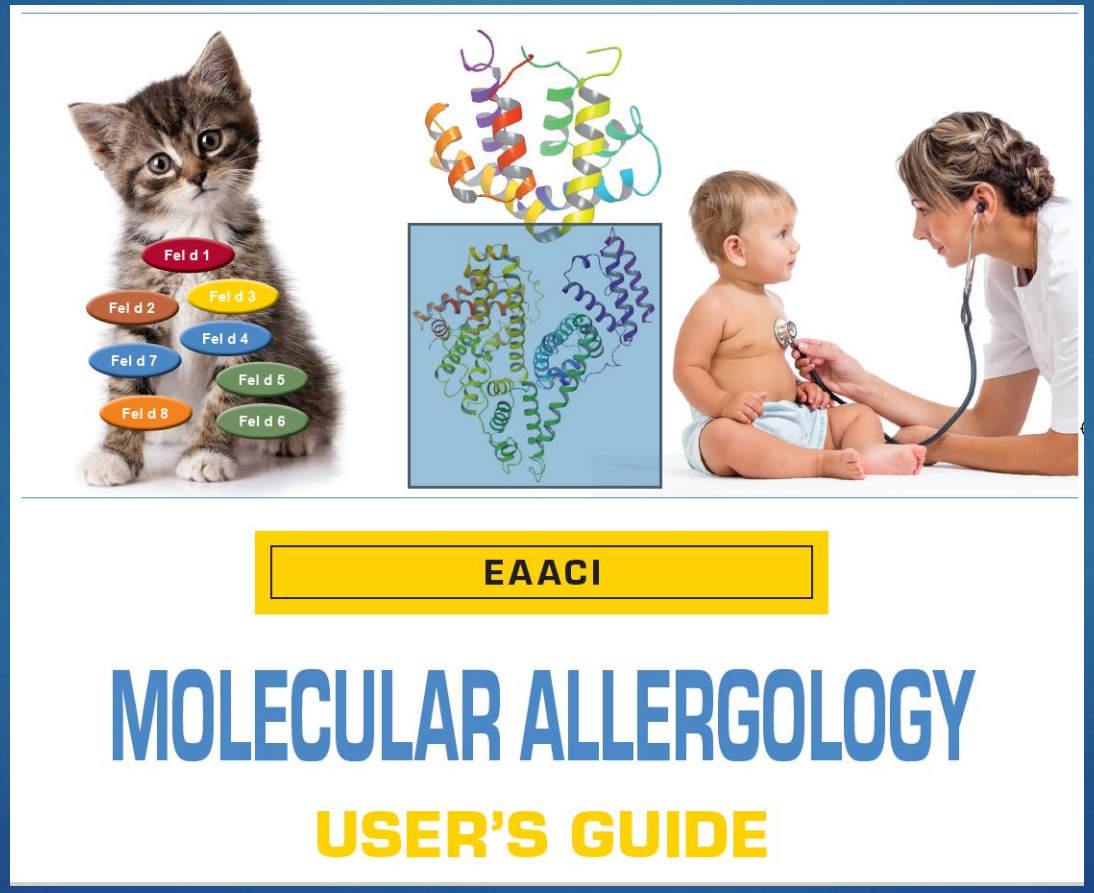
Table 2 The major genuine sensitizers from the relevant allergenic sources

Allergen source	Species	Specific allergen molecules
House dust mite	Dermatophagoides pteronyssinus	Der p 1 Der p 2
	Dermatophagoides Farinae	Der p 23 Der f 1
	Blattella Germanica (cockroach)	Der f 2 Bla g 1
Pet dander	Felis domesticus (Cat)	Fel d 1, Fel d 2
	Canis familiae (Dog)	Can f 1, Can f 3
Grass Pollen	Phleum pratense (Timothy grass)	Phl p 1, Phl p 5, Phl p 6
	Cynodon dactylon (Bermuda grass)	Cyn d 1
	Lolium perenne (Ryegrass)	Lol p 1, Lol p 5
	Poa pratensis (Meadow)	Poa p 1, Poa p 5
	Dactylis glomerata (Cocksfoot)	Dac g 1, Dac g 5
	Holcus lanata (Velvet grass)	Hol l 1, Hol l 5
Tree Pollen	Betula verrucosa (Birch)	Bet v 1
	Olea europoea (Olive)	Ole e 1
	Alnus glutinosa (Alder)	Aln g 1
	Cryptomeria Japonica (Japanese cedar)	Cry j 1
	Cupressus arizonica (Cypress)	Cup a 1
Weed pollen	Ambrosia artemisifolia (Ragweed)	Amb a 1
	Artemisia vulgaris (Mugwort)	Art v 1
	Parietaria judaica (Wall pellitory)	Par j 1 Par j 2
Molds	Alternaria alternata	Alt a1
	Aspergillus Fumigatus	Asp f 1
	Cladosporium herbarum	Cla h 1

