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Lectures

# Selected Lectures of the Workshop of Pediatrics in collaboration with the Italian Society of Pediatric Allergology and Immunology (SIAIP)

## CAGLIARI (ITALY) · OCTOBER 20TH, 2023

The Meeting "Workshop of Pediatrics" is a Satellite Meeting of the 19th International Workshop on Neonatology and Pediatrics, Cagliari (Italy), October 18th-21st, 2023. The Meeting has been organized in collaboration with the Italian Society of Pediatric Allergology and Immunology (*Società Italiana di Allergologia e Immunologia Pediatrica* – SIAIP).

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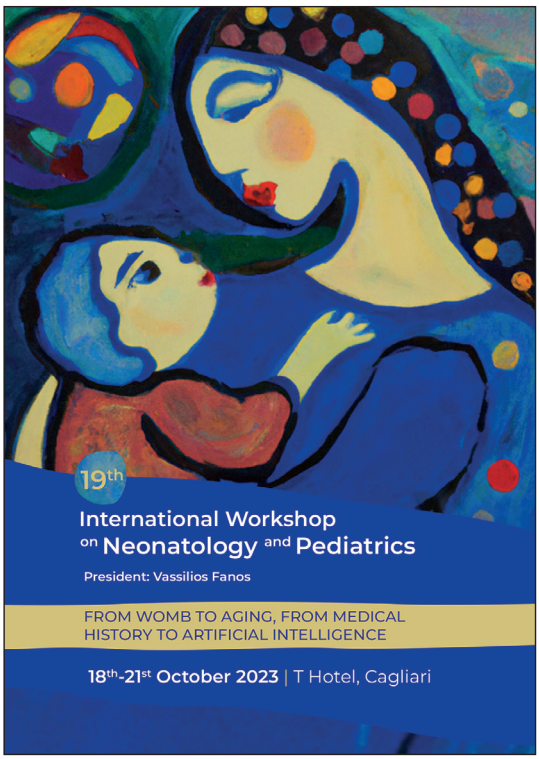


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**LECT 1**

## LONG SHADOW OF COVID-19 IN CHILDREN AND ADOLESCENTS

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For over 3 years, all of humanity has been facing the COVID-19 pandemic. The knowledge accumulated to date regarding SARS-CoV-2 infection is insufficient and there are many questions that await answers, but what is seemingly obvious is that there is a low incidence and low severity of infection with COVID-19 in children. Most children with SARS-CoV-2 infection are asymptomatic or have mild symptoms and usually recover without sequelae. Morbidity and mortality from COVID-19 are very low in children compared to adults [1]. However, in rare cases, children may also develop severe forms of the disease. The long-term effects of the novel coronavirus infection in the pediatric population began to be noticed and subsequently mentioned in literature starting mid-2021. Almost all published studies on "long COVID" in this age group have considerable limitations. Among these, we mention: including children without confirmation of SARS-CoV-2 infection in the study groups, the lack of appropriate control groups, etc. [2, 3]. The most common long-term complications of SARS-CoV-2 infection in children are: myocarditis, multisystem inflammatory syndrome in children/pediatric inflammatory multisystem syndrome (MIS-C/PIMS) and long COVID. Infection with SARS-CoV-2 is associated with a 30-fold increased risk of myocarditis in children under 16 and 16-fold in the general population, according to data from the Centers for Disease Control and Prevention (CDC). Furthermore, scientific data suggest that myocarditis in the context of SARS-CoV-2 infection, both in severe COVID-19 disease and in multisystem inflammatory syndrome, presents a much higher risk than that derived from vaccination with a COVID-19 mRNA vaccine.

The definition of long COVID varies according to the different sources (National Institute for Health and Care Excellence [NICE], World Health Organization [WHO], CDC). An important element is the duration of symptoms, which varies between 4 and 12 weeks after the acute infection.

Thus, the definition developed by NICE [4] mentions the presence of signs and symptoms lasting for more than 4 weeks after infection with SARS-CoV-2. The definition makes a distinction between:

- symptomatic COVID-19: signs and symptoms of COVID-19 disease lasting from 4 to 12 weeks;
- post-COVID syndrome: signs and symptoms that develop during or after an infection that are consistent with the definition for COVID-19 disease; they persist for > 12 weeks and are not explained by another diagnosis.

