JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS
Volume 36 n.1 (Suppl 1), January-February 2022
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IN THE PEDIATRIC EMERGENCY DEPARTMENT"

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Special issue: "Focus on infectious disease in the pediatric emergency department"

The infectious disease in pediatric patients may represent an emergency for the children's health. This special issue provides a rich blend of news, reviews, and clinical studies on many aspects of the understanding, management, and prevention of some of the most common infectious diseases in the pediatric emergency department. Primary care in the pediatric emergency department, principally, must concern early detection of infections, management, and the prevention of complications. With these goals, the themes presented in this "mosaic" of topics include:

- Respiratory infections.
- Cardiovascular infections.
- Infections that require surgical interventions.
- Neurological Infections.
- Urinary tract infection.
- Sars-CoV-2 infection.
- Sepsis.
- Ocular infection.

I thank the researchers and professors of Catanzaro, Napoli, Catania, Pavia, Brescia, Taormina, and Messina University for their interesting and up-to-date scientific contributions, and I hope the information gathered in this issue will be helpful to the reader.

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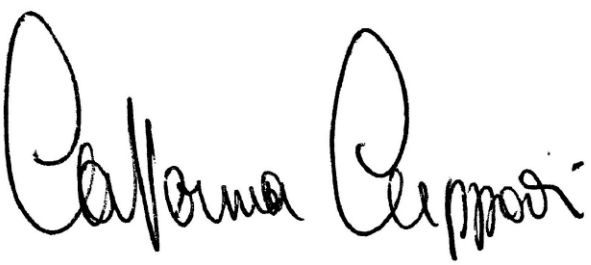

Overview of Guillain-Barrè Syndrome

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Guillain-Barrè syndrome (GBS) refers to a group of immune-mediated, rapidly progressive polyneuropathies. Before the clinical onset, most patients present with potentially triggering events in their history, including infections and vaccinations. The commonly accepted pathogenic mechanism is an autoimmune response targeting the axons and myelin of peripheral nerves. Diagnosis is based on clinical criteria, though it may be supported by consistent laboratory, neurophysiological and neuroradiological findings, increasing diagnostic reliability. Due to its sudden and invalidating onset, GBS represents a possible cause of access to the emergency room. Therefore, physicians must take into account GBS clinical heterogeneity to promote an early diagnosis and promptly start acute phase treatment and supportive therapies.

Guillain-Barrè syndrome (GBS) refers to a group of immune-mediated, rapidly progressive polyneuropathies, to date considered the most common cause of acute flaccid paralysis in childhood (1-3). Its incidence in the pediatric population ranges from 0.34 to 1.34 per 100.000 person/year, and it appears to be more frequent in males than in females, both in childhood and in adulthood (3-5).

The two main key aspects of classic GBS are an acute (monophasic) and progressive (ascending) bilateral weakness of the limbs, and the concomitant areflexia, also known as "Landry palsy", with the nadir of motor impairment occurring within 4 weeks of onset (6, 7). In addition, for 1-6 weeks preceding the onset of symptoms, potential trigger events are reported in 50% to 82% of children (3, 4).

However, GBS antecedents, clinical features, and extent may be highly heterogeneous. Therefore, different classifications, including a wide range of clinical variants, have been proposed (5, 6, 7).

Finally, due to its sudden and invalidating onset, sometimes even life-threatening (i.e., when respiratory muscles are involved), GBS represents a possible cause of access to the emergency room. Thus, physicians need to be fully aware of GBS clinical heterogeneity to promote an early diagnosis and

Keywords: cerebrospinal fluid, flaccid paralysis, Guillain-Barrè Syndrome, immune-mediated, Landry's paralysis, neuropathy, poliradicoloneuropathy

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0393-974X (2022)

promptly start treatment, reducing the odds of poor outcomes (8).

Antecedent events and pathogenesis

Among the antecedent events, upper respiratory or gastrointestinal infections, vaccinations and, rarely, malignant diseases, treatments with immune checkpoint inhibitors, and surgery have been reported in the literature (3, 5, 9).

In post-infectious cases of GBS, many pathogens have been variably brought into play worldwide (depending on the endemic presence in diverse countries, or outbreaks), among which Campylobacter Jejuni, Cytomegalovirus, Haemophilus Influenzae, Mycoplasma Pneumoniae, Epstein-Barr virus, Hepatitis E virus, Influenza A virus, Zika virus. In addition, during the COVID-19 pandemic, an increase of GBS incidence was reported, supporting the hypothesis of a pathogenic role of the SARS-CoV-2 infection. Among the recent literature data on the topic, several cases of GBS following COVID-19 have also been described in the pediatric population, sharing similar pathogenesis and the same clinical presentation of classic GBS (5, 10, 11 12).

GBS onset has also been reported in some individuals who had previously received vaccines for several infectious agents (Table I) (6, 13) and, very recently, case reports have been reported, suggesting a correlation with the Sars-CoV-2 vaccine (14, 15). Again, however, controversial data exist on the topic, and data from the literature clearly show that, overall, the benefits deriving from vaccinations significantly exceed the risks (16, 17, 5, 18).

It is commonly accepted that GBS is an immune-mediated disease targeting the axons and myelin of peripheral nerves. However, a molecular mimicry mechanism has been proposed: pathogens may induce the production of different (auto)antibodies that, due to structural similarity between pathogens and gangliosides of peripheral nerves, may end up targeting their axons, and particularly the nodes of Ranvier. This mechanism determines a pure motor impairment in most cases, namely the "Acute Motor Axonal Neuropathy" (AMAN). However, to date, a more severe variant may also include a sensory involvement, known as "Acute motor-sensory axonal neuropathy" (AMSAN) (19, 1, 20). On the other hand, when myelin is damaged, the autoimmune response

Table I. *Main causes of GBS (43, 44, 13, 5).*

INFECTIONS
Bacterial infections
Campylobacter Jejuni *Haemophilus Influenzae* *Mycoplasma Pneumoniae* *Salmonella species*
Viral infections
Influenza viruses *Cytomegalovirus (CMV)* *Epstein-Barr virus (EBV)* *Varicella Zoster virus (VZV)* *Human Immunodeficiency virus (HIV)* *Hepatitis E virus (HEV)* *Zika virus (ZIKV)* *Dengue virus (DENV)* *Chikungunya virus (CHIKV)* *Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)*
VACCINATIONS
Influenza vaccine Quadrivalent meningococcal vaccine (MCV4) Human Papilloma virus (HPV) vaccine Measles/Mumps/Rubella (MMR) vaccine Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) vaccine
OTHER RARE CAUSES
Malignant diseases (non-Hodgkin lymphoma, lung cancer, bladder cancer, colon cancer, skin cancer, *etc.*) Immune checkpoint inhibitors (*Ipilimumab*) Surgery

appears to be mediated by complement-activated T-cells and macrophages. These latter determine the Schwann cell membranes destruction, hence segmental demyelination, causing the variant commonly known as "Acute Demyelinating Inflammatory Polyneuropathy" (AIDP) (1, 21, 22). In the damage of myeline at node of Ranvier, also genetic factors need to be taken into account. Genetic neuromuscular and/or developmental disorders with infantile-onset include a variety of monogenic conditions, with expanding clinical differential diagnosis, genetic heterogeneity, and associated disease mechanisms (23-26). These can include genetic nodopathies that, opposed to immunomediated disease, usually start in infancy and are associated with central demyelination and frequent neurodevelopmental impairment (27). The molecular dissection of these conditions and the underlying genetic and/or immuno-mediated are interesting examples to explain the frequent intricated multisystemic, metabolic and neurological abnormalities associated with a wide array of (both rare and common) pediatric disorders (28- 35).

Diagnosis

To diagnose GBS, flaccid weakness of the limbs and/or motor cranial-nerve involvement, evidence of a monophasic course (with an interval of 12 h to 4 weeks to reach the plateau), and the absence of an alternative diagnosis, are strictly necessary. However, additional data may support the diagnosis, increasing the level of diagnostic certainty: according to Brighton criteria, cerebrospinal fluid (CSF) albuminocytological dissociation (namely CSF white cell count <50/ul and raising proteins after week 1) and conduction slowing/block in neurophysiological studies strongly increase the diagnostic reliability (6, 36).

As previously mentioned, the GBS spectrum is really wide and encompasses several clinical variants (Table II). In this regard, over the years, many classifications have been proposed, taking into account both clinical presentation and consistent laboratory, neurophysiological and neuroradiological findings (37, 6, 36).

Shahrizaila et al. (2021), in a very recent review on the topic, distinguished three main clinical groups/entities: GBS spectrum, Miller-Fisher syndrome (MFS) spectrum, and Bickerstaff brainstem encephalitis (BBE). According to this classification, the GBS spectrum gathers all those conditions variably comprising weakness of the limbs and/or sensory involvement, hyporeflexia/areflexia, with or without cranial nerve or bulbar dysfunction. On the contrary,

Table II. *Clinical features of GBS (5,15,36).*

	Upper limbs weakness	Lower limbs weakness	Sensory involvement	Ataxia	Cranial nerve involvement	CNS involvement
GBS spectrum						
Classic	+	+	+/-	+/-	+/-	-
Pure motor	+	+	-	-	+/-	-
Paraparetic	-	+	+	-	-	-
Facial diplegia and paresthesia	-	-	+	-	+	-
Pharyngeal-cervical-brachial	+	-	+/-	-	+	-
Acute bulbar palsy	-	-	+	-	+	-
MFS spectrum						
Classic	-	-	+	+	+	-
Acute ophthalmoplegia	-	-	+/-	-	+	-
Acute ataxic neuropathy	-	-	+/-	+	-	-
Acute ptosis	-	-	+/-	-	+	-
Acute mydriasis	-	-	+/-	-	+	-
BBE						
Classic	-	-	+/-	+	+	+
Acute ataxic hypersomnolence	-	-	-	+	-	+

in the MFS spectrum, cranial nerve involvement is a constant (except in the so-called "acute ataxic neuropathy" subtype) and is usually associated with ataxia or sensory involvement, without evidence of limb weakness. BBE is instead characterised not only by the possible presence of sensory impairment, ataxia and cranial nerve dysfunction but also by CNS involvement, determining drowsiness and/or hyperreflexia. In light of this, despite being a unique entity, it is usually considered related to GBS due to the overlap among these clinical entities (5).

Treatment and outcome

Patients suffering from GBS need a multidisciplinary and individually-tailored approach, with particular attention to the disease stage (1, 5). The acute phase treatment is based on intravenous immunoglobulins (usually 2g/kg over 5 days) and/or plasmapheresis (50ml/kg plasma per session) regardless of the variants (5, 37). Protocols including steroids have also been proposed proving beneficial when combined with immunoglobulins (39). Overall, immunoglobulins should be considered the first-line treatment in children (2).

During the progressive phases, particular attention must be paid to indirect complications of GBS (i.e., thrombosis, pain, respiratory failure, hypertensive crisis, *etc.*); hence supportive therapy becomes imperative (2, 40-42).

Children with GBS show more favourable outcomes than adults since most fully recover. However, a minority of them may develop neuropsychiatric sequelae (i.e., motor impairments, fatigue, poor coordination and concentration, *etc.*) or even die (2, 5).