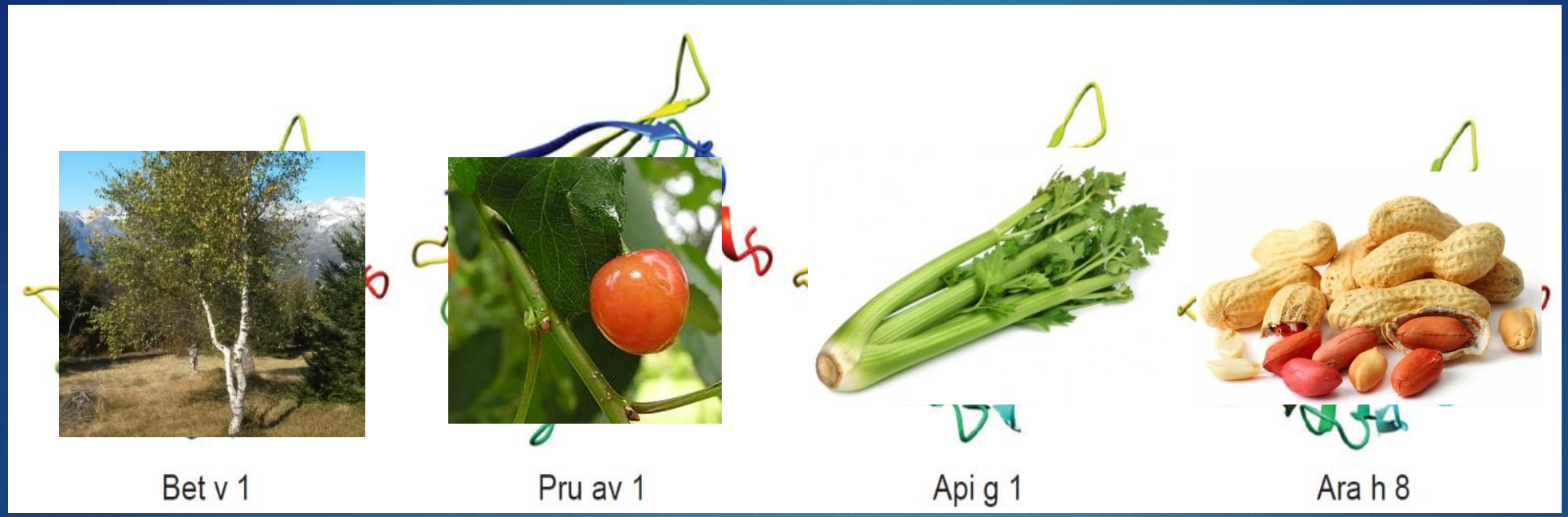
Pajno et al. Italian Journal of Pediatrics (2017) 43:13

Clinical practice recommendations for allergen-specific immunotherapy in child

DA MOLTI A UNO



FAMIGLIA PR-10



- ESPRESSE IN ALTE CONCENTRAZIONI IN VARI FRUTTI E VEGETALI
- Betulla, Prunus, Apium, Arachis

AIT is recommended in allergic rhinitis/conjunctivitis with/without allergic asthma, with an evidence of specific IgE-sensitization towards clinically relevant inhalant allergens

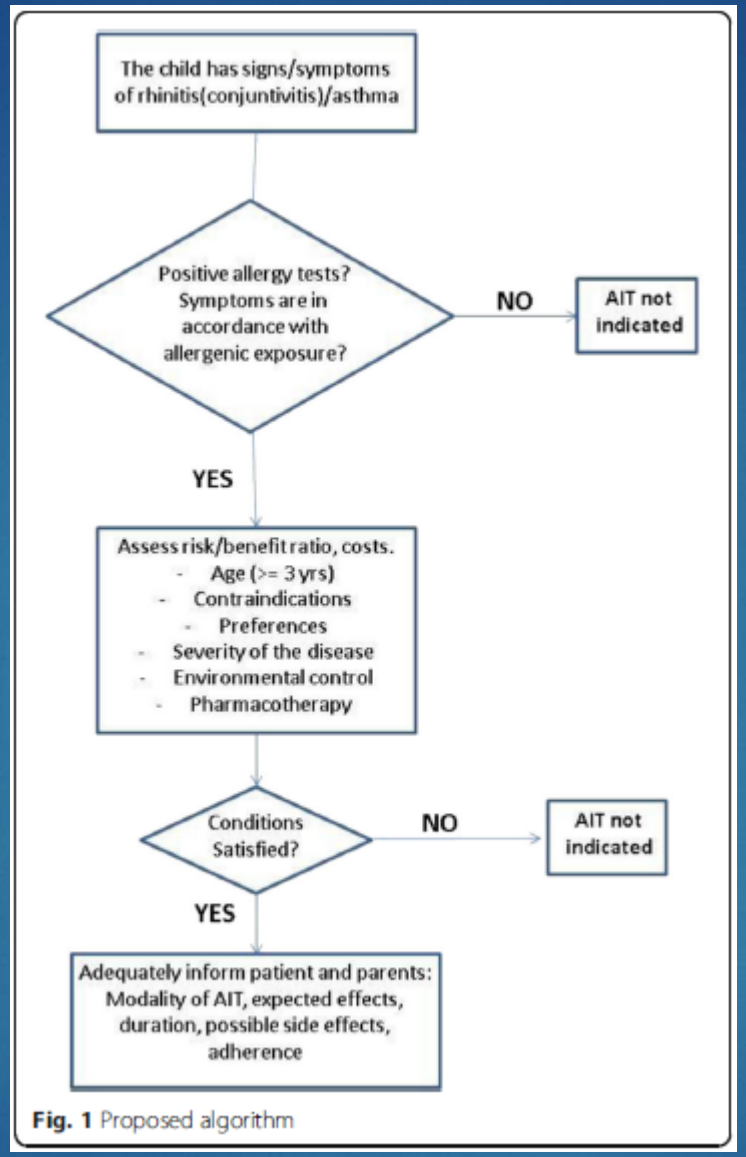


Fig. 1 Proposed algorithm

Pajno et al. Italian Journal of Pediatrics (2017) 43:13

Clinical practice recommendations for allergen-specific immunotherapy in children: the Italian consensus report