The definition used by the WHO [5] specifies that the symptoms of long COVID occur in people with a history of probable or confirmed SARS-CoV-2 infection with symptoms that last ≥ 2 months and cannot be explained by an alternative diagnosis.

A person of any age who has had SARS-CoV-2/COVID-19 infection may later develop a post-COVID condition, even if the acute illness was mild or asymptomatic. Severe COVID-19 disease is rarely found in children compared to adults. However, there are 2 possible long-term consequences in children: long COVID and MIS-C/PIMS.

The symptoms of long COVID are diverse and very heterogeneous. Thus, the patient may exhibit: fatigue, breathing difficulties, myalgia, headache, cognitive dysfunction, thromboembolic events, gastrointestinal disorders and MIS-C (in patients aged < 21 years) [5-7]. Symptoms generally have an impact on daily activity; they may start following an initial recovery from the COVID-19 disease or they can persist after the initial episode; in addition, symptoms may fluctuate or recur over time [5]. Evidence regarding the prevalence and spectrum of symptoms related to the post-COVID status in children, especially in the very young, is limited as a result of: the inability of young children to verbalize their symptoms, the small number of clinical studies that included children, the absence of control groups, the presence of symptoms in children without knowing the status of SARS-CoV-2 infection. In an article published by Stein et al. [1], it is stated that the incidence of symptoms suggesting long COVID can reach up to 30%, depending on the population evaluated, data collection method and time elapsed

since the acute illness. In another online surveillance study undertaken in the UK on a cohort of 297,743 people aged over 2 years (by self-report or parental reporting of long-term COVID symptoms) it was observed that 4 weeks after the acute illness, 0.2% of children between the ages of 2 and 11 and 0.9% of those aged 12-16 showed symptoms of long-term COVID [1]. A study conducted in Israel on a group of 13,834 children with proven SARS-CoV-2 infection showed that residual symptoms after acute infection, at 6 months, were found in 1.8% to 4.6% of patients [1]. The top 5 clinical manifestations of long COVID in children and adolescents were mood disorders (16.50%), fatigue (9.66%), sleep disorders (8.42%), headache (7.84%) and respiratory disorders (7.62%) [8].

Although SARS-CoV-2 infection is relatively mild in children compared to adults, a multisystemic hyperinflammatory syndrome continues to be reported in this population. MIS-C/PIMS associated with SARS-CoV-2 infection was first described in April 2020 in the UK [9, 10]. Clinical manifestations occur approximately 2-4 weeks after infection with SARS-CoV-2 and include: persistent fever, gastrointestinal signs and symptoms, muco-cutaneous and cardiac symptoms, and elevated inflammatory markers [10]. Some signs and symptoms are similar to those seen in Kawasaki disease, toxic shock syndrome, and/or acute COVID-19 disease. 26 references published in the last 2 years document 1,136 cases of MIS-C with a mean age of 6-11 years [11]. At the end of October 2022, the total number of MIS-C patients meeting the case definition in the US was 9,006 [11]. More than half (56%) of these cases were found in boys. Most children who developed this long-term complication of SARS-CoV-2 infection had no comorbidities [1]. 53-80% of children diagnosed with MIS-C had cardiac involvement and 20% required mechanical ventilation [1]. Children with MIS-C are at increased risk for developing prolonged signs/symptoms. Thus, at 6 months, persistent symptoms were reported by 35% of the children who presented MIS-C and 21% of their parents [12].

In conclusion, it can be stated that post-COVID manifestations in children seem to be less frequent compared to adults. A UK survey found that 7-8% of children with COVID-19 disease reported ongoing symptoms > 12 weeks [13]. Manifestations suggesting long COVID can also occur after mild infections up to severe forms of the disease and after MIS-C. The most common symptoms include fatigue, headache, insomnia, difficulty concentrating, muscle and joint pain, and cough [14, 15]. Prolonged persistence of symptoms has an important impact on the quality of life of the little patients (limitations of physical activity, a feeling of suffering due to symptoms, mental health issues, school absenteeism).

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## LECT 2

## A "BREATH OF HOPE": NEW INSIGHTS ON PEDIATRIC SEVERE ASTHMA IN THE OMICS ERA

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### INTRODUCTION

Severe asthma is a life-threatening, medical-costly disease, impacting 2-5% of children and adolescents globally. This condition includes difficult-to-treat asthma and severe therapy-resistant asthma (STRA), depending on whether or not there are coexisting treatable traits [1]. In pediatrics, STRA exhibits distinct features and specific challenges. Omics sciences have played a crucial role in shedding light on these aspects, with the promise of further insights in the future.