CONCLUSION

GBS refers to a wide group of heterogeneous immune-mediated disorders with an acute onset and life-threatening clinical features. Although it is considered a rare disease, in classic and atypical variants, its clinical presentation must be considered in patients accessing the emergency room.

A thorough anamnesis and a rapid recognition of the symptoms may lead the physician to a proper diagnosis and an early treatment, crucial requirements to prevent poor outcomes, especially in children.

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Management of croup in children

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Croup or laryngitis is the most common cause of acute stridor in childhood. It accounts for 15% of pediatric respiratory admission in the emergency department (ED). Most children presenting with an acute onset of barky cough, stridor and indrawing have croup. The differential diagnosis, among other forms of acute obstruction of the larynx (e.g., epiglottitis, foreign body, angioneurotic oedema of the epiglottis), is essential and lifesaving. Corticosteroids are the mainstay of treatment in children with croup of all levels of severity. Nebulized epinephrine is an accepted treatment in patients with moderate to severe croup. Patient management remains of fundamental importance, is necessary to keep children calm by ensuring a relaxed and reassuring atmosphere to minimize oxygen demand and respiratory muscle fatigue.

In recent years, a large number of omic-based studies revealed an expanding complexity underlying pediatric emergencies associated with acute presentations (1). The molecular dissection of these conditions and the possible underlying genetic and non-genetic causes may explain the frequent multisystemic, metabolic and neurological abnormalities associated with a wide array of pediatric conditions (2, 3-8).

Many novel molecular factors have been identified with consequent benefits in refining clinical phenotypes, valuable prognostic information, detailed imaging studies, and targeted therapies for children admitted to the Pediatric Department for various reasons (9-16).

Pediatric disorders at the pediatric emergency department may implicate several molecular pathways, including regulation of transcription, translation, and post-translational processes at the cellular and sub-cellular levels (17-28). In addition, such disorders may include a variety of monogenic and polygenic/genetically complex and/or environmental conditions with expanding clinical differential diagnosis, molecular heterogeneity and associated (underlying) disease mechanisms (29-33). The molecular dissection of these conditions and the underlying factors are interesting examples to explain the frequent intricated multisystemic, metabolic and neurological abnormalities associated with a wide

Keywords: croup, children, stridor

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0393-974X (2022)

array of (both rare and common) pediatric disorders and useful etiological targeted treatments (34-38).

Croup represents one of the most frequent medical emergencies in the pediatric population. The course of illness is typically the result of acute subglottic region obstruction, and genetic and/or immunomediated mechanisms associated with this condition have not yet been fully elucidated. It is typically mild and self-limiting but may be severe and, rarely, life-threatening (39). Most children can be managed at home, while moderate and severe respiratory distress require hospitalization and supportive care. Mortality is extremely low, affecting less than 0.5% of intubated children (40, 41). Following discharge, approximately 5% returns to the Emergency Room (ER) within 1 week due to the recurrence of symptoms (42).

Clinical manifestations and diagnosis

Croup affects about 3% of children per year, usually between 6 months and 6 years of age, with a peak incidence between 7 and 36 months (5%). Among the wide variety of clinical presentations of croup, the most frequent ones are acute laryngitis and laryngospasm (or "spasmodic croup"). Viral infections usually cause acute laryngitis: parainfluenza virus and rhinovirus are the most common etiologic agents; enterovirus, respiratory, syncytial virus, influenza virus, and human bocavirus has also been detected (43). As a consequence of viral aetiology, the disease occurs mainly in the autumn-winter period within the 6 months to 3 years age group.

Viral tissue invasion causes inflammation, hyperaemia, and oedema of laryngeal mucosa, resulting in narrowing the subglottic region (44). Typical clinical manifestations are barking cough, inspiratory stridor and hoarseness of voice with or without respiratory distress (45). The episodes have a gradual onset; prodrome symptoms (usually coryza) are described due to inflammation of the upper airways in the previous days. Finally, fever may occur within the first 24 hours.

Conversely, spasmodic croup occurs in the presence of spasmodic contraction of larynx muscles probably due to allergic stimuli, gastroesophageal reflux or other environmental factors, but, frequently, the cause remains unknown (46). The main age is between 1 and 5 years. Clinical symptoms have a sudden night-time onset and are characterized by a metallic cough without fever. Often child parents and/or the patient are affected by allergic diseases.

Diagnosis of croup is clinical. Investigations, indeed, could unnecessarily distress the child and worsen symptoms. Laboratory and imaging evaluation are not essential but may rule out other illnesses in selected patients with an atypical or severe presentation. Although chest radiography cannot diagnose croup, it can rule out other pulmonary conditions when the diagnosis is unclear in a child with stridor (47).

Assessment

In clinical practice, the most used scoring system is the one suggested by Westley et al. (48) evaluates the severity of croup by assessing five factors: level of consciousness, cyanosis, stridor, air entry, and retractions.

MILD: Occasional barking cough (no audible stridor at rest), no or mild respiratory distress at rest, normal SpO2, no cyanosis, alert.

MODERATE: Frequent barking cough (audible stridor at rest), moderate respiratory distress, normal SpO2, no cyanosis, little or no agitation.

SEVERE: Persistent stridor at rest (may be expiratory), severe respiratory distress, SpO2 < 93% or cyanosis, fatigue or altered mental state.

LIFE-THREATENING: Audible stridor may be quieter, exhausted, have poor respiratory effort, SpO2 < 93% or cyanosis, lethargy, or decreased level of consciousness.

Risk factors for severe croup include: age less than six months, underlying structural upper airway condition (e.g. tracheomalacia, subglottic stenosis), history of previous severe croup, unplanned representation to ED within 24 hours of first croup presentation, trisomy (21).

Differential diagnosis

The differential diagnosis is essential and lifesaving and must include the other acute obstructive illnesses involving the larynx, such as epiglottitis, foreign body aspiration, angioneurotic oedema of the epiglottis (49) (Fig. 1).

Epiglottitis is characterized by inflammation of the supraglottic area mainly caused by Haemophilus influenzae type b. It usually occurs in preschool children with sudden onset, high fever, lethargy, pharyngodynia, paleness, profuse salivation, severe dyspnea associated with retractions, and forced posture ("tripod") with the chin pushed forward and a reluctance or refusal to lie down (50). Due to the high rate of immunization against H. influenzae type b in the pediatric population (51).

Foreign body oedema and angioneurotic oedema usually occur suddenly, without fever or other signs and symptoms of infection.

Bronchitis and pneumonia may be characterized by respiratory distress and signs of lower-airway involvement (crackles, air trapping, wheezing, and radiological signs of pneumonia) (52).

Treatment

Children with croup should be made as comfortable as possible, in the arms or knees of the mother, without shaking them with unnecessary procedures. Take special care not to distress the child as this may exacerbate symptoms. Keep children calm by ensuring a relaxed and reassuring atmosphere to minimize oxygen demand and respiratory muscle fatigue; melatonin may be used (53-59).

Therapy is aimed at decreasing airway oedema and providing supportive care. In children, treatment for stridor includes supportive care, nebulized epinephrine, and steroids (Fig. 2). Mist or humidified oxygen treatment for stridor is, however, not recommended. All experts now routinely recommend Corticosteroid therapy (60) (for children with mild to severe croup). Steroids reduce respiratory distress in approximately 30 minutes (61), more quickly if given via a nebulizer (62). There is improvement within 2-3 hours of administration, and the effect persists for 24-48 hours. High mobility group box 1 can be used as a biomarker of inhaled corticosteroid treatment response in children with croup (63-64).

Oral administration is recommended whenever possible. Advantages of oral administration over other methods include: less pain and distress for the child, low cost and ready availability, ease of administration.

Dexamethasone: 0.15 - 0.6 mg/kg (max 12 mg/day)
Betamethasone: 0.15 mg/kg (max 4 mg/day)
Prednisolone: 1 mg/kg (max 40 mg/day)
Nebulised budesonide: 2 mg nebulized with oxygen (consider for a child who repeatedly vomits the oral medication).

The beneficial effects of systemic corticosteroids in croup have been summarized: reduction in the intensity of symptoms related to upper airway obstruction (at 6, 12, and 24hr after treatment); decreased use of nebulized epinephrine; reduced length of stay in the emergency department; fewer hospital admissions or return visits to the emergency department.