TOP-DOWN

- ESECUZIONE DI TEST ALLERGOLOGICI ESTRATTIVI
- VALUTAZIONE DEI POSITIVI
- EVENTUALE APPROFONDIMENTO

BOTTOM-UP

- ESECUZIONE DI TEST ALLERGOLOGICI MOLECOLARI
- VALUTAZIONE DEI POSITIVI
- EVENTUALE APPROFONDIMENTO

MULTIPLEX VS SINGLEPLEX

Singleplex IgE Antibody Assay	Multiplex IgE Antibody Assay
Performance Related Advantages (pro)	
* Increased assay analytical sensitivity (lower Limit of Quantitation, LoQ) * Potentially more precise quantification and precision, facilitating comparisons between different allergen reagents (extracts versus molecules) * More established internal and external quality control measures (proficiency testing)	* Increased speed of analysis and reduced result turn-around time * Conservation of sample volume facilitating pediatric testing
Assay Design and Cost Related Advantages (pro)	
* Traceable of allergen-specific IgE values to a total human IgE International Reference Preparation * Similar units for total IgE and allergen-specific IgE due to heterologous calibration (permits calculation of allergen-specific IgE/total IgE-ratio) * Global availability in many countries * In case of limited number of samples more cost efficient * Minimizes unneeded testing	* Greater simplicity * Reduced cost due to fewer required reagents * Reduced technician intervention * Optimal design applications for point of care tests
Performance Limitations (con)	
More costly due to increased need for reagents More technical intervention Limited answers in case of few samples per subject Expensive in case of large scale screening (i.e. multi-sensitized subjects)	* Potentially lower analytical sensitivity for each analyte specificity measured (higher limit of detection, LoD) * Reduced ability to accurately quantify each IgE antibody * Encouragement of abusive testing which involves the measurement of unwanted or unneeded IgE antibody specificities
Assay Design and Cost Related Limitations (con)	
* More serum required, particularly in case of many samples * Potentially slower analysis * Likely more sophisticated assay format	* Less global availability * Cost of the new instrumentation and reagents * Greater challenge in managing different levels of non-specific binding * Enhanced challenges in optimizing, balancing and standardizing assay reagents and assay quality control * Potential greater inter-lot variability

Hamilton RG **EAACI Molecular Allergology User´s Guide**

Arguments raised pro a TOP-DOWN approach or against a BOTTOM-UP approach.

Extracts are essential screening tools that most doctors are accustomed to; they cannot be removed as routine diagnostics since doctors would loose basic tools for proper allergy work-up.

Microarrays are too complex and detailed; doctors are not yet prepared to interpret them properly.

Microarrays offer useful information – if linked to individual clinical symptoms - but also information on sensitizations not being linked to any symptoms. This extra information can generate conceptual, ethical and legal problemS.

Arguments raised pro a TOP-DOWN approach

or against a BOTTOM-UP approach.

The use of individually selected reagents, covering extracts and molecules for testing, facilitates the use of the singleplex approach to molecular diagnosis, which is inductive and less expensive than the use of microarrays.

Extract-based reagents contain more molecules than those included in a microarray or in catalogues for singleplex tests. Some of these missing molecules are essential for a diagnosis.

SPT based on extracts offer information on biological function of IgE antibodies, not only their presence/absence.

Degranulating cells in polysensitized patients (the vast majority) react to complex protein mixtures in the allergen extract and not only to individual molecules.

Arguments raised pro a BOTTOM-UP approach or against a TOP-DOWN approach

Patients should be examined with a multiplexed approach (microarray) to get a global evaluation of their individual IgE repertoire.

Any step or approach using extracts is outdated and unreliable, because extracts are not always properly characterized and standardized; different extracts tend to give different results leading to different decisions in the same patients.

Patients should be examined with a multiplexed approach (microarray) for a global evaluation of the entire sensitization profile, then the physician should properly address the clinical history, and make a comprehensive evaluation of the atopic conditions.

Positive results in a microarray, even if not clinically relevant at present, may predict future allergic symptoms.

Matricardi P. EAACI Molecular Allergology User´s Guide

Arguments raised pro a BOTTOM-UP approach or against a TOP-DOWN approach

The bottom-up approach, performed with a microarray, would reduce the number of contacts patient-doctor-lab while a singleplex "reflex" approach would require several consultations (and therefore higher costs) before all the molecules relevant for a final diagnosis are tested.

Broader use of microarrays will induce companies to reduce the prices in view of a broader distribution and increase the likelihood of reimbursement from insurance companies and public health systems.

The resistance of allergists to the progress of molecular allergology, which may reduce their earnings based on SPT, should be overcome.