### NEW INSIGHTS ON: PATHOGENESIS

Recent research into STRA genetics and the collective effects of multiple environmental stimuli globally called exposome, has significantly reshaped our comprehension of asthma's pathogenesis, leading to the epithelial barrier hypothesis. Accordingly, genetic predisposition, airways dysbiosis and environmental factors adversely affect the airway epithelium, resulting in reduced repair capabilities and heightened permeability to harmful or allergenic stimuli [2]. Notably, the airway epithelial cells may undergo a specific pre-commitment, initiating either a type 2-high or type 2-low local response [2]. Such response involves both the innate and the adaptive immunity, resulting in different STRA endotypes. However, the longitudinal stability of those endotypes and their clinical significance in the pediatric setting have yet to be clarified.

### NEW INSIGHTS ON: BIOMARKERS

Exploring biomarkers is a thriving field in pediatric STRA. In fact, biomarkers offer affordable, specific, non-invasive tools helping to assess the long-term response to anti-asthmatic therapies, the early identification of incoming asthma attacks or the definition of STRA endotypes needing early additional treatments. Classic asthma biomarkers include the blood and sputum eosinophilia, the serum immunoglobulin E (IgE) levels and the fractional exhaled nitric oxide (FeNO). However, recent years have seen the ongoing discovery of numerous new biomarkers. These encompass urinary metabolites, exhaled breath condensate (EBC) compounds, products of oxidative reactions and multi-transcriptome analysis across various biological specimens [3]. Currently, the application of these new biomarkers is largely restricted to research settings. Since a single biomarker alone is not sufficient for pinpointing distinct asthma endotypes and outcomes, further studies are needed, focusing on the use of multiple omics panels.

### NEW INSIGHTS ON: THERAPIES

Recent improvements in understanding the pathogenesis of STRA have resulted in the introduction of the new concept of theratypes. Theratypes are pragmatically defined based on the patient's real clinical response to specific therapies. Using such theratypes in managing pediatric STRA will facilitate therapeutic adjustments, ultimately reducing asthma costs and side effects.

### CONCLUSIONS

The synergistic application of different omics sciences will enable the personalized care of children and adolescents with STRA, facing their individual needs.

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## LECT 3

## THE "OMNIPOTENT" VITAMIN D

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Several studies have pointed out that vitamin D plays an important role in the general regulation of the immune system. Many organs express vitamin D receptors. Examples are T and B lymphocytes, monocytes, antigen-presenting cells (APCs) including macrophages and dendritic cells. Vitamin D, therefore, exerts its effects on the immune system, especially increasing the expression of catelicidines, an important defense factor against pathogens of the respiratory tract. The antiallergic effects of vitamin D are due to the action on dendritic cells, favoring the production of IL-10 and reducing the production of IL-12. As for the relationship between vitamin D and wheezing, a prenatal deficiency of vitamin D predisposes to both wheezing and subsequent asthma, negatively affecting the development of the lung and the immune system. An adequate intake of vitamin D during pregnancy shows a protective action.

In conclusion, recent evidences suggest a positive immunomodulating action of vitamin D, in particular when administered in the newborn and in the toddler. It is important for pediatricians to pay attention to the levels of vitamin D in allergic and asthmatic children for an early supplementation.

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## LECT 4

## CORRELATION BETWEEN ORAL AND LUNG MICROBIOTA

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Probiotics are live and viable microorganisms, capable of conferring health benefits on the host when taken in adequate quantities. Efficacy, stability and safety are the key characteristics to ensure that a given strain can be used in a context of prevention and therapeutic support; further characteristics necessary for correct use are given by resistance and balance with the external environment and the absence of transmission of antibiotic resistance. The available strains are multiple and distinguishable according to the organ target: most are represented by strains with intestinal action, while others are strains with non-intestinal action, which act at the organ level. The evidence of action of the strains with systemic involvement, with an influence on the oral microbiota and on respiratory infections, mainly concerns *Lactobacilli* and *Bifidobacteria*, which colonize the intestinal tract during administration. Some studies have shown how protracted treatment is often able to modify, even if transiently, the proportions of microbiological genera at the level of the oral cavity, with an increase in saprophytic antagonists of cariogenics and a reduction in the pro-inflammatory component, especially spindle bacteria and *Prevotella spp.* It would also seem that the use of specific strains of probiotics is able to prevent infections, especially in preschool age, both gastrointestinal and respiratory, even if the indications are controversial [1, 2]. The action of strains not obtained from intestinal derivation and not necessarily colonizing the intestine, can offer the host an advantageous action of "bacterial interference" at the organ level, the result of a bactericidal and/or competitive action receptor against microorganisms colonizing the same tissue. Colonization of organs (i.e., the oral cavity), with commensal and non-pathogenic agents able to produce particular substances such as, for example, bacteriocins with natural antibiotic action, can represent a real prophylaxis measure to prevent the