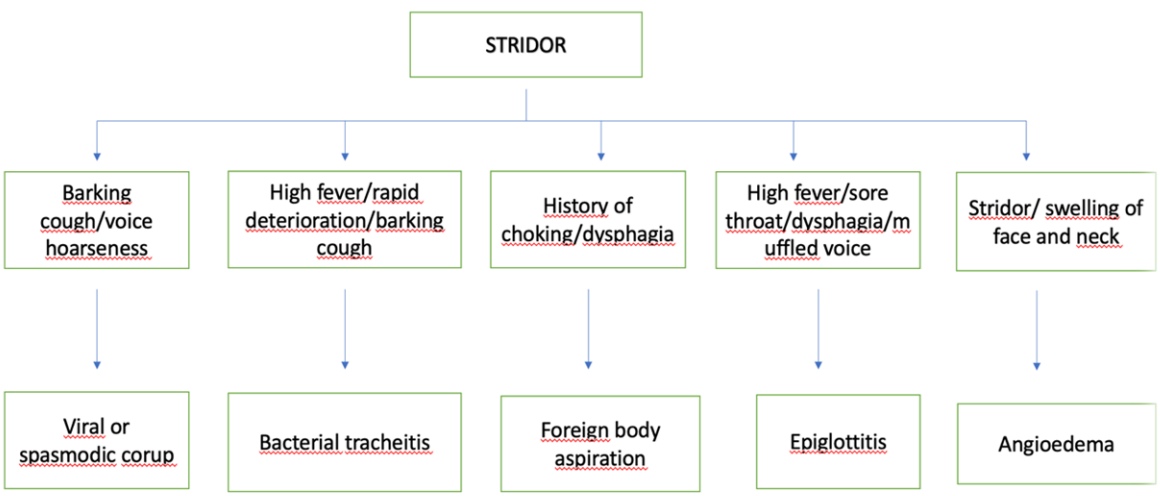

Fig. 1. *Clinical differential diagnosis of stridor in children.*

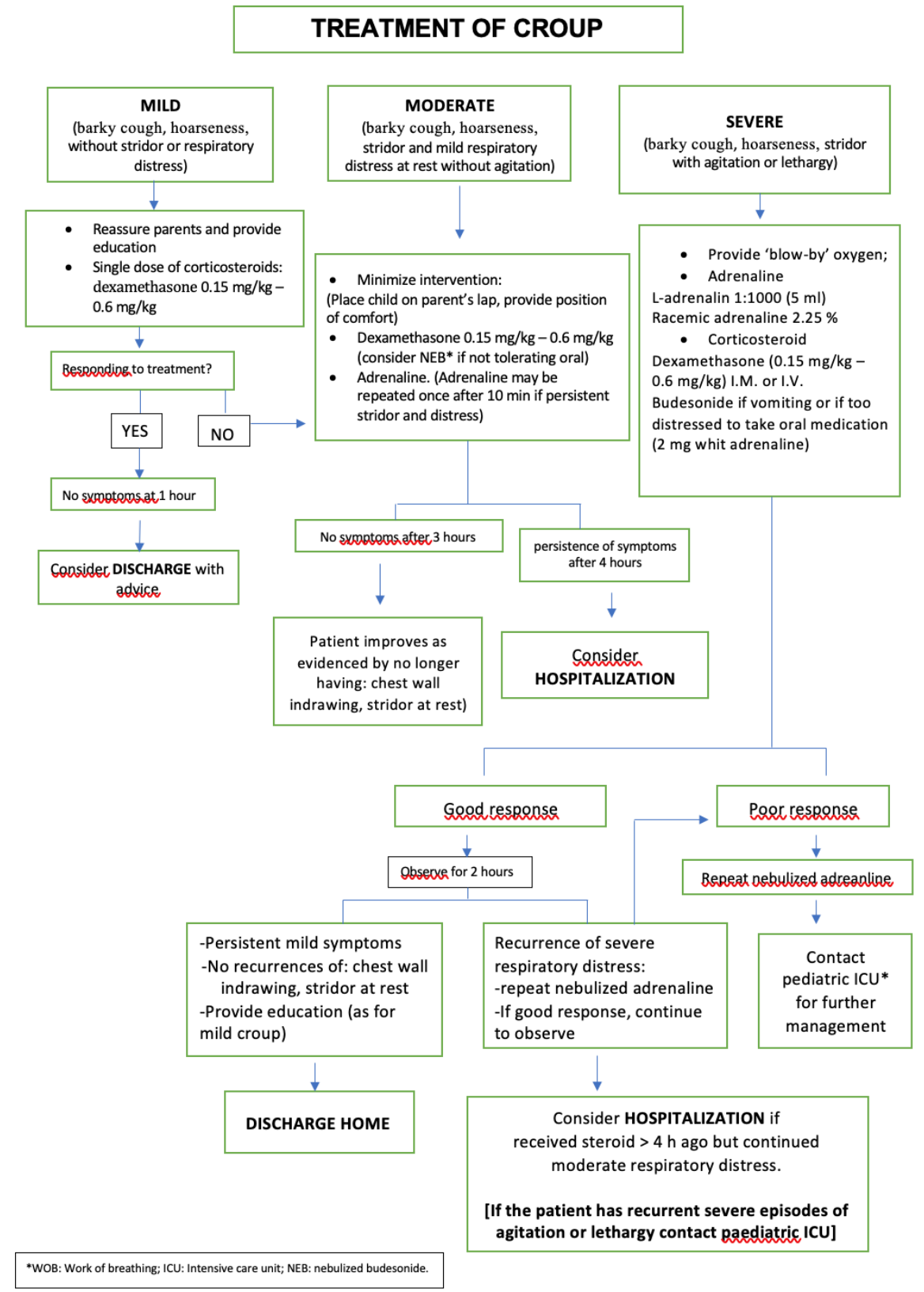

Fig. 2. *Flow-chart of croup treatment.*

Nebulized adrenaline is considered at the forefront of treatment in any child with persisting inspiratory stridor (at rest) and marked respiratory distress. Symptoms improve in the first 30 minutes (65). The effectiveness lasts about 2 hours (66). The recommended dose is 0.05 mL per kg (maximal dose: 0.5 mL) of racemic epinephrine 2.25% or 0.5 mL per kg (maximal dose: 5 mL) of L-epinephrine 1:1,000 via nebulizer (67). It is important not to discharge the patient before 4 hours to monitor heart rate and breath rate (it can lead to hypertension and tachycardia) and, above all, for a possible return of symptoms, as it does not confer long-term benefits (68).

Together with corticosteroids and adrenaline, oxygen therapy is reserved for children with hypoxia and significant respiratory distress. It should never be forced on a child, particularly if it results in significant agitation. 'Blow-by' administration of oxygen through a plastic hose with the end opening held within a few centimetres of the child's nose and mouth is often the most beneficial way of administration (69) for children with severe croup and SpO2 less than 93%, at a minimum of 10 L/min flow rate. Antibiotics, steam inhalations, heliox treatment, antitussives or decongestants are not recommended (Fig. 2).

Indications for hospitalization are:

severe croup on arrival in PS with reduced air entry, altered level of consciousness and imminent upper airway obstruction;

persistent symptoms (e.g., respiratory distress or stridor at rest) three hours after treatment;

age less than six months.

unplanned representation to ER within 24 hours following diagnosis of croup at first presentation.

Discharge criteria are:

no respiratory distress or stridor at rest post-treatment (minimum three hours post-administration of nebulized Adrenaline or 1 h post-oral corticosteroids);

croup remains the primary diagnosis after consideration of differential diagnoses;

parent/caregiver has access to further doses of any required prescribed medication, received education and are comfortable with what to do if symptoms recur, access to transport or emergency services in the event of deterioration.

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Fever of unknown origin

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Fever of Unknown Origin (FUO) is defined as the febrile condition persisting for at least 2 weeks despite an initial clinical investigation. The infective etiopathogenesis is the most common cause of FUO, followed by cases without a diagnosis, inflammatory or systemic diseases, rheumatological and onco-haematological pathologies. Therefore, a diagnostic algorithm must evaluate potential diagnostic clues (PDCs) pointing towards a possible diagnosis and correct treatment. Further studies are necessary to understand the trends in mortality and overall outcomes associated with pediatric FUO.

Fever is the increase in central body temperature above normal limits not caused by changes in environmental temperature, unlike hyperthermia. According to the World Health Organization, the central body temperature is generally between 36.5°C and 37.5°C, but it can vary depending on various factors such as age, sex, physical activity, menstrual cycle phases, circadian oscillations and anatomic site of measurement. Fever is a common clinical manifestation of many illnesses in humans, and it is determined by the activation of immune cells (macrophages and dendritic cells) by specific microbial products (such as bacterial lipopolysaccharide – LPS, and pathogen-associated molecular patterns-PAMPs). Specific receptors recognize LPS and PAMPs, called toll-like receptor 4 (TLR-4). The activation of these cells in the lungs and/or liver causes the release of prostaglandins E2 (PGE2) and pyrogenic cytokines (IL-1, IL-6 and TNF), responsible for the early and late stage of fever (1).

Fever of unknown origin (Fever of Unknown Origin - FUO) is a prolonged fever whose origin is not established. In 1961 Petersdorf and Beeson suggested criteria to define FUO based on an analysis of 100 cases (2). In the past, FUO was defined by a temperature

Keywords: fever, children, Fever of Unknown Origin

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0393-974X (2022)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

above 38.3°C, that occurred on 3 or more occasions with three weeks of malaise, at least. The diagnosis was not made after one week of hospital admission. However, FUO has now been defined as a febrile condition that persisted for at least 2 weeks, despite a thorough investigation, either on an outpatient or in-hospital basis. Its origin is difficult to identify at an initial clinical evaluation (3). The incidence and prevalence of FUO are still now unknown (4).

Etiology

A systematic review of the literature on the causes of FUO has recently been conducted in pediatric patients (1). From the analysis of some American, European and Asian cases, infections are the most common causes of FUO (51%), followed by 23% of cases without a diagnosis. Other causes of FUO are:

11% miscellaneous (inflammatory diseases or systemic onset);

9% rheumatological diseases (in particular juvenile idiopathic arthritis, systemic lupus erythematosus and others);

6% onco-haematological pathologies (leukaemias, lymphomas and neuroblastoma) (5).

Antoon et al. distinguished infectious and non-infectious causes of FUO in 2015. Among the infectious causes, there are bacterial, viral and mixed forms. Tuberculosis is the most frequent diagnosis; other infectious etiologies are intra-abdominal abscesses, infective endocarditis, osteomyelitis, Epstein–Barr virus (EBV) mononucleosis. Minor infectious etiologies are brucellosis, malaria, cytomegalovirus (CMV), visceral leishmaniasis (kala-azar), typhoid-enteric fevers.

Among the non-infectious causes, there are the oncological, autoimmune, rheumatological and other causes. Hodgkin's disease and non-Hodgkin's lymphoma are the most frequent oncologic etiology of FUO. Non-infectious inflammatory diseases are adult Still's disease, giant cell arteritis, polyarteritis nodosa, Takayasu's arteritis, Behcet's disease, granulomatosis with polyangiitis (Wegener's). Other conditions are non-inflammatory conditions such as endocrine diseases, drug use such as antibiotics, anticonvulsants, neuroleptics, antiarrhythmics, vasodilators and nonsteroidal anti-inflammatory drugs (6,7).

A further classification on the etiology of FUO was made in 2018 by Attard et al., which allowed to associate the various clinical and physical signs to different causes of FUO (8). It is also possible to make a differential diagnosis based on the age of onset of fever**.** Infections are always present in all ages. However, between 6 and 14 years of age, autoimmune diseases are also associated (23%), and in the age above 14 years, neoplastic diseases are present in 19% of cases.

Initial evaluation

An accurate history and objective examination guide the diagnosis in a high percentage of FUO cases (9). It must investigate the following aspects:

family history of infectious diseases (TB, HIV, viral hepatitis, etc.), autoimmune diseases or self-inflammatory, onco-haematological, IBD, etc.;

personal history of infections (consider any immunodeficient spies especially for children 1 year);

exclude recurrence of the episode;

duration and type of fever

evaluate associated signs and symptoms

concurrent pharmacological therapies (drug fever) and/or ailments

travel to developing countries

Objective examination mainly aimed at auxological parameters, examination for apparatus, an inspection of the skin and mucous membranes (mucositis, foot-and-mouth disease, teeth condition, etc.) and all stations and explorable lymph nodes. Red flags are cutaneous-mucous manifestations, organ dysfunction, bone pain, anaemia and/or piastrinopenia. However, the normality of the objective examination points to a benign cause of fever (10).

Laboratory and imaging test

Laboratory and imaging tests should be required in stages, starting with the most straightforward, least expensive and least invasive. Laboratory investigations to be carried out in FUO cases shall include blood count with leukocyte formula, routine tests including immunoglobulins, lactate dehydrogenase, bilirubin and liver enzymes, urinalysis and/or urine culture, blood-culture (3 samples), peripheral blood smear, anti-nucleus antibodies, HMGB1, detection of human calprotectin,

CMV, EBV and human immunodeficiency virus (HIV) serology. Instrumental examinations include chest X-ray and Mantoux test and abdominal ultrasound (abdominal abscesses? peritoneal effusion? pyelonephritis?) (11-16).

Through the medical history, objective examination and laboratory tests, the physician should try to frame the cause of FUO.

It is important to evaluate all signs, symptoms, and abnormalities (potential diagnostic clues - PDC) pointing towards a possible diagnosis in a diagnostic algorithm. When PDCs are present, the various types of fever should be distinguished. In the case of continuous fever, diagnostic tests are guided by specific signs and symptoms. Invasive radiological investigations (CT, scintigraphy and MRI), additional serologies or bone marrow biopsy are carried out when the diagnosis is not possible. Conventional diagnostic imaging is often ineffective in revealing the underlying cause of FUO. The fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG-PET/CT) is a highly sensitive method for detection of the underlying cause of FUO in patients without disease-specific symptoms on one examination (17). Genetic tests (MEFV, MVK, TNFRSF1A) are helpful in case of recurrent fever and present PDC. Follow up should be continued in case of negative tests. Monogenic autoinflammatory syndromes presenting as periodically recurring fevers are cryopyrin-associated periodic syndrome, familial Mediterranean fever, mevalonate kinase deficiency syndrome, tumor necrosis factor receptor-associated periodic syndrome (18).