IgE Specifiche E CLINICA

- La presenza di IgE specifiche dimostra sensibilizzazione non allergia
- Un test negativo generalmente esclude l'allergia (VPP circa 90%)

  (Fatto salvo IgE Totali > 20 KU/L e specifiche caratteristiche dell'allergene utilizzato=
- Test fortemente positivi (> 20 IU/ml) correlano con una maggiore probabilità di reazione e non con una maggiore gravità
- L'utilizzo di allergeni molecolari consente di migliorare i tassi di rilevamento delle positività (Tri a 19, hev b 5), di inquadrare meglio le cross-reattività (Bet v 1), di prevedere le reazioni gravi (Ara h 1, 2, 3)

Scott H. PEDIATRICS Volume 129, Number 1, January 2012

Hamilton RG EAACI Molecular Allergology User´s Guide

IgE Specifiche E CLINICA

Sia con un approccio TOP-DOWN che con uno BOTTOM-UP è necessario un attento inquadramento clinico del paziente per verificare, anche mediate test di provocazione, l'effettivo livello di tolleranza all'allergene.

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CELIACHIA

La malattia celiaca è una patologia immuno-mediata che si manifesta in soggetti geneticamente predisposti, a seguito dell'assunzione di alimenti contenenti glutine. Il glutine è un complesso proteico contenuto in frumento, farro, segale, kamut, orzo e altri cereali minori; il glutine si trova quindi in tutti gli alimenti prodotti utilizzando queste farine come pane, pasta, biscotti, pizza etc. Tra i cereali che non contengono glutine ci sono il mais ed il riso.

1. Linee guida per la diagnosi di laboratorio e istologica della malattia celiaca E. Tonuttia et al. RIMeL / IJLaM 2005; 2 110-123
2. Linee guida per la diagnosi ed il monitoraggio della celiachia e relative patologie associate e complicanze. Ministero della Salute Comitato Nazionale Sicurezza Alimentare(C.N.S.A.)

FORMA TIPICA

La forma tipica della malattia si manifesta nel bambino durante lo svezzamento. Con l'introduzione di alimenti contenenti glutine si manifestano sintomi quali diarrea cronica, vomito e ritardo dell'accrescimento dovuto alla malnutrizione. L'addome appare molto dilatato in contrasto con la magrezza di braccia, gambe e glutei.

FORMA ATIPICA

Nella forma atipica si ha una prevalenza dei sintomi extraintestinali, con assenza di diarrea e frequente ricontro di stipsi. Le manifestazioni cliniche includono: bassa statura, anemia da carenza di ferro o di acido folico, rachitismo, osteoporosi, dolori addominali ricorrenti, aftosi recidivante, ritardo puberale, aumento dei valori delle transaminasi, sindromi emorragiche, alopecia. Sono note inoltre **forme silenti e forme latenti**, identificabili solo mediante test diagnostici.

PREVALENZA

La prevalenza della celiachia sia nei bambini che negli adulti è attualmente stimata intorno all'1%. **Ne risulta quindi affetta una persona su cento.** La celiachia è strettamente associata alla presenza dell'antigene HLA DQ2 di classe II e più del 90% dei soggetti celiaci esprime questo marcatore; i pochi pazienti DQ2 negativi sono DQ8 positivi. La prevalenza dell'antigene DQ2 nella popolazione generale europea è però compresa tra il 20% e il 30% e solo una piccola parte di questi individui svilupperà la MC. Il valore predittivo positivo di DQ2, considerando una prevalenza della malattia nella popolazione generale dell'1%, sarebbe quindi compreso tra il 3.0% e il 4.5%, mentre il valore predittivo negativo con DQ8, sarebbe vicino al 100%. **Questo significa che con test genetico negativo è possibile escludere la malattia con alta probabilità, mentre un test genetico positivo consente solamente di stabilire che il soggetto ha una modesta predisposizione a sviluppare la malattia**.