infectious disease from manifesting itself and above all recurring periodically. In this regard, a well-characterized example in the literature is provided by the *genus Streptococcus salivarius*, whose strains (15 reported in the literature) are saprophytes constituting the oral cavity microbiota and are the dominant members from birth [3]. Some strains are able to produce bacteriocins, which not only represent a defense mechanism, antagonizing the growth of pathogens for the host, but also stabilize the oral microbiota "system". It follows that the treatment with probiotics of this type will be able to lead to a non-intestinal colonization, which would determine a local competition with the pathogens and which can be proposed as a prophylaxis for recurrent respiratory pathologies, with less use of antibiotic therapy over time.

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## LECT 5

## INSECTS IN THE DISH: FUTURE OR PRESENT?

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The risks associated with the consumption of insect proteins can be of various kinds [1]. The microbiological risk is related to the possibility that insects act as vectors for some microorganisms. The chemical risk depends on the hypothesis that insects may be carriers of pesticides or other environmental contaminants. The physical risk occurs exclusively for workers in companies that produce insects for food use. The allergic risk involves children also following the intake of allergenic proteins in food. Indeed, the marketing of partially defatted cricket flour and mealworms has recently been authorized. If food production is to be achieved using current techniques, increased emissions of greenhouse gases and ammonia, deforestation, soil erosion, loss of plant biodiversity and water pollution are set to continue. For these reasons, it is essential to find food sources that can have a minimal environmental impact. Some insects can satisfy these requirements. It is estimated that the consumption of insects is regularly practiced by at least 2 billion people worldwide. The consumption of insects could lead to the onset of food allergies even in our population, which usually does not eat them. We know that in some Countries, where insect consumption is very common, high reactivity frequencies are reported. For example, in China, 18% of food-induced anaphylaxis reactions are caused by the ingestion of insects [2]. Now, the sale of foods based on insect proteins may potentially lead to allergic reactions. In our Country, the risk of food allergy for insects is evaluated for 2% of Italian citizens. For this reason, about 800,000 patients, with the allergy to dust mite, seafood and shellfish, can cross react with the tropomyosin that is present in insects, also. To protect people at risk, it is important that the commercial food reports specific information clearly, regarding the presence of insect proteins, before being placed on the sales shelves. The presence of cricket flour must be shown clearly by a small graphic representing an insect or by the scientific name of the cricket: "*Acheta domesticus*". But in which children should we think that the clinical symptoms may depend on an allergy to insect proteins? The diagnosis should be suspected in non-EU children especially, with sensitization to tropomyosin and who maintain, at least in part, their previous eating behaviour, through the purchase of food containing insects in ethnic stores. The molecular diagnostics aimed at identifying specific serum IgE allows the diagnosis of sensitization to insect proteins. The IgE to be identified is directed towards tropomyosin and arginine kinase. The tropomyosin is a protein highly resistant to heat and the various types of food preparation are not able to modify it in the slightest [3]. The arginine kinase is susceptible to heat treatment or thermal food processing and is less heat resistant than tropomyosin.

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## LECT 6

# TUBERCULOSIS IN CHILDREN: A FORGOTTEN DISEASE?

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## INTRODUCTION

Even today, about 1.1 million children get tuberculosis (TB) each year; they represent 1.1% of the entire population. It is currently considered one of the top 10 causes of death in childhood.

During the COVID-19 pandemic in 2020, there was a sharp decline in access to diagnostic and treatment services (63%) of children aged 5-15 years and a sharp reduction (72%) in prophylaxis for TB infection in children aged < 5 years. The reduction in access to hospital facilities consequently led to a significant increase in new cases in subsequent years and deaths [1].