Treatment

In the absence of a diagnosis, empirical therapy should be limited only to those with stability conditions; otherwise, it could mask the diagnosis of relevant pathological conditions. Antibiotic therapy is not always helpful; in fact, bacterial diseases can cause FUO and viral, oncological, autoimmune. Interleukin-1 receptor antagonist, anakinra, can be tried in patients with a suspected autoinflammatory disorder (19-21). If it is ineffective after two weeks of treatment, the drug should be stopped (5,8).

DISCUSSION

Recently, several experimental studies aimed to dissect the molecular complexity underlying pediatric neglected or less understood conditions associated. FUO is part of a large group of diseases in which laboratory investigations have not been yet identified the exact molecular pathophysiology for a large proportion of cases. The molecular dissection of neglected pediatric conditions and the possible genetic and/or environmental factors may often highlight different metabolic, genetic, and neurological defects that underlie many pediatric diseases (22-27). Many molecular factors have been recently identified in association with different neglected diseases; this allows clinicians to reach defined clinical phenotypes and precious prognostic data and possibly etiologically-targeted treatments for a wide array of pediatric conditions (28-37). Pediatric diseases are often associated with pathways that regulate important homeostatic processes at the sub-cellular level (38-42).

These conditions can result from a faulty gene or a more complicated gene-environment interaction, resulting in enlarging differential diagnoses and a combination of molecular mechanisms and disease phenotypes (43-50).

CONCLUSION

The increasing understanding of the pediatric disorders associated with multisystemic, metabolic and genetic abnormalities can shed new light on future precision medicine strategies in the Paediatric field (51-55). Notably, many pediatric FUO resolve spontaneously, but others remain undiagnosed; thus, there is a great deal to do in terms of genetic and mechanistic studies. Interestingly, the prognosis is better in children than in adults due to differences in etiologies. A particular form of empiric therapy is antipyretic therapy used as a symptomatic approach (56-60); however, prognosis depends on diagnostic delay and the nature of the underlying disease. This condition continues to be a clinical challenge for physicians, and further studies are needed to

understand the trends in mortality and overall outcomes associated with pediatric FUO (5,8,61).

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Immunoglobulin A (IgA) deficiency and clinical manifestations

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To the Editor,

Selective absence of serum and secretory IgA is the most common form of human immunodeficiency. Although often asymptomatic, it is important to identify patients with SIgAD, given the various diseases this population is predisposed to. This review summarizes the main features of SIgAD diagnosis and treatment and its link with recurrent infection. We also emphasized the potential association with Covid 19 infection. A comprehensive search of published literature using the PubMed (HTTP:// www.ncbi.nlm.nih.gov/pubmed/) database was carried out to identify all articles published in English in peer-reviewed journals. The search terms included ("IgA Deficiency ") AND ("Young" OR "Children" OR "Adolescence" OR "Adolescents" OR "Childhood").

Immunoglobulin A (IgA) is the most prevalent immunoglobulin in the body that plays an important role in mucosal immunity. It has two subclasses, IgA1 and IgA2; IgA1 can be found in monomeric form in blood circulation; instead, IgA2 is mainly found as a dimeric form in the gastrointestinal and respiratory tracts, saliva, tears, and breast milk. Secretory IgA plays a primary role as the first line of defence in immune exclusion of pathogenic microorganisms, binding multiple antigenic epitopes on the surface of viruses and bacteria, neutralizing their effect by prevention adherence and other processes.

DISCUSSION

Immunoglobulin A (IgA) deficiency is the most common primary immunodeficiency, defined as a decreased serum level of IgA in the presence of normal levels of other immunoglobulin isotypes. There is a remarkable variability of prevalence among different ethnic groups, ranging from 1:600 in the Caucasian population to 1:5000 in the Asian population (1). According to the European Society for Immunodeficiencies, the diagnosis is usually established in children older than 4 years whose blood levels of IgA are below 7 mg/dl but have normal levels of IgG and IgM. These children have a normal antibody response to vaccines. Other causes of hypogammaoglobulinemia or T- cell defect should be excluded. IgA deficiency may include enhanced susceptibility to infections, especially respiratory and concomitant autoimmune diseases (2,3).

Keywords: Immunoglobulin, IgA, deficiency

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0393-974X (2022)

Most cases of IgA deficiency appear to be sporadic, but many familial cases have been reported, and genetic defects were shown. Chromosomal abnormalities, cytogenetic defects, mutations in JAK3, RAG1, RAG2, TACI, CXCR4, and STAT1 are individuated. Identification of genetic alteration may lead to new and specific treatment strategies. Among secondary causes, anti-epileptic drugs, non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, angiotensin-converting enzyme inhibitors, and even antibiotics are described (4-8). Usually, drug-induced IgA deficiency is reversible; however, IgA deficiency could be persistent after cyclosporine treatment (9).

In the SIgAD, the B cell development is blocked before they mature into IgA-secreting plasma cells. Several pathways have been implicated in abnormal B cell maturation, particularly low serum levels of transforming growth factor beta (TGF-β), which directs to isotype switching and differentiation of B lymphocytes into IgA-secreting plasma cells. Moreover, multiple cytokines such as IL-4, IL-6, IL-10 and IL-21 are involved in IgA production. In addition, HMGB1 serum level may be altered (10-12). Additionally, it has been demonstrated that some patients with SIgAD have decreased proportions of FoxP3 regulatory T cells (Tregs), which may play a role in patients who develop concomitant infection and autoimmune disease such as celiac disease, thyroiditis, autoimmune cytopenia, juvenile rheumatoid arthritis and systemic lupus erythematosus (13-16).

Patients with IgA deficiency can be categorized into different phenotypes as asymptomatic or symptomatic with minor infection, allergy, autoimmune and severe diseases. Most of the patients are asymptomatic; if symptomatic, the patients could be present recurrent sinopulmonary infections, allergic disease, particularly severe allergic rhinitis or asthma. Less common but more severe complications are malignancies, such as adenocarcinoma of the stomach and lymphoma, which arise particularly in older patients (17). The infections that affect the upper and lower respiratory tract are common, including recurrent sinusitis, pneumonia or otitis media, due to bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. A primary immunodeficiency should be suspected in children with four or more sinus or ear infections or two or more episodes of pneumonia within one year. In addition, a recurrent respiratory infection could result in chronic lung damage such as bronchiectasis.

In patients with a concomitant IgM or IgG subclass (IgG2, IgG3), deficiency is reported a higher risk of recurrent infection than patients with isolated IgA deficiency. (8,18,19). In SIgAD, infection of the gastrointestinal tract has also been reported, above all protozoa, such as Giardia lamblia, that can adhere to the epithelium, proliferate, and cause infection. This condition may lead to malabsorption due to intestinal villi damage, and it is often involved in nodular lymphoid hyperplasia (20).

The association between SIgAD and celiac disease is well known. It is reported that intestinal villi damage could facilitate the entrance of some molecules through the subepidermal and submucosal tissue. This process may promote antibody production against some antigens and intolerance to certain foods. Moreover, some patients with selective IgA deficiency could be reported to be associated with Inflammatory bowel diseases, mostly ulcerative colitis (18, 21).

IgA and Covid-19

The recent literature has been reported a potential association between immunodeficiency and the Covid-19 spread cases around the world. The low Covid 19 infection rate in Japan, where the incidence of selective IgA deficiency is extremely low, may suggest that it could be a contributing factor. Conversely, countries with an upraised frequency of selective IgA deficiency, such as the USA, have several Covid-19 infections increased proportionately. The reason for this correlation can be found in the pathophysiology of Covid-19 infection. The lack of secretory IgA in the respiratory tract may promote virus attachment and penetration. Therefore, it is suggested to check the blood IgA level to take more care of who is deficient, as part of the new strategies to face Covid-19 infection. Other efforts should concern preventing the virus from entering human cells (22). Therefore, a recent study suggests using probiotics (Lactobacillus delbrueckii ssp. and Bulgaricus R-1) to increase IgA production in saliva and increase resistance to viral infections Covid 19 infection in the general population (23).

Management

There is not a recommended specific treatment for all patients with SIgAD. Each patient should be managed individually according to his/her clinical features. Asymptomatic patients with SIgAD do not require treatment. Instead, patients with recurrent upper respiratory tract infections may benefit from prophylactic antibiotics. It is generally suggested the use of broad spectrum antibiotics. Additional immunization with pneumococcal vaccines should be evaluated to increase immunity in some patients. If the patient is enduring recurrent infection despite antibiotic prophylaxis and pneumococcal vaccines, immunoglobulin therapy, intravenously or subcutaneously, should be considered, especially if patients continue to experience infections in spring and summer (24) melatonin administration may be a beneficial treatment (25-30).

Immunoglobulin serum levels and blood cell count should be periodically evaluated in patients receiving antibody replacement therapy. Finally, patient education and awareness are very important to prevent anaphylactic reactions secondary to blood transfusion and/or its product. For the same purpose, it is suggested to perform a screening test for anti IgA antibodies to assess patients risk for future infusion reactions to blood products. It is very important to remind that in patients with SIgAD, certain live vaccines (specifically oral polio vaccine, Bacille Calmette-Guerin, and yellow fever) should be avoided in patients with concomitant IgG deficiency (31).

Prognosis

The prognosis is generally good in patients with selective IgA deficiency, and particularly in young patients, spontaneous resolution could be reported. Though the possible progress into CVID is well known. Therefore, this condition, once identified, requires a regular clinical and biochemical follow up (18,21)

CONCLUSION

In the era of omic-based sciences, many basic and clinical investigators tried to dissect the pathophysiological mechanisms underlying different pediatric immunodeficiencies of variable severity, including milder forms such as IgA deficiency. The molecular pathophysiology of immunodeficiencies and other not common and not yet fully understood pediatric conditions, using next-generation and other recent technologies, showed an increasing proportion of monogenic causes underlying these childhood diseases (32-37). Several different genes and cellular mechanisms have thus been identified in recent years, and this highlighted the importance of omic sciences in defining the clinical spectrum and the therapies tailored to many disorders of infancy (38-43). Importantly, genes that regulate response to environmental factors or influence metabolic substrate or sub-cellular events have been revealed as causative for previously neglected disorders (44-52).

Our understanding of the processes associated with rare and/or multisystemic abnormalities will be pivotal for targeted treatments and the development of personalized paediatrics, according to several examples (53-59). Although IgA deficiency is the most common primary immunodeficiency, little is known about its genetic basis and the cellular and molecular mechanisms involved in IgA physiology, and future research in this field will be fundamental. Studies are necessary to investigate more about this condition to increase treatment options based on precise molecular and genetic profiles.