DIAGNOSI DI LABORATORIO

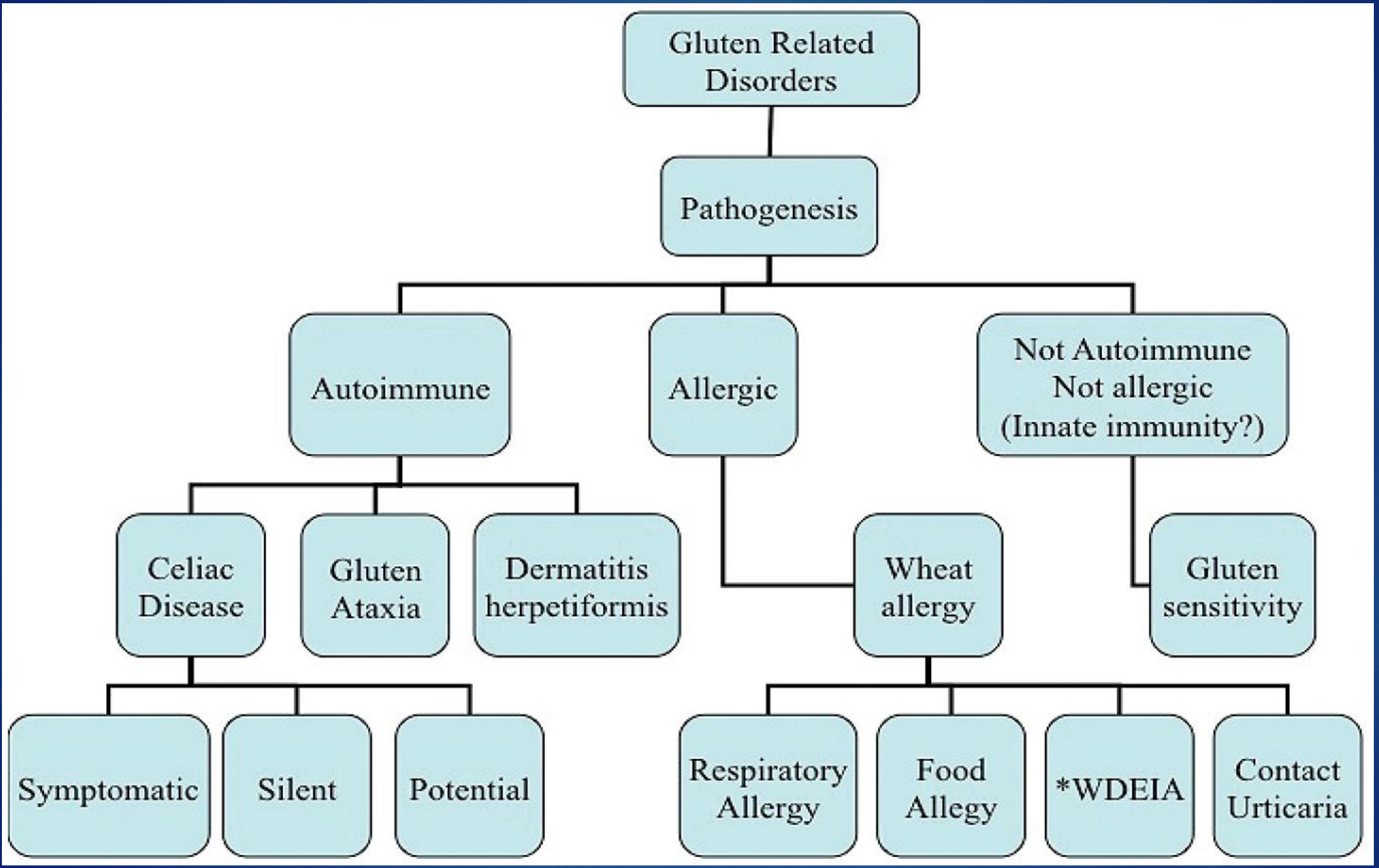
Per i bambini al di sotto dei 5 anni le linee guida suggeriscono la determinazione degli anticorpi **anti Trasglutaminasi IgA (tTG)**, degli anticorpi **anti Endomisio IgA (EMA)** e degli anticorpi **anti Gliadina Deamidati (AGA)**. Deve essere eseguito inoltre il dosaggio delle **IgA totali**, per escludere un deficit che potrebbe rendere negativi i primi due test (< 5mg/dl). In tal caso sono utilizzabili i dosaggi specifici per anti Transglutaminasi IgG e anti Endomisio IgG. In caso di positività agli EMA e ai tTG la diagnosi è praticamente certa. Recenti linee guida consentono di porre diagnosi anche se nza biopsina in specifiche condizioni.

In caso di positività ai soli AGA, in particolare per titoli modesti, è consigliabile un monitoraggio a distanza di qualche mese.

Non-Celiac Gluten Sensitivity

Non-celiac gluten sensitivity (NCGS) is a term that is used to describe individuals who are not affected by celiac disease or wheat allergy yet who have intestinal and/or extraintestinal symptoms related to gluten ingestion with improvement in symptoms upon gluten withdrawal. The prevalence of this condition remains unknown. It is believed that NCGS represents a heterogenous group with different subgroups potentially characterized by different pathogenesis, clinical history, and clinical course. There also appears to be an overlap between NCGS and irritable bowel syndrome (IBS). Hence, there is a need for strict diagnostic criteria for NCGS. The lack of validated biomarkers remains a significant limitation in research studies on NCGS.

Sapone A **PRACTICAL GASTROENTEROLOGY • JUNE 2015**


Gluten Related Disorders
Pathogenesis
Autoimmune
Allergic
Not Autoimmune Not allergic (Innate immunity?)
Celiac Disease
Gluten Ataxia
Dermatitis herpetiformis
Wheat allergy
Gluten sensitivity
Symptomatic
Silent
Potential
Respiratory Allergy
Food Allegy
*WDEIA
Contact Urticaria

PATOGENESI

- The pathophysiology of NCGS remains largely undetermined. A study by Sapone et al. has found that NCGS subjects have normal intestinal permeability compared to CD patients, intact level of protein expression that comprise intestinal epithelial tight junctions and a significant reduction in T-regulatory cell markers compared to controls and CD patients.4 Moreover, NCGS patients have an increase in the a and β classes of intraepithelial lymphocytes (IELs) with no increase in adaptive immunity-related gut mucosal gene expression. These findings suggest an important role of the intestinal innate immune system in the pathogenesis of NCGS without an adaptive immune response.8 Unlike duodenal mucosa from CD patients exposed to gliadin *in-vitro*, intestinal mucosa from NCGS patients do not express markers of inflammation. Newer techniques such as examination of basophil activation in response to gluten or wheat stimulation might suggest alternative pathogenic mechanisms for NCGS.

CLINICA

- The clinical symptoms of NCGS are elicited soon after gluten exposure, improve or disappear with gluten withdrawal and reappear following gluten challenge, usually within hours or days. While this finding could be attributed to a placebo/nocebo effect, the 2011 study by Biesiekierski et al. argues for the existence of a true NCGS disorder. In a double-blind randomized, placebo-controlled study design, the authors found that IBS-like symptoms of NCGS were significantly higher in the gluten-treated group (68%) than subjects treated with placebo (40%).6

SINTOMI

NCGS is dened as a condition in which ingestion of foods containing wheat, rye, and barley leads to one or more of a variety of immunological, morphological or symptomatic manifestations in people in whom CD has been excluded.