Children are more vulnerable to TB infection because of their immunological immaturity characterized by reduced defense of pulmonary innate immunity. Children < 2 years of age are more susceptible to infection and more severe forms of the disease; about 80% of pediatric deaths occur in this age group.

## DIAGNOSIS

Even today, diagnosis in children is often difficult, as the signs and symptoms, especially in the early years of life, may be confused with other diseases. Diagnosis is based on:

1. history;
2. clinical criteria;
3. bacteriological tests;
4. skin tests;
5. new diagnostic tests;
6. radiological investigations.

### *History*

Clinical history of contact with known cases of TB allows suspicion of infection or disease.

### *Clinical criteria*

In children, and particularly those < 2 years of age, symptomatology is often unspecific and easily confused with other diseases of bacterial, viral or fungal etiology, making diagnosis more difficult. Simultaneity of certain symptoms such as (a) persistent cough, (b) listlessness, (c) weight loss have predictive value in 80% of children with pulmonary TB. Pulmonary localization appears to be the most frequent (71.8%), while extrapulmonary localization is present in about 20-30% of cases and includes localization of infection in different districts: central nervous system (meninges, parenchyma, spinal cord), lymph nodes (lymphadenitis present in 30-40% of cases in laterocervical, supraclavicular, axillary, mediastinal, abdominal sites), skeleton (spondylitis, osteomyelitis arthritis), genitourinary system (present in 3% of cases, expression of disease reactivation), kidney (presence of pyuria in sterile urine), gastrointestinal system, skin (scrofuloderma).

### *Bacteriological tests*

The diagnostic gold standards are definitely *Mycobacterium tuberculosis* (MTB) culture and polymerase chain reaction (PCR), but the difficulty in obtaining appropriate specimens in children for examination for different reasons is the major obstacle to this investigation, as the disease is often paucibacillary and children unlike adults hardly produce sputum and in them the use of bronchoscopy has significant limitations.

### *Skin tests*

The conventional skin test (Mantoux) is certainly the oldest test that has enabled both diagnostic and epidemiological screening. It is simple to perform and requires only minimal operator attention. Positivity appears 2-10 weeks after the onset of infection and is considered positive in relation to the size of the pompho.

### *New diagnostic tests*

Recently, 3 new tests have been developed for the diagnosis of MTB:

a. Diaskintest®;
b. C-Tb skin test;
c. Ec-test.

These tests are based on the presence of 2 MTB antigens: CFP-10 and ESAT-6; they have the advantage over the traditional skin test of differentiating between infection-positive and vaccination-positive.

Interferon-gamma release assay (IGRA) tests have been widely used in the last decade.

Two second-generation tests, QuantiFERON®-TB Plus and T-SPOT®.TB, are currently on the market, and differ from their predecessors in that they use new antigens that can stimulate both CD4 and CD8+.

Recentrly, new diagnostic tests have been developed:

- MTB nucleic acid amplification test (NAAT) and the Xpert® MTB/RIF Ultra test, based on the rapid determination of MTB DNA in the test sample;
- the lateral flow urine lipoarabinomannan (LF-LAM) test, based on the identification of the

LAM lipoxacaride antigen present on the MTB cell wall.

THERAPY

The main goals of therapy are to prevent progression from infection to disease and to cure the active form, in view of the fact that the risk of the onset of severe forms remains high even 2 years after infection and is particularly high in children < 2 years of age.

Children, compared with adults, have fewer therapy-related side effects; the risk of hepatoxicity is higher only in obese children (hepatic steatosis) and those on anticonvulsant drug therapy.

Recently, it has been recommended to increase the dosage of some of these drugs: isoniazid 10-15 mg/kg, rifampicin 10-20 mg/kg, pyrazinamide 30-40 mg/kg, ethambutol 15-25 mg/kg [1].

Resistance to antitubercular drugs is rare in children; it is estimated that about 25,000-32,000 children worldwide may develop drug resistance each year (2%).

Several studies conducted in Europe have shown that increased immigration has coincided with an upswing in resistant TB cases.

Recently, the WHO has authorized the use of 2 new drugs: bedaquiline and delamanid. Both are bactericidal agents, the former can be used in children of all ages for 9-12 months, the latter is recommended for 6 months and can be used for < 18 months maximum.