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Fever and tick-borne disease

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Tick-borne diseases are increasing compared to the past. This group includes various clinical conditions such as Lyme disease, Rocky Mountain spotted fever (RMSF), ehrlichiosis, anaplasmosis, babesiosis, tularemia, Colorado tick fever, and relapsing tick-borne fever. The difficulty is that the symptoms of many tick-borne infections are similar to the common childhood illnesses. Therefore, identifying the location of exposure or the feature of the rash or the identification of tick vector can help us identify the specific disease and recognize, treat, and prevent the complications of these pathologies.

Tick-borne diseases pose a serious threat to individuals of all ages, and the incidence has increased dramatically over the past decade. Since most children have nonspecific signs and symptoms such as fever, ache, rash, headache, nausea, vomiting, and malaise, other more common conditions in childhood include gastroenteritis, pharyngitis, or viral infection are often suspected. In addition, the exposed areas, identification of the specific tick vector, and evaluation of a rash, if present, support identifying the specific disease (1-2).

Overview

This group of infections includes Lyme disease, and Rocky Mountain spotted fever (RMSF), ehrlichiosis, anaplasmosis, babesiosis, tularemia, Colorado tick fever, and relapsing tick-borne fever.

Lyme disease is the most common tick-borne disease, caused by the spirochete Borrelia burgdorferi and transmitted by ticks such as *I. scapularis* or *I. pacificus*. Fever, erythema migraines, and arthralgia are the diagnostic triad for Lyme disease. The tick must be attached for 36-48 hours to transmit the disease. A migrant erythematous (EM) eruption begins at the tick bite site by three to thirty days in 70-80% of patients and lasts for three to four weeks. If an infected tick bite is not detected or treated, Lyme disease can lead to early and late disseminated manifestations that include neurologic, musculoskeletal, and cardiovascular symptoms (3).

Prophylaxis may be considered within 72 hours of tick removal if the vector is being identified, if the

Keywords: fever, tick bite, children, tick-borne disease

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0393-974X (2022)

tick has been attached for more than 36 hours, and if the patient has visited a region where the infection is endemic (4, 5). A single dose of doxycycline for adults and children eight years of age and older as prophylaxis is indicated after a tick bite. Doxycycline is contraindicated in children younger than eight years of age due to the risk of permanent tooth staining or enamel hypoplasia. Since Lyme disease is easily treatable, even after symptoms develop, watchful waiting over prophylaxis is preferred, particularly in young children (6). The diagnosis of Lyme disease is exclusively clinical; serological tests are indicated only in patients with possible exposure to ticks, who have suggestive but not typical signs of the disease of Lyme. Doxycycline, amoxicillin, or cefuroxime axetil are recommended as first-line Lyme disease treatments. Macrolides are an alternative for patients allergic to penicillin or unable to take tetracycline. People with late neurological symptoms require intravenous ceftriaxone or benzathine penicillin G (6, 7).

Rocky Mountain Spotted Fever (RMSF) is the deadliest of tick-borne diseases. RMSF is a systemic, small-vessel vasculitis that presents fever, headache, rash, nausea, vomiting, and myalgias, typically five to seven days after inoculation. Macular rash begins on the wrists, forearms, and ankles, then spreads to the trunk, and sometimes to the palms and soles of the feet, and eventually becomes petechial. Late manifestations include pulmonary haemorrhage and edema, acute respiratory distress syndrome, myocardial inflammation, acute renal failure, and cerebral edema. If high clinical suspicion is present, empirical treatment with doxycycline is recommended for all patients, including children. The gold standard for serologic diagnosis of RMSF is the indirect immunofluorescence antibody (IFA) test, but negative serologic test result from the acute phase does not exclude the diagnosis. In addition, Thrombocytopenia, hyponatremia, elevated transaminases, and hyperbilirubinemia can be found in the presence of untreated disease. The first-line treatment for RMSF is doxycycline. If doxycycline is contraindicated, Chloramphenicol could be used (8, 9).

Ehrlichiosis and anaplasmosis occur mainly with gastrointestinal symptoms, fever, chills, headache and myalgia. Diffuse maculopapular or petechial erythema occurs in up to one-third of people infected with *Ehrlichia Chaffeensis*, most frequently in children, while anaplasmosis rarely presents with a rash. Late manifestations of the untreated disease include meningoencephalitis, respiratory failure, uncontrolled bleeding, and organ failure. The mortality rate with ehrlichiosis and anaplasmosis is 3% and less than 1%. Therefore, the diagnosis should be considered in patients with flu-like symptoms, predominantly gastrointestinal. Also, for ehrlichiosis and anaplasmosis, the treatment is doxycycline in pregnant women and children. Patients who cannot tolerate tetracyclines can be treated with a course of rifampicin (4).

Babesiosis, caused by the parasite *Babesia microti*, has reached an endemic spread in the last 15-20 years. In addition to being transmitted by tick bites *I. scapularis*, it can also be transmitted through a blood transfusion or perinatal infection. Whereas most vector-borne cases of babesiosis occur during late spring, summer, or fall, transfusion or vertical transmission associated cases can occur year-round. Clinical manifestations develop one to nine weeks after exposure and result from erythrocyte lysis. They include fever, myalgia, splenomegaly, hepatomegaly, or jaundice. In addition, congenital infection with nonspecific manifestations suggestive of sepsis, including fever, hypothermia, irritability, and poor feeding, has been reported (10-12).

Complications can be acute respiratory failure, disseminated intravascular coagulation, renal failure, and splenic infarction (13, 14). Severe infection is more likely in immunocompromised or asplenia patients and is associated with a 10% mortality rate (15).

Microscopic identification of Babesia with the characteristic tetrad ("Maltese cross") on Giemsa staining of thin blood smears is pathognomonic but requires a high parasitic load, while PCR allows diagnosis even when the parasitemia is low. Mild to moderate babesiosis is preferentially treated with atovaquone plus azithromycin. Intravenous clindamycin plus oral quinine is recommended for more severe infections, and exchange transfusion

may be required, particularly in cases of high parasitemia or hemodynamic instability (16).

Tularemia is caused by *Francisella tularensis* and can be transmitted by a tick, handling infected tissue, eating undercooked meat, infected animals, or inhaling dust or aerosols. The clinical features vary between ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonia and typhoidal tularemia. Ulceroglandular disease, the most common form of tularemia in children, appears like a maculopapular lesion at the tick bite site with subsequent ulceration and painful lymphadenopathy, usually in the armpit or groin. The diagnosis can be obtained from a culture of the infected tissue and alternative tests like PCR, immunohistochemistry staining or serology. HMGB1 serum level may be altered (17-23), and the treatment is based on the use of aminoglycosides (Streptomycin or gentamicin) or doxycycline or ciprofloxacin in mild tularemia (24).

Colorado tick fever is a self-limited viral disease transmitted from an infected tick Rocky Mountain wood. This disease typically starts three or five days after inoculation, presenting high fever biphasic, myalgias, headache, pharyngitis, photophobia or disseminated intravascular coagulation (25). There is no specific treatment, and the diagnosis is possible thanks to serologic testing, PCR and laboratory findings like leukopenia and thrombocytopenia. (24)

Tick-borne Relapsing fever is a rare infection transmitted by Ornithodoros tick species that usually live in rustic rodent-infested cabins in the mountains, in mountain areas of the western United States. This disease is characterized by high fever, headache and myalgias for about three days, seven days without fever and another three days with fever. This process can repeat if antibiotic treatment is not used (26). Uncommon complications include meningitis, ARDS, ocular manifestation (iritis, uveitis) or myocarditis (27-31).

The diagnosis consists of observing spirochetes in a dark field microscopy or with Wright or Giemsa stain. Tetracyclines are the first-line treatment for adults, rather than children younger than eight years and pregnant erythromycin. In patients with central nervous system events, it is used ceftriaxone intravenous or *Penicillina G*. It is also possible the Jarisch-Herxheimer reaction that occurs in the first time of treatment, and is due to the cytokine release with fever, rigours and hypotension. Melatonin administration may be a beneficial treatment (32-37).

DISCUSSION

In the last decade, omic-based and experimental investigations yielded an increasing molecular and pathway complexity as causative of several pediatric infectious diseases, including tick-borne disease (38); this is observed in the context of advances brought by basic sciences regarding the molecular understanding of many pediatric conditions, both rare and common, associated with either genetic or non-genetic etiologies (39-46). Several pathomechanisms have been identified, allowing better pediatric patient care in clinical diagnosis, prognosis and treatment (47-56). Pediatric disorders can often result from the combination of immuno-mediated and genetic factors, and their aberrant interplay may lead to multisystemic involvement and metabolic and neurological abnormalities as part of the clinical phenotype in some cases (57-60). These etiologically complex disorders may include a variety of monogenic and polygenic/genetically complex and/or environmental conditions with increasingly widened molecular heterogeneity and implicating a wide array of disease mechanisms that could be potentially drug-targeted (61-68).

CONCLUSION

In conclusion, we can affirm that tick-borne diseases have a not yet fully understood pathophysiology regarding the disease outcome and prognosis and an increasing epidemiological relevance. These diseases can only be diagnosed clinically, and laboratory tests can give false negatives. Antibiotic therapy in an initial study is generally decisive to avoid complications, especially in more fragile subjects such as pregnant women, the elderly and children. For prevention, it is essential to avoid direct contact with vegetation wear light-coloured, protective and repellent clothing that allows you to easily identify and remove ticks and carry out checks and washing.

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Management of complicated acute appendicitis

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Appendicitis, denoting the appendix inflammation, is the most common emergent condition in children (1). The global incidence of acute appendicitis (AA) ranges between 76 and 227 cases per 100,000 population annually (2). The acute appendicitis lifetime risk varies from 7-8% in the USA (3) to 16% in South Korea (4). All age groups could be affected, but there is a peak incidence in children of 10 to 20 years of age (5).

Pathophysiology

Acute appendicitis results from obstruction of the appendiceal lumen, usually by lymphoid hyperplasia but in some circumstances by a foreign body or a fecalith, foreign body (5). The obstruction cause distention and often also bacterial overgrowth and severe inflammation, which may lead to ischemia. If a prompt surgical evaluation and treatment are not possible, complications may occur, including necrosis, gangrene, and occasionally perforation. Recently, some genetic factors have been identified as potentially causative of severe acute appendicitis (6). The genetic factors may also contribute and interact with additional environmental causes, including metabolic and immunomediated causes (7). This intricated pathophysiology reflects how complex molecular mechanisms underlie different pediatric emergency conditions (8-16). In fact, in recent years, next-generation sequencing technologies, including exome and mRNA sequencing studies, have been essential in dissecting the pathophysiology of a wide array of childhood disorders (17-19), this led to a better understanding of the sub-cellular alterations underlying diseases, including several regulatory networks and genetic together with non-genetic factors (20-24).

Diagnosis

Based on the appendix's histological evaluation or macroscopic appearance, AA may be defined as uncomplicated appendicitis (UA) or complicated appendicitis (CA), the latter defined by a gangrenous, necrotizing or perforated appendix on pathology examination or the presence of purulent peritonitis. The incidence of CA varies between 15% and 50% of all patients with AA. (25) Despite all the excellent papers and research in literature, clear and definitive guidelines about the AA diagnosis are not well established.