The majority of symptoms associated with NCGS are to a certain extent subjective, and may include both gastrointestinal as well as extra-intestinal symptoms: abdominal pain, diarrhea, nausea, headache, "brain fog," tingling and/or numbness in hands and feet, fatigue, and musculoskeletal pain. More severe neurologic and psychiatric conditions including schizophrenia and cerebellar ataxia have also been claimed to be associated with NCGS.

Ludvigsson JF, Leer DA, Bai JC, et al. e Oslo definitions for celiac disease and related terms. Gut 2013;62:43-52.

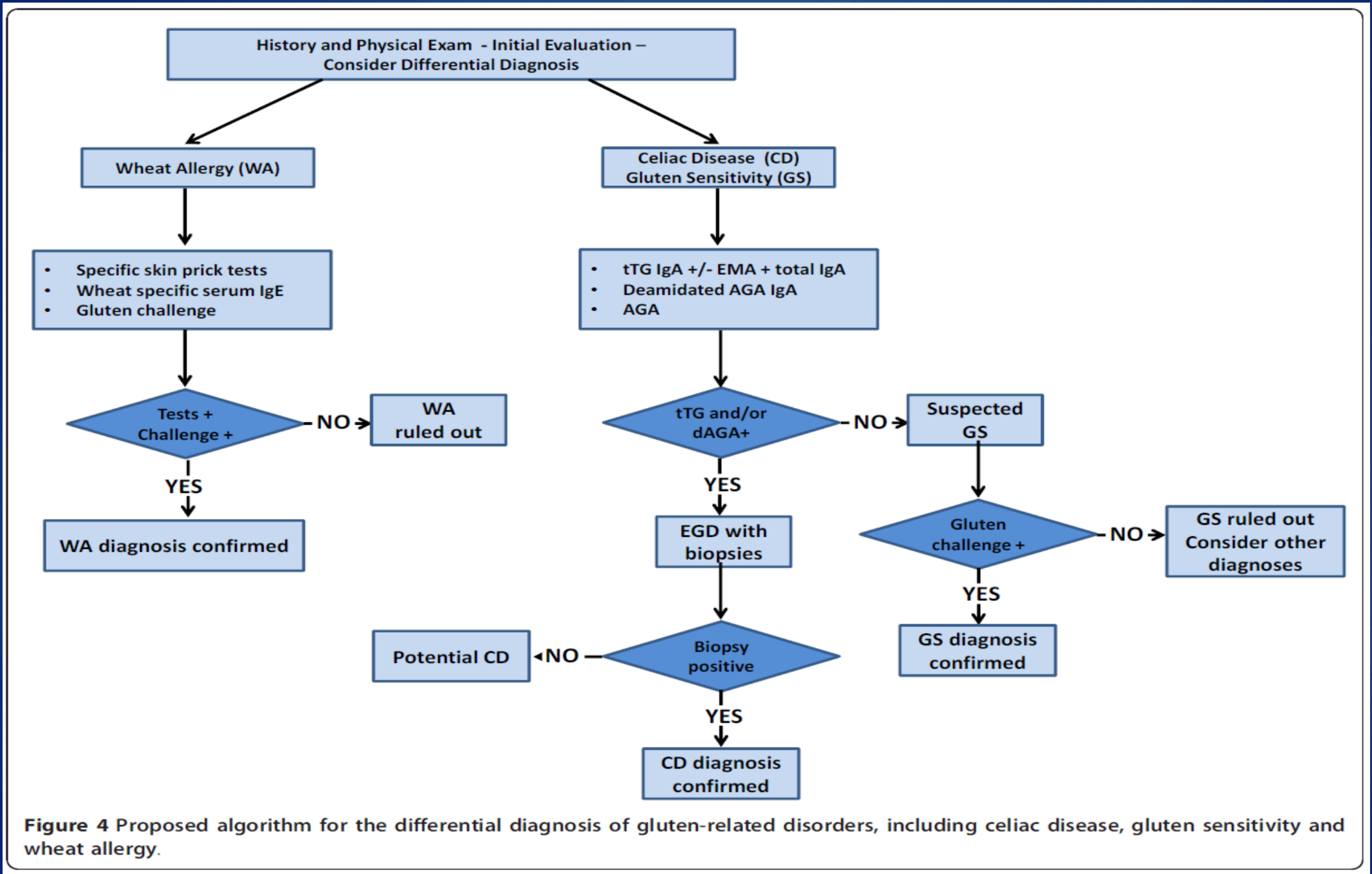


Figure 4 Proposed algorithm for the differential diagnosis of gluten-related disorders, including celiac disease, gluten sensitivity and wheat allergy.