VACCINES

Today, several new vaccines derived from killed *Mycobacteria*, live attenuated vaccines by genetic modification, and recombinant vaccines with viral vectors that have yet to complete the testing cycle to be approved, which are expected to replace the traditional BCG vaccine, are in trials.

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## LECT 7

## CYSTIC FIBROSIS: FROM CLINIC TO METABOLOMICS

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Cystic fibrosis (CF) is an autosomal recessive inherited disease caused by a mutation in the CF transmembrane conductance regulator (*CFTR*) gene, which codes for the protein of the same name, whose main function is to act as a chlorine channel. Dysfunction of this protein is responsible for the increased density and viscosity of the mucous secretions of the secreting epithelia. It is a chronic and progressive disease with multi-organ involvement and an inauspicious prognosis. The mutations causing CF are extremely numerous, and this means that the disease can express itself with equally variable clinical pictures, ranging from very severe to paucisymptomatic or even asymptomatic forms. The classic phenotype is represented by chronic developmental bronchopneumopathy, exocrine pancreatic insufficiency and elevated electrolyte levels (Na and Cl) in sweat. In Sardinia, the most frequent mutations are the T338I mutation and the F508del mutation. The latter is responsible for severe clinical pictures, when in homozygosis or compound heterozygosis with another severe mutation, characterized mainly by chronic developmental bronchopneumopathy and pancreatic insufficiency. In contrast, patients with the T338I mutation, whether in homozygosity or compound heterozygosity (even with severe mutations, such as F508del), present a mild clinical picture with no or mild lung involvement and normal pancreatic function. We studied subjects with CF who carry the two most frequent mutations in Sardinia, investigated the urinary metabolome of homozygous T338I, homozygous F508del and compound heterozygous F508del/T338I patients, with the aim of identifying the metabolic differences and metabolites characterising these populations and defining any correlation between the urinary metabolomic profile, genotype and phenotype. Metabolomics, by identifying metabolic profiles that are the end product of the interaction between genetic and environmental factors, allows the molecular complexity of a disease to be investigated and is, therefore, a valuable tool for studying complex diseases with variable expressivity, such as CF.

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## LECT 8

## PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS, ADENITIS (PFAPA): WHAT

## WE LEARNED AND WHAT METABOLOMICS TEACHES US

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PFAPA (periodic fever, aphthous stomatitis, pharyngitis, adenitis) is a syndrome characterized by recurrent episodes of fever, aphthous, sore throat and lymphadenitis, affecting the pediatric population aged between 2 and 5 years without a prevalence of gender (M = F). Recent studies support the role of inflammasome-related genes in the onset of PFAPA. An aberrant innate immune response to exogenous triggers as well as a familial recurrence are factors that cannot be excluded in the PFAPA pathogenesis [1]. Globally, PFAPA shows a good prognosis and frequently it disappears during childhood; however, this syndrome impacts negatively on the health and quality of life of little patients and their families, especially due to the lack of a specific treatment. Glucocorticoids are the first-line treatment in PFAPA thanks to their ability to suppress the attacks readily and completely in most patients; however, their use does not prevent further attacks, and sometimes their administration has been associated with an increase in the frequency of the attacks [2]. Recently, the discovery of the immune mechanisms involved in PFAPA pathogenesis has paved the way to novel therapeutic approaches promising new advances in managing and treating patients with PFAPA. In this regard, pidotimod, a synthetic dipeptide molecule, showed biological activities both in the adaptive and the innate immune mechanisms, resulting in a significant improvement of PFAPA's clinical outcomes [3]. Authors demonstrated that patients receiving pidotimod plus betamethasone showed a significant decrease in the frequency of fevers, episodes of pharyngitis, aphthous stomatitis, and betamethasone use on need when compared to patients receiving only betamethasone on need. Interestingly, the treated group did not report any adverse event during the treatment period. We performed a very preliminary metabolomic analysis to specific urinary metabolites associated either with PFAPA or with the pharmacological treatment [3].