Classic symptoms of AA, such as pain in the right iliac fossa, vomiting, fever, occur in less than half of the patients; in the other half of children, many further symptoms overlapping with other pathologies could appear, leading to a more challenging diagnosis. (25) It is evident that a good clinical examination, with physical signs investigation, is fundamental to orientate

Keywords: necrotizing Enterocolitis, full-term, intestinal disease, neonatal disease, microbiome, early diagnosis

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0393-974X (2022)

the diagnosis. Laboratory tests could help AA diagnosis and management, even if there is not a specific blood marker for AA. White Blood Cells (WBC) combined with C-Reactive Protein (CRP) do not predict AA directly, but they increase the odds. (26) Furthermore, WBC and CRP together with Procalcitonin (PCT) are usually used to differentiate UA from CA; moreover, CRP level greater than 10 mg/L and leucocytosis greater than 16,000/mL are solid predictive factors for appendicitis in pediatric patients. (27). HMGB1 serum level may be altered (28-29).

Imaging is often required to support symptoms, clinical examinations, and laboratory tests in the diagnosis. Ultrasound (US) is the first-line imaging because of its wide availability, low cost, and absence of ionizing radiation. It usually allows to diagnose AA, sometimes even differentiating UA from CA. The US diagnosis relies on direct signals for AA, such as a diameter greater than 6 mm, a wall thickness greater than 3 mm, non-compressibility of the appendix, appendicolith, or US Color Doppler hypervascularity in early stages, along with indirect signs for local inflammation, such as free fluid surrounding the appendix, abscess formation, increased echogenicity of local mesenteric fat or enlarged local mesenteric lymph nodes. (30) In all the situations where the diagnosis is still unclear, a Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) could be used.

Complicated appendicitis management

CA could be managed differently with operative management, including early appendectomy at presentation and initial antibiotic treatment followed by delayed appendectomy, or conservative management, using antibiotic treatment alone without any appendectomy. Predicting values for CA are age < 5 years, symptoms duration more than 24h, WBC greater than 12,000/ml, CRP greater than 10mg/l (30).

Loss of submucosal layer, hyperechoic periappendiceal fat greater than 1 cm, maximum outside diameter more than 6 mm, presence of an appendicolith are significantly associated with CA (31, 32).

Operative management

Early appendectomy at presentation is adopted as treatment of choice in many centres because it reduces adverse events and unplanned readmissions. Appendectomy, the surgical removal of the appendix, is performed primarily as an emergency procedure, usually within the first 24 h of hospitalization, to treat acute appendicitis (33). Appendectomy could be performed using an open or laparoscopic approach. However, lower postoperative pain, fewer postoperative complication, and better quality of life seem to be associated with minimally invasive surgery (MIS), therefore for both UA and CA, laparoscopic should be preferred over open appendectomy where MIS equipment and expertise are available (27).

Early appendectomy is reported as the treatment of choice for CA in many studies, but few studies with substantial scientific evidence are available at this time. However, even if the timing for surgical management in CA is still debated, a recent metanalysis underlined that early appendectomy reduces the unplanned readmissions, complications, and hospitalization costs (34).

An appendectomy performed 6 to 8 weeks from the initial diagnosis is called “interval”. Operating when peritoneal contamination has resolved seems advantageous for the patient because it potentially results in fewer intraoperative and/or postoperative complications (35).

Analyzing the type of CA, Fugazzola et al. found that immediate operative management is the favoured option if perforated appendicitis without abscess is suspected. At the same time, delayed appendectomy should be performed in CA with abscess or phlegmon; when the imaging-based greater risk of operative complications is suspected (36, 37).

Non-operative management

However, the usual indications that an urgent surgical intervention is required for CA has changed in the last decade. Antibiotic therapy could be used to manage CA without significantly compromising complications, return to the emergency department, readmission, failure of non-operative treatment requiring surgery. Pennell et al. conducted a prospective study to implement the non-operative CA management, founding that a standardized protocol for a conservative approach reduces resource

utilization without affecting the outcomes (38).

A European survey revealed that 96% of surgeons administrate a preoperative antibiotic therapy for CA, and most of them choose a triple therapy (aminoglycoside, b-lactam and an anaerobe covering) (39). However, broad-spectrum single (piperacillin/tazobactam) or double (ceftriaxone and metronidazole) therapy seems to be similarly effective and less costly than triple therapy, and they are recommended from the guidelines of the American Pediatric Surgical Association (40). The antibiotic therapy duration should be based on clinical (pain, fever, bowel function) and laboratory criteria (white blood cell count); 5 days intravenous antibiotics and 2 days oral antibiotics (7 days in total) should be the administration (40).

When a conservative approach with antibiotic therapy is chosen, it should also be considered a treatment option for intra-abdominal or pelvis abscess, which seems to be associated with CA in 3.8% of patients (41). Beneficial results for complications and patient recovery have been found with percutaneous abscess drainage (42). In contrast, significant complications, such as intestinal and bladder perforations, have been reported for patients undergoing the drainage procedure compared with the interval appendectomy group, where a phlegmon treated with antibiotic therapy was the only concern. (43, 44). In order to reduce the occurrence of complication rate, Gasior et al. proposed to drain only abscesses greater than 20 cm^2 (45). Therefore, although disagreements for percutaneous abscess drainage, there is evidence that, in selected patients, it may have positive results; in fact, non-operative management with antibiotics and percutaneous drainage, when available, for CA with a periappendicular abscess, is suggested in settings where laparoscopic expertise with a very low conversion rate is not available (27). Melatonin administration may be a beneficial (46-50).

CONCLUSION

In conclusion, the type of antibiotics and its length for CA conservative treatment needs further investigation with RCTs. Early operative management is favoured for CA without abscess, while delayed non-operative management is preferred for CA with abscess or phlegmon. Nevertheless, because of the lack of high-quality studies, there is a need for more RCTs to delineate the specific management strategy for CA.

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Intussusception, infection, and Rotavirus vaccine: a review of literature

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Rotavirus (RV), an icosahedral RNA virus in the family of Reoviridae, is the most common cause of gastroenteritis in infants and children <5 years old. RV was first discovered in stool specimens from children with gastroenteritis by Ruth Bishop and collaborators in 1973 (1,2). In Europe, more than 700,000 medical consultations, 87,000 hospitalisations and 20 to 40 deaths per year for RV illness were estimated (3). Instead, in most developing countries, RV gastroenteritis occurs all year round and is responsible for considerable medical and societal costs besides being a major cause of morbidity and mortality, influencing the drive to develop a vaccine (4).

Intussusception (IS) is the most common cause of intestinal obstruction, usually occurring between 4 and 10 months of age. It is a medical emergency that may become life-threatening if not promptly and adequately treated (5). IS can occur in different parts of the gut, but the most frequent site is the ileocolic valve, through which ileus tends to telescope inside the ascendant colon, leading to vessels compression, ischemia and bleeding, hesitating in the typical sign: "red currant jelly stool" (4). Other clinical features are periodic abdominal pain, nausea and bile vomiting (5). The natural history of the illness is to progress in necrosis, perforation and, sometimes, death. The majority of cases do not recognise a definite cause, remaining referred to as idiopathic. Different potential risk factors are considered leading pathological points such as Meckel's diverticulum, duplication cysts, polyps, hematomas, Henoch-Schonlein purpura, nephritic syndrome and lymphomas (4, 6). Viral infections, such as adenovirus and rotavirus, seem to cause the IS process. While an association with adenovirus infection has been demonstrated, evidence of an association with RV infection is still unclear. (7)

This brief narrative review summarises the possible role of infection and rotavirus vaccine in causing IS in children.

Pathophysiology of Rotavirus-related intussusception

Even if historically, adenovirus is the primary pathogen frequently individuated, some studies revealed an association with RV infection and IS. (8-10) Viral infections, which infect intestinal lymphatic components, have been shown to cause hypertrophy of Peyer's plaques, *Primum movens*, to trigger intussusception. Regarding RV, the infection has been associated with increased distal ileum wall thickness and lymphadenopathy; through this, a pathophysiological mechanism, RV infection, could cause IS (11). However, in evaluating this relationship worldwide, conducted analyses gave conflicting results (12). Bines et al. studied risk factors for the development of IS in infants in Vietnam and Australia. They concluded that the incidence of intussusception

Keywords: Rotavirus, children, gastroenteritis

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0393-974X (2022)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

was significantly higher in Vietnam than in Australia. A strong association between adenovirus infection and IS was identified in both countries, while no correlation with rotavirus infection was found (13). Willame et al. investigated the risk of IS after rotavirus gastroenteritis in the first year of life, concluding that there was a temporal association between IS and RV infection (7).

Other studies in emerging countries do not demonstrate a clear relationship between RV-infection and IS (14-18). A study on the annual trend of hospitalisations for IS comparing it with that of rotavirus gastroenteritis (GARV); was conducted in Italy to evaluate IS and GARV incidence rates in children hospitalised between 2005 and 2014. The authors stated that the distribution of the hospitalisation rates by months of hospitalisation showed the absence of seasonality, in contrast to hospitalisation rates for GARV (19). In addition, a study was performed with viral testing of stool samples from patients with IS to determine which viruses may have an association. This study showed that IS is associated with non-enteric adenovirus infections and Enterovirus B infections.

A statistical association with rotavirus and IS was observed, but it was impossible to prove whether this was related to the vaccine strain or the rotavirus wild type (20). Interestingly, some genetic factors have been proposed as causative and/or contributory intussusception following the Rotavirus vaccine (21). The genetic factors likely interact with additional environmental causes, including metabolic and immunomediated causes, similar to what occurs in many complex multifactorial pediatric conditions (22-27). This intricated pathophysiology reflects how complex molecular mechanisms underlie different pediatric and/or emergency disorders (28-32). In fact, in recent years, emerging technologies, including DNA sequencing and additional omic-related studies, have been revealed as important in understanding the mechanisms of several diseases of childhood, with important consequences for patient diagnoses, prognosis and possible treatment (33-36).

Rotavirus vaccine and intussusception

Many papers are reported on the association between the rotavirus vaccine and intussusception. Different types of anti-RV vaccine are available worldwide including: monovalent (RV1) (Rotarix), pentavalent (RV5) (RotaTeq), monovalent human-bovine (116E) (Rotavac), oral bovine pentavalent (BRV-PV) (Rotasiil) and human neonatal (RV3-BB). A human reassortant rotavirus tetravalent vaccine (RRV-TV) (Rotashield) was released in 1998. During the post-license evaluation period, numerous IS cases after administration of RRV-TV have been reported. Consequently, immunisation with RRV-TV was suspended, and the manufacturer voluntarily removed the vaccine in October 1999 (4).