Table 2. Laboratory Criteria for Non-Celiac Gluten Sensitivity (NCGS)

Diagnostic Test	NCGS	CD	WA
Celiac Disease Serology			
Anti-tissue transglutaminase	Negative	Positive	Negative
Anti-endomysial antibody	Negative	Positive	Negative
Anti-deamidated gliadin peptide	Negative	Positive	Negative
Anti-gliadin (IgG) antibody	Positive (~56%)	Positive	Negative
Duodenal Histology	Negative (Marsh 0-1)	Positive	Negative
Other Histologic Findings	Activated circulating Basophils Eosinophilic infiltration in small intestine, colon		
HLA Haplotypes (DQ2 and DQ8)	Absent/Present (50%)	Present	Absent
IgE-based Assays	Negative	Negative	Positive

INTOLLERENZA AL LATTOSIO

Il lattosio è il principale zucchero contenuto nel latte e nei latticini. Nei soggetti non intolleranti il sistema digerente è in grado di scindere il lattosio in glucosio e galattosio due zuccheri semplici assimilabili dall'intestino grazie all'azione dell'enzima **lattasi**. I neonati producono fin dalla nascita questo enzima che consente quindi di nutrirsi del latte materno. In numerose popolazioni però la produzione di lattasi diminuisce con l'avanzare dell'età. Una consistente quota della popolazione adulta italiana presenta quantità molto basse di lattasi nel proprio intestino.

Lactose Intolerance Vesa T., Marteau P., Korpela R. J.
Am. Coll. Nutr. (2000) 19; 165S-75S.

SINTOMATOLOGIA

In questi soggetti, definiti intolleranti, il lattosio non viene digerito e prosegue il proprio transito intestinale senza essere assorbito. Giunto nel colon, la flora microbica locale lo fermenta con produzione di gas (idrogeno, metano, ed anidride carbonica), dando origine ai tipici **fenomeni dell'intolleranza al lattosio (meteorismo, flatulenza, nausea e dolori addominali).** Poiché questi sintomi sono riconducibili ad un ampia varietà di intolleranze e disturbi gastrointestinali la diagnosi di intolleranza al lattosio può essere posta solo in presenza di un test specifico. Il latte infatti costituisce un prezioso nutriente per gli adulti, in particolare in età avanzata, grazie all'elevato contenuto di calcio e la privazione immotivata deve essere evitata.

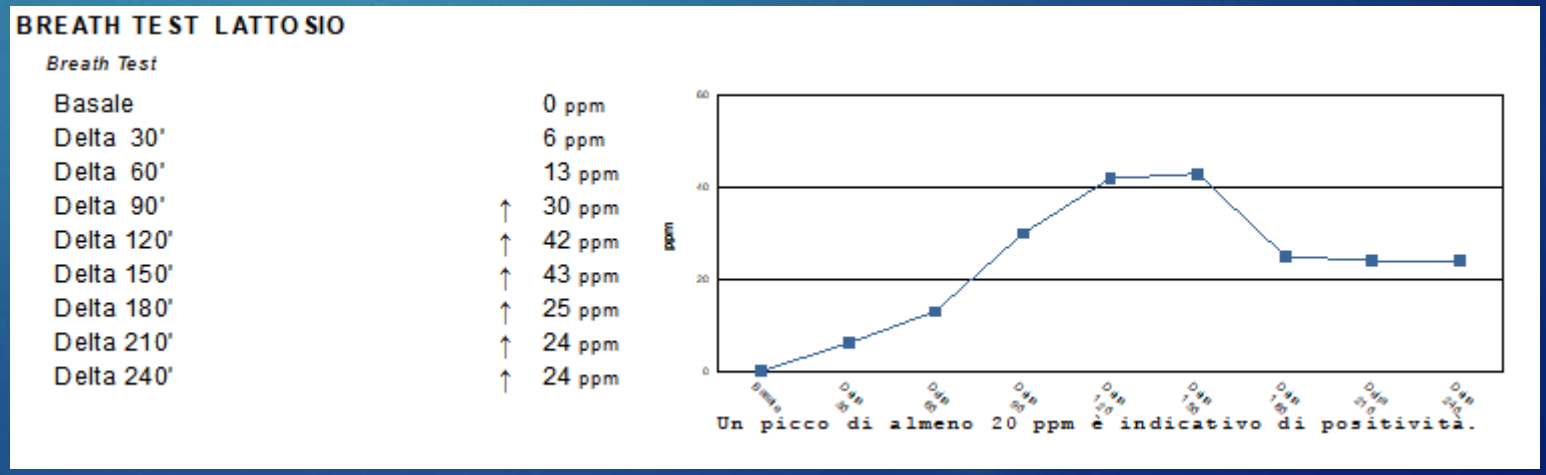
DIAGNOSTICA

L'incapacità di digerire il lattosio può essere semplicemente diagnosticata con l'ausilio di un ***Breath Test* al lattosio** (letteralmente "Test del respiro"). Il test si esegue somministrando al paziente 20 gr di lattosio disciolti in acqua. In presenza di una scarsa digestione dello zucchero i batteri normalmente presenti nell'intestino fermenteranno il lattosio. I gas prodotti saranno in parte assorbiti dal flusso sanguigno e riemessi nell'espirato. Il test si basa sulla ricerca nell'espirato della forma molecolare dell'idrogeno (H2) in maniera da massimizzare la sensibilità e la specificità.

Gasbarrini A, Corazza GR. Methodology and indications of H2-Breath testing in Gastrointestinal Diseases : the Rome Consensus Conference . Alimentary Pharmacology &Therapeutics .ol 29, suppl 1 May 2009.