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## LECT 9

## PEDIATRIC ALLERGY HIGHLIGHTS

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We will focus on the main news in pediatric allergy, concerning asthma, food and drug allergies and omics technologies. Caruso studied *S. aureus* enterotoxins as biomarkers in asthma phenotyping, finding a role in predicting prognosis, comorbidities and guiding treatment [1]. He found an association between staphylococcal enterotoxin B-IgE and gender, chronic rhinosinusitis and chronic rhinosinusitis with nasal polyposis, younger age and earlier onset. Lee-Sarwar studied the relationship between the early-life fecal microbiome and childhood asthma phenotypes, finding maternal and infant fecal microbial taxa a longitudinal profile associated with asthmatic phenotypes [2]. Through the metabolomic study of newborn blood, Gürdeniz identified biomarkers of fish oil intake in pregnancy, able to reduce the risk of childhood asthma [2]. The metabolomic profiles differed in infants whose mothers had taken fish oil compared with the placebo group. Fish oil metabolomic profile and a biomarker, CMPF, were associated with reduced risk of asthma at age 6, and CMPF was inversely associated with pulmonary symptoms and infections in the first 3 years. CMPF could be a screening tool for childhood asthma in the newborn. Binder characterized the features of α-gal syndrome [2]. The mean age at onset was 53 years, 56% were female, 95% White race, 86% with history of tick bite, and 75% met criteria for anaphylaxis. Dairy intolerants were significantly less likely than the tolerant ones to have isolated mucocutaneous symptoms, had more frequent gastrointestinal symptoms and lower

sIgE α-gal. Kauppila studied the different responses to milk oral immunotherapy (OIT) [2]. The baseline Ig milk molecule profiles and responses to OIT differed in subjects with different OIT outcomes: lower casein sIgE was associated with better outcome, sIgG4/IgE ratio earlier distinguished the long-term OIT outcome, and higher casein IgA was associated with high-milk dose in the long term. Doña showed that 15% of patients with suspected allergic reaction to penicillin and negative skin tests (ST) and provocation tests were then positive to the ST, with a higher positivization percentage in previous anaphylaxis [1]. Before considering the patient as non-allergic, it is necessary to repeat the ST if initially negative, to avoid reactions after subsequent drug intakes. EAACI issued a *Position paper* on the use of omics technologies, that, integrated with non-omics data in the research of allergies and asthma, allow to study the biochemical systems and pathophysiological processes, leading to a precision medicine useful in stratification of patients, accurate prognosis and treatment decision [3].

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## LECT 10

## EXPERIENCES FROM REAL WORLD PRACTICE IN THE LOW DOSE APPROACH TO SEASONAL ALLERGIC RHINITIS

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The purpose of this clinical trial conducted in 167 patients (89F, 78M) aged 5 to 14 years (mean age 9 years and 4 months) is to evaluate the efficacy and tolerability of a low dose (LD) protocol vs. a standard therapy (ST) reference in the prevention and treatment of seasonal allergic rhinitis. The LD protocol uses the cytokines INFγ and IL-12, a Galium aparine-based compound remedy and a histamine-based compound remedy of the most common allergens underlying seasonal allergic manifestations as pre-seasonal preventive therapy, while the ST protocol involves the use of levocetirizine dihydrochloride. During the acute phase of allergic manifestations, the two protocols are modified by suspending the administration of cytokines in the LD one and adding purely symptomatic drugs in both by general and local routes (nasal and ocular instillation). Patients in both groups underwent 4 visits: the first at enrollment, in January, 2 months before the presumed onset of acute symptomatology; the second at the time of the onset of acute symptomatology, immediately before the start of symptomatic therapy, or – if no symptoms were present – in any case within 90 days of the start of preventive therapy; the third after 4 weeks; and finally, the fourth after 8 weeks. To evaluate the results, a clinical questionnaire was used, where with a score ranging from 0 (absence of symptoms) to 3 (symptoms of considerable severity) nasal, ocular and general symptoms were repertorized. Analysis of the results shows that the LD protocol, both in pre-season and acute phase therapy, has superior efficacy to the ST reference protocol; in fact, better preventive efficacy and a more rapid and lasting disappearance of symptoms during the acute phase are observed, in the absence of side effects. This leads us to propose large-scale use of such an LD protocol that is effective, safe, and excellently tolerated.

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## LECT 11

## METABOLOMICS IN CLINICAL PRACTICE

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Metabolomics refers to the study of metabolites and their pathways in biological fluids and tissues of humans, animals, plants.

Pioneering studies date back to 1951, when Roger J. Williams observed that residents of mental hospitals exhibited characteristic metabolic patterns in their urine. In detail, Williams postulated that the presence of specific urine metabolic patterns might predispose toward schizophrenia, and observed that the tendency toward excessive alcohol consumption was marked by a specific, characteristic metabolic pattern [1]. Williams concluded that "the metabolic patterns of individuals are crucial in connection with their susceptibility to disease".