Following a thorough pre-authorisation safety assessment regarding IS, two second-generation RV vaccines (HRV, monovalent Rotarix; GSK and HBRV, pentavalent RotaTeq) has been recommended by World Health Organization (WHO) since 2009 for use worldwide in routine RV vaccination programs in infants. In a meta-analysis of 5 studies on the effects of different RV vaccines, evidence suggests an increased risk of IS during the 7-day post-dose period 1 for HRV and 5.5 for HBRV and to a lesser extent during the 7-day post-dose period 2 for HRV and 1.7 for HBRV. These data suggest a class effect for both RV vaccines regarding the risk of IS after immunisation. However, the WHO Global Vaccine Safety Advisory Committee stressed that the benefit-risk profile of both licensed RV vaccines remains favourable, with the benefits outweighing the risk of IS. The risk of IS attributable to RV vaccination depends on age, and recent evidence suggests that the absolute risk of IS is only slightly increased by vaccination (increased risk attributable to vaccination was 1.7 for HRV and HBRV) when the vaccines are administered within the recommended time window of <3 months.

A surveillance study conducted in seven African countries reported a relative risk of 0.25 (<0.001–1.16) and 0.76 (0.16—1.87) during 1-week post-dose 1 and post-dose 2 of HRV, respectively when administered in children <3 months of age (37). Reddy et al. reported their results after administration of Rotavac, an oral monovalent produced in India. They conclude that Rotavac does not increase the incidence of IS in Indian infants (38). The introduction of RotaTeq vaccination in New Zealand resulted in a significant reduction in rotavirus hospitalisation and a halving

of hospitalisations for all-cause gastroenteritis. Furthermore, the study highlights no changes in the incidence of IS, although this complication occurred in the risk period immediately following the vaccine (39).

A recent study suggests that introduction of rotavirus vaccination in England has resulted in a downward shift in the age at which intussusception occurs in infants, with no overall increase in hospital admission rate or disease severity. These findings support the view that the benefits of rotavirus vaccination outweigh the small increased risk of intussusception in the early post-vaccination period (40).

Considered the role of hypertrophied Peyer's patches in the pathogenesis of IS, Willame et al. highlight that the administration of the first dose of RV-vaccine might be performed in the first weeks postnatally when Peyer's patches are not yet fully developed. (4) Moreover, Clark et al. suggest administering the first dose of RV vaccine at birth to avoid all vaccine-related cases of ISS and reveal the exigency to evaluate the benefits and risk of RV vaccines at the national level due to variability of ISS incidence by country and region (41). In support of this evidence, Cho et al. argue that the incidence differences were due to genetic predisposition, environmental differences, including circulating pathogens, dietary pattern of infants, breastfeeding, and accessibility to medical care (42). Lu et al., in a recent systematic review and meta-analysis, analysed 25 randomised clinical trials (RCTs) including 200.594 participants (104.647 received vaccine), revealed no association between RV-vaccine and IS among neonates or infants (43). Benninghoff et al. argue that the meta-analysis mentioned above, including only randomised clinical trials, is inadequate to identify a potential increased IS risk. Additionally, the safety profiles of newer RV vaccines in clinical studies with limited sample size were considered comparable with that of the well established and widely used RV vaccines, RotaTeq and Rotarix evaluated.

The Authors believe that extensive and updated evidence from post-marketing surveillance indicates a slightly increased risk of IS, mostly within 7 days of RV vaccination, with a benefit/risk profile assessment favouring RV vaccination (44).

This mini-review allows us to conclude that RV infection is closely linked to its prevention with the vaccine. This problem is even more important for developing countries where RV mortality is still high. At the same time, we believe it useful that active surveillance clinical trials are conducted by government public health on a large pediatric population to define with certainty the correlation between RV immunisation and IS.

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Necrotising enterocolitis features in full-term infants: an update

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Necrotising enterocolitis (NEC) is among the most common and most destructive diseases occurring in neonatal intensive care, assessing as sepsis is the third leading cause of neonatal mortality (1) and affects both pre-term and full-term babies, although different incidence rates. NEC might be considered a spectrum of different diseases or endotypes and, even if it is one of the most investigated pathologies, not much progress has been made in its treatment or prevention, allowing it to still have dangerously high morbidity and mortality (2). The authors aim to provide a concise update on the NEC in full-term newborns through this review.

Epidemiology

Although the majority of cases of NEC occur among premature infants, a small subset of babies born at term or shortly before developing NEC-like gastrointestinal signs and symptoms, frequently in association with other conditions (3).

Approximately 90% of cases of NEC occur in pre-term infants, with an incidence of about 12% in infants weighing less than 1500 grams at birth, while about 10% of NEC cases occur in full-term babies (gestational age greater than 37 weeks) (4, 5).

Risk factors and pathogenesis

Even if no studies have found a clear genetic phenotype strongly associated with NEC, studies show a familial predisposition for the disease (6).

While pre-term neonates are more susceptible to intestinal injury due to the underdeveloped nature of their intestine, proposed risk factors for NEC in term infants include cyanotic heart disease, perinatal asphyxia, hypoglycemia, polycythemia, respiratory distress syndrome, protracted diarrhea, pre-eclampsia, cocaine abuse during pregnancy, cows' milk allergy and anti-C Rhesus incompatibility (7).

Congenital heart disease (CHD)

Several studies identified a connection between CHD and NEC in term infants. The frequency of this connection ranged from 7.6% to 38% of term infants with NEC (7).

It has been suggested that a common feature in many congenital heart lesions associated with NEC is the combination of a widened pulse pressure and low diastolic pressures (8-10), which could trigger poor intestinal perfusion, contributing to the onset of NEC (11).

Perinatal asphyxia and Meconium Aspiration Syndrome (MAS)

The finding that the risk for NEC is correlated with asphyxia also strongly supports the notion that insufficiency in mesenteric flow is a risk factor for

Keywords: Necrotizing Enterocolitis, full-term, intestinal disease, neonatal disease, microbiome, early diagnosis

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0393-974X (2022)

NEC development. In addition, MAS is considered a major cause of respiratory failure, implying higher levels of neutrophils and pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, and TNF-α also (12) contributing to NEC development.

Perinatal sepsis

Early perinatal infection is considered a major risk factor for NEC. On 39 full-term NEC, Short et al. (13) documented bacteraemia in 64% of patients, including *Klebsiella, Escherichia coli, Clostridium difficile*, *Treponema pallidum* and Rotavirus.

Congenital Diseases

Bolisetty et al. workgroup (14,15), analysing twenty-nine infants with NEC, found out that about 66% of them suffered from congenital illnesses and, among other things, 17% was affected by endocrinal dysfunctions such as congenital adrenal hyperplasia, hypopituitarism, hypoparathyroidism and/or hypothyroidism.

Hypoglycemia

Diazoxide, an agonist of pancreatic β-cell K-ATP channel employed in neonatal hypoglycaemia treatment, often determine severe G.I. disturbances, like feeding intolerance and NEC signs (16).

Feeding

NEC severity resulted in being affected by both complete withholding of feeds in the immediate afterbirth period (because of intestinal atrophy and increased microbic translocation) (17) and early enteral nutrition (because the faultily digested formula is hypothesised to provide a substrate for microbial proliferation and higher risk of tissues hypoxia related to the increase of oxygen demand during nutrient digestion) (7).

Some authors showed a direct relationship between initiation of feedings (reported as earlier in F.T. babies) and NEC occurrence (18). In this regard, it is hypothesised that (a) incompletely digested formula can provide a substrate for bacterial proliferation, and (b) feeding increases intestinal oxygen demand during nutrient absorption, thus increasing the risk of intestinal tissue hypoxia that can cause mucosal injury and bacterial invasion (5).

Drugs early employment

According to Haque (17), clinicians should care about the use of certain molecules during the first months of life:

Antibiotic therapy: this may adversely affect the gut innate and adaptive immune systems, playing a role in NEC pathogenesis;

H2 blockers: in reducing gastric acidity, these drugs seem to empower gut bacterial increasing the risk to develop NEC;

Blood transfusions: neonatal enterocytes are proven to express A.B. blood group epitopes whose, becoming a check of serum antibodies, may lead to the so-called “post-transfusion NEC”.

Pathogenesis

NEC-onset timing seems to be noteworthy concerning clinical features and mortality. Qiu-Yu et al. (19), reviewing medical records of 253 full-term infants affected by Early-Onset NEC (EO-NEC) (median 2 days) and Late-Onset NEC (LO-NEC) (median 14 days), showed that vaginal delivery was identified more frequently in EO-NEC than LO-NEC full-term infants. The results of prior studies (20) have indicated that vaginal delivery was also associated with an increased risk of EO-NEC in pre-term infants. Recently, genetic and non-genetic factors have been identified as potentially causative of severe NEC (21, 22).

In the context of this disease model, genetic factors may contribute and interact with additional environmental causes, including metabolic and immunomediated causes. This complex pathophysiology reflects how complex pathophysiological mechanisms underlie different pediatric and/or surgical conditions (23-29).

Importantly, in recent years, next-generation sequencing technologies, including exome and mRNA sequencing studies, are essential in understanding the pathophysiology of a wide array of childhood- or pediatric-onset disorders (30-32), this led to a better understanding of the sub-cellular alterations underlying diseases, including several regulatory networks and genetic together with non-genetic factors (33-37).

Also, in NEC, the determinant role seems to be played by the Gut Microbiome; as displayed in Table I, F.T. infants are first colonised by aerobes bacterial species, whose oxygen consumption allows subsequent colonisation of anaerobic ones (38).

Mai et al. postulated that NEC patients have an increase in Proteobacteria and a decrease in Firmicutes between 1 week and <72 h prior to detecting clinical NEC (39). Changing the microbiome is a promising target for future therapies (40).

Shielding factors

Due to the lack of effective treatments for NEC, prevention has been included in the focus of researchers. Specifically speaking, evidence underlined the role of maternal milk feeding and probiotics.

Maternal milk feeding

Thanks to the presence of beneficial immune mediators in high concentration within colostrum and mother's own or banked milk, to early expose infants to them has been shown to supply bacterial and anti-inflammatory protection, as well to boost the gastrointestinal development, through substances as specific oligosaccharides and prebiotics, nitrate and nitrite antioxidant factors, L-arginine secretory IgA,

Table I. *Comparison of microbiome between full-term and pre-term infants.*

COMPARISON OF MICROBIOME BETWEEN FULL-TERM AND PRE-TERM NEONATES

FULL-TERM INFANTS	PRE-TERM INFANTS
Streptococcus, Staphyococcus, Escherichia coli, Lactobacillus and Enterobacter as first colonization	⇑ Facultative anaerobes
Clostridia	⇓ Bifidobacterium
Bifidobacteria	⇑ Staphylococcus
Other Firmicutes	⇓ Bacteriodes
	⇑ Escherichia coli, Klebsiella, Staphylococcus

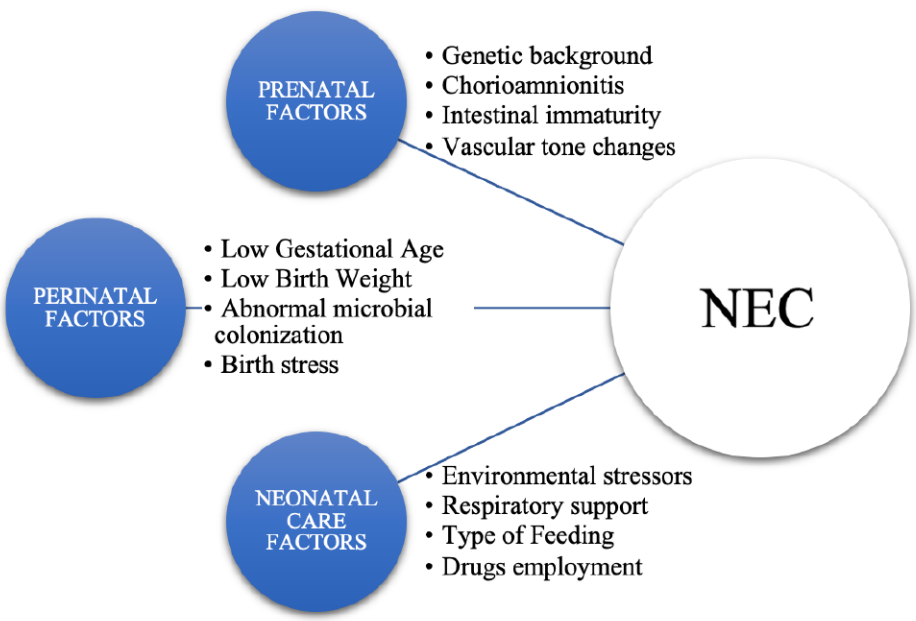

Fig. 1. *Risk factors for NEC.*

acetylhydrolase, Platelet-Activating Factor (PDA) and lactoferrin (41).