BREATH TEST

Il test prevede la misura della concentrazione di H2 per 4 ore con raccolte di espirato ogni 30 minuti in maniera da poter valutare efficacemente anche i pazienti con transito alterato. Nei pazienti con intolleranza al lattosio si osserva, tipicamente entro 120 minuti, un innalzamento della concentrazione di H2 nell'espirato, che si protrae per circa un ora. Il test si considera positivo se la concentrazione di H2 supera di 20 ppm il livello basale misurato prima della assunzione di lattosio.



Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus

Rezaie A The North American Consensus group on hydrogen and methane-based breath testing

Table 1. Preparation before breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We recommend that antibiotics should be avoided for 4 weeks prior to the breath test.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊕
2. A firm position statement cannot be reached due to lack of conclusive data on stopping or continuing pro/prebiotics prior to breath testing.	Uncertain (44.4% agree, 44.4% uncertain, 11.1% disagree)	⊕⊙⊙⊙
3. We suggest that, if tolerated by the patient, promotility drugs and laxatives should be stopped at least 1 week prior to breath testing.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊙⊙⊙
4. We suggest that fermentable foods such as complex carbohydrates should be avoided on the day prior to breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
5. We suggest that the fasting period for breath testing as part of preparation should be 8–12 h.	Agree (77.8% agree, 0% uncertain, 22.2% disagree)	⊕⊕⊙⊙
6. We recommend that smoking should be avoided on the day of breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊕
7. We recommend that physical activity should be limited during breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊕
8. We suggest that it is not necessary to stop proton pump inhibitors prior to breath testing.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙

Table 2. Indications for breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. Current small bowel culture techniques are not satisfactory for the assessment of SIBO.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
2. If culture is considered for diagnosis of SIBO, based on the current evidence, we suggest the threshold of $>10^3$ c.f.u./ml for the definition of SIBO	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙
3. We suggest breath testing in the diagnosis of small intestinal bacterial overgrowth.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
4. Until a true gold standard is established, we suggest breath testing in assessing the presence of antibiotic-responsive microbial colonization of the gastrointestinal tract.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊕⊙
5. We suggest to evaluate for excessive methane excretion on breath test in association with clinical constipation and slowing of gastrointestinal transit.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊙
6. We suggest that breath testing should not be used for assessment of orocecal transit time.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊕⊙
7. We suggest breath testing for the diagnosis of carbohydrate maldigestion syndromes.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊕⊙
8. We suggest breath testing in the assessment of conditions with bloating.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊙⊙

Table 3. Performance of breath tests

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We suggest that the correct dose of lactulose for breath testing is 10 g with or followed by one cup of water.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
2. We suggest that the correct dose of glucose for breath testing is 75 g mixed with or followed by one cup of water.	Agree (88.9% agree, 11.1% uncertain, 0% disagree)	⊕⊕⊙⊙
3. We suggest that the correct dose of lactose for breath testing is 25 g mixed with or followed by one cup of water.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
4. We suggest that the correct dose of fructose for breathe testing is 25 g mixed with or followed by one cup of water.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊕⊙
5. We suggest that fructose and lactose breath test should be performed for at least 3 hours.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
6. We suggest that the presence of bacterial overgrowth should be ruled out before performing lactose or fructose breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊕⊙
7. We recommend that hydrogen, methane and carbon dioxide should all be measured simultaneously during breath testing.	Agree (77.8% agree, 22.2% uncertain, 0% disagree)	⊕⊕⊕⊕

Table 4. Interpretation of breath testing

Consensus statement	Percentage of agreement	Quality of evidence (GRADE)
1. We suggest that a rise of ≥20 p.p.m. from baseline in hydrogen during the test should be considered positive for fructose and lactose breath testing.	Agree (100% agree, 0% uncertain, 0% disagree)	⊕⊕⊙⊙
2. We suggest that until better data are available, for clinical and research purposes, a rise of ≥20 p.p.m. from baseline in hydrogen by 90 min should be considered a positive test to suggest the presence of SIBO.	Agree (77.8% agree, 11.1% uncertain, 11.1% disagree)	⊕⊕⊙⊙
3. We suggest that two peaks on breath test are not required for the diagnosis of SIBO.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
4. Until further data is available, we suggest that a level of ≥10 p.p.m. be considered positive for methane on a breath test.	Agree (88.9% agree, 0% uncertain, 11.1% disagree)	⊕⊕⊙⊙
5. A firm position statement cannot be reached due to lack of conclusive data on the definition of abnormal methane on to be ≥3 p.p.m.	Uncertain (44.4% agree, 44.4% uncertain, 11.1% disagree)	⊕⊕⊙⊙

WHAT IS CURRENT KNOWLEDGE

- ✓ Breath testing represents an important, simple and safe test to diagnose carbohydrate maldigestion syndromes and small intestinal bacterial overgrowth (SBIO).
- ✓ There is significant heterogeneity in test performance/preparation, the indications for breath testing and the interpretation of results.

WHAT IS NEW HERE

- ✓ Consensus doses for lactulose, glucose, fructose and lactose breath tests are 10, 75, 25 and 25 g, respectively.
- ✓ Breath testing is useful in the diagnosis of carbohydrate maldigestion, methane-associated constipation but not in the assessment of oro-cecal transit.
- ✓ For glucose or lactulose breath tests for SIBO, a ≥20 p.p.m. rise in hydrogen by 90 min is considered positive.
- ✓ Methane levels ≥10 p.p.m. are considered methane-positive.
- ✓ For assessment of carbohydrate maldigestion, a rise in hydrogen of ≥20 p.p.m. above baseline during breath testing is considered positive.