Only 48 years later, the term metabonomics was defined as "the dynamic metabolic response of living systems, resulting from the interplay between genome, microbiome, and environment, to perturbations over time, mapped by appropriate analytical and statistical techniques" [2].

In 2002, metabolomics was defined as "the comprehensive analysis of small molecules involved in metabolic pathways of living organisms, enabling to assess the composition and concentration of metabolites within a tissue or a biofluid" [3].

Metabonomics and metabolomics are two interchangeable terms, being analytical and modeling procedures identical. Almost all studies use the term metabolomics.

Over the past 20 years, the number of studies based on metabolomics has dramatically increased; by using the keyword "metabolomics", the PubMed database shows 8 publications in 2000 and 12,746 in 2022.

Three factors strongly contribute to the impressive growth of publications on metabolomics:

a. the availability of high-throughput technologies generating large-scale data related to "omics" analysis, including metabolomics;
b. the need to unravel the molecular processes induced by the complex interplay between the human genome and environmental factors;
c. the application of individualized medicine based on the system biology approach.

Most articles consist of experimental *in vitro* or *in vivo* studies in animal models and plants, as well as clinical research in human diseases.

Regrettably, most discoveries have not yet been translated into the clinical setting, and thus, there is the need to move metabolomics from clinical research to clinical testing.

The goal is to implement metabolomics in the routine of clinical laboratories and using metabolic results in clinical practice.

By this way, metabolic results should impact clinical decision-making, similarly to any other laboratory test result.

In Italy, our laboratory (Valsambro Clinical Laboratory, Bologna) has applied metabolomics in clinical practice: urine samples of our patients are analyzed by gas chromatography or liquid chromatography, both coupled with mass spectrometry.

By using the targeted approach, we explore several metabolic panels through the measurement of key metabolites, including microbial metabolites, mitochondrial and oxidative stress-derived metabolites, neurotransmitters, purines and pyrimidines, essential, non-essential, and conditionally essential, amino acids, various organic acids, and, ultimately, lipidomics.

Each test result is represented by a colored graph indicating the reference range and the position of the result within the measuring range; in addition, results are associated with a specific, individualized narrative interpretation.

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## LECT 12

## PEDIATRIC AND NEONATOLOGY ATLAS THROUGH IMAGES

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## INTRODUCTION

For several decades, no text has been published that can stimulate and remind the reader of the importance of "visual semiotics" as the initial approach to the patient: a human approach, personalized by empathetic narrative support.

## PRESENTATION

Our experience has taught us that an image or direct observation can be naturally useful for a rapid "face to face" with the patient. It can certainly be helpful in recognizing symptoms and signs, which are valid and valuable for a proper diagnostic orientation.

This premise is not intended as an indication of returning to the past, but as a reconsideration and reevaluation of traditional systems, in light of knowledge and modern technological investigations.

The old semiotics and narrative not only represent a glorious and ancient past but are still the "*primum movens*" for framing a case. Careful narration, observation and inspection, palpation and percussion, auscultation and tactile vocal fremitus detection can certainly aid us in formulating a correct diagnosis. Unfortunately, this prestigious and challenging aspect of the medical art is increasingly being replaced by a fleeting approach to the patient and by the request for countless investigations. This "cultural transition" in the approach to the patient has been favored by the progressive neglect of semiotics during university studies.

Therefore, having at our disposal countless images accumulated over decades of work, and with the collaboration of other colleagues, we have created this Atlas (titled *Atlante di Pediatria e Neonatologia per immagini*).

A beautiful image represents an expressive language to describe more evident clinical signs. It can be a means of rapid intuition and communication that strikes the observer, helping them to become familiar with the subject.

Furthermore, the visual memory of the detected clinical signs will guide them to plan more precise and appropriate investigations to verify and confirm the initial diagnostic orientation. Nothing remains impressed like things seen, and for this reason, we have given the utmost importance to iconography, for almost all of the original cases, coming from our daily practice.

## CONCLUSIONS

In this book, we have explored many areas, from practice to research, from the history of the discipline to its links with culture since antiquity, and with the humanities. Patient inspection is the very first step in the pediatric clinical examination. We hope this book will be useful for students and Colleagues.

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