Probiotics

The core is the role of probiotics down-regulating pro-inflammatory gene expression, upregulating cytoprotective genes and regulating cellular immunity (42).

Wang et al. (21), in a meta-analysis of 20 randomised controlled trials, have shown that probiotics content of Lactobacillus and Bifidobacteria was able to reduce both the incidence and mortality from NEC, in contrast with probiotics containing only Bifidobacteria, which reduced the NEC incidence but with no effect on mortality rate.

Clinical onset and diagnosis

Clinical presentation of the infants in full-term babies is similar to that described for pre-term infants, including abdominal distension, vomiting, bloody stools and septic appearance (7).

Necrotising enterocolitis tends to have an earlier and more rapid onset after feeding in the F.T. infant (i.e. there is an inverse relationship between gestational age and age of onset of NEC). However, there are fewer systemic manifestations and perforation or transmural bowel necrosis, with classical abdominal X-ray findings occurring less frequently in the full-term neonate (43,44). In addition, infants with OE-NEC seems to be greater in gestational age than LO-NEC infants (19). Therefore, suspicion of NEC is based on Modified Bell's Staging Criteria, explained in Table II.

Urboniene et al. (45) have evaluated the Resistive index (R.I.) and the pulsatility (P.I.) of the superior mesenteric artery in pre-term infants and found 96.3% of infants with NEC had an R.I. of >0.75 whereas 88.9% of infants with NEC had a P.I.> 1.85, suggesting Doppler measurements of intestinal blood flow as a useful bedside tool for predicting and diagnosing NEC. In addition, portal Vein flow lower than 37 ml/min was present in 89.7% of patients with NEC (45).

A new diagnostic strategy called GutCheckNEC was recently developed for the timely recognition of NEC. This tool kit creates a weighted composite risk score for NEC taking into account prior mentioned risk factors and protective ones. (46).

The differential diagnosis of NEC includes ileus secondary to septicaemia, primary bowel pathology, spontaneous bowel perforation of intestines, congenital intestinal obstruction (pyloric stenosis, ileal atresia, Hirschsprung's disease), neonatal appendicitis and colitis (46).

Biomarkers for early diagnosis

Research effort has focused on discovering biomarkers capable of predicting and differential-diagnose NEC (11). Among them, the most promising is the Fecal Calprotectin (a reported sensitivity ranges from 76 to 100%, and the specificity ranges from 39 to 96%) (47), Intestine-Fatty Acid Binding Protein (I-FABP) (28), Claudins, TreFoil Factor 3 (TFF3) (47).

Management

NEC requires medical or surgical management based on the clinical presentation. Close clinical observation is recommended when a NEC is suspected of increased abdominal distention or unexpected feeding intolerance. Moreover, it can be considered a bowel decompression using a nasogastric tube and brief discontinuation of feeding (e.g., 24 hr). Also, in this stage, serial abdominal radiograph, monitoring of white-cell, differential, and platelet counts (decreases suggest progression of the disease), blood cultures and a short course of intravenous antibiotics are suggested (48).

If a NEC is confirmed (without sign of bowel perforation), medical intervention typically includes abdominal decompression, bowel rest, discontinuation of enteral feeding for 7-10 days, broad-spectrum intravenous antibiotics (for 7-10 days), and intravenous hyperalimentation. In addition, close radiological monitoring and strict monitoring of white-cell, differential, and platelet counts, blood culture is strongly recommended (49).

Surgical treatment of NEC (exploratory laparotomy with resection if necessary or placement of drain) is necessary in case of free intraperitoneal air on abdominal radiograph or persistent ileus pattern, abdominal distention, and radiographs that show an absence of bowel gas, coupled with deteriorating clinical and laboratory values (e.g., decreasing neutrophil and platelet counts) (50). melatonin administration may be a beneficial treatment (51-55).

Prognosis

Overall survival rate did not differ between the groups (65% vs 69% for F.T. and P.T., respectively) (18). Short et al. (13), in a two-centre retrospective review of 39 NEC-affected babies, assessed overall mortality of 18%; multivariate analysis revealed the late onset of NEC to be an independent predictor of mortality.

The results of the subgroup analysed by Qiu-Yu (19) indicate that peritonitis and kidney failure were independent risk factors for mortality in infants with EO-NEC and that peritonitis and respiratory failure were independent risk factors for mortality in LO-NEC infants.

NEC results to be the most common cause of Short Bowel Syndrome in children, following the resections of ischaemic intestinal segments and resulting in inadequate nutrition absorption and other related complications. Moreover, infants who come through NEC and its medical or surgical treatment are proven to be at increased risk to tackle neurological and cognitive alterations, like psychomotor impairment and cerebral palsy, due to brain susceptibility to early-in-life insults (41).

Summarising, Necrotizing Enterocolitis can be considered among the most destructive diseases in the neonatal period based on the difficulty of early diagnosis, management and outcome, which often has upshot an enormous disease burden and poor life quality.

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Infective endocarditis in children: state of the art

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Infective endocarditis (IE) is an infection of the endocardium and/or heart valves. Although more common in adults than in children, it is important to identify and treat IE because of its significant morbidity and mortality. This review summarizes the main features involved in the IE pathogenesis and treatment to reduce and prevent IE risk. A comprehensive search of published literature using the PubMed (HTTP:// www.ncbi.nlm.nih.gov/pubmed/) database was carried out to identify all articles published in English in peer-reviewed journals. The search terms included ("Infective Endocarditis") AND ("Young" OR "Children" OR "Adolescence").

Infective endocarditis (IE) is an infection of the endocardium and/or heart valves less common in children than adults. It can lead to thrombus formation (vegetation) on the endocardium that may cause valvular stenosis, abnormalities in the conduction system, myocardial abscesses and mycotic aneurysms. The annual incidence rate of IE had not a significant trend. In the United States, from 2003 to 2010 is between approximately 0.05 and 0.12 cases per 1000 children. Moreover, the annual frequency of IE among children has increased in recent years. It may be related to the increasing congenital heart defect (CHD) corrective surgery and/or increasing hospitalized newborn infants (1-3).

In the past, rheumatic heart disease was the predominant underlying condition predisposing the development of IE. However, over the last three decades, there has been a constant decline of its incidence in developed countries and an increase of IE in cases with early surgical correction of CHD, palliative shunt procedures and/or complex intracardiac repairs. The cardiac surgery of CHD has lengthened the survival of children affected by heart diseases and has increased the risk of IE substantially during the postoperative period of cardiac surgery. In healthy children with normal heart structure, invasive techniques during neonatal and pediatric intensive care are another high-risk factor of IE. In addition, the use of central venous catheters (CVC) carries to bloodstream infections and endocardium infection. In

Keywords: endocarditis, children, infection

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0393-974X (2022)

adults, other known risk factors of IE, rarely found in the pediatric population, are intravenous drug abuse and degenerative heart disease (4).

Pathogenesis

Endothelial damage is the first factor contributing to the development of IE and is caused by direct or indirect trauma. The use of intravascular catheters near the heart or intracardiac devices in intensive care causes direct damage to the endothelium. The inflammation or turbulent jets of blood due to stenotic or regurgitant valves in CHD or acquired cardiac abnormalities cause indirect trauma. The damaged endothelium is necessary for the host response and initial pathogen colonization. Indeed, the tissue injury forms the initial platelet and fibrin deposit, known as nonbacterial thrombotic endocarditis (NBTE). Then the bacteria or fungi colonize on this nidus and form a dense endocardium mass (vegetation), visible at echocardiographic examination (4,5).

Dental procedures are frequent causes of bacteremia. Microorganisms also enter through the skin of percutaneous catheters or catheter lumen, mucosal surfaces and/or other focal infection sites. These pathogens float in the bloodstream and deposit in the heart valves on preexisting NBTE. Some organisms seem to bind to fibronectin, laminin, and collagen components of damaged endothelium; other pathogens such as *S. aureus* may bind directly to endothelial cells. The virulence factors involved in bacterial adherence are proteins, so-called adhesins, that interact with host proteins in a site of endothelial damage and could be operative in bacterial persistence (6).

The most frequent pathogens of IE in children are gram-positive cocci. *Streptococci viridans* is more frequent after dental procedures; *Staphylococcus aureus* in children with CHD and *Enterococcus species* after invasive procedures in the terminal tract of the intestine or genitourinary apparatus. Negative coagulase staphylococci are common in the presence of a permanent central venous catheter. Other gram-negative microorganisms are defined as the HACEK group of organisms (*Haemophilus species, Aggregatibacter species, Cardiobacterium hominis, Eikenella corrodens, and Kingella species*) are involved less frequently, and in about 6% of cases, the blood cultures are negative. Fungal infections are very rare (7).

Furthermore, in recent times genetic components have been identified underlying severe infective endocarditis in children (8). Thus, genetic factors may contribute and likely interact with further factors from the environment and induce the risk of the disease as part of complex multifactorial pathophysiology. These complex disease models reflect how molecular and aetiological mechanisms underlie a large number of pediatric and/or emergency illnesses (9-15). DNA sequencing and omic-related technologies, such as mRNA sequencing studies and biomarker profiling, have been revealed to be crucially involved in the underlying pathophysiology of various childhood- or pediatric-onset diseases (16-20). These advances provide us with a better comprehension of the fine alterations causing pediatric diseases, such as the intricate interplay between both genetic and non-genetic (e.g., immunomediated, endocrinological, metabolic) factors (21-28).

Clinical manifestation

The clinical findings in children are often mild and non-specific (high temperature or low-grade fever with protracted history of weight loss, anorexia, arthralgias, myalgias or general malaise). This cluster of non-specific symptoms requires careful evaluation for IE in patients with CHD and infant newborns with no underlying structural heart disease but who have undergone invasive procedures or use of indwelling lines. The typical clinical findings of IE are correlated to different phenomena. Fever, anorexia, and malaise are related to bacteremia or fungemia. The appearance of heart murmurs with new or changed characteristics and/or congestive heart failure are related to inflammation of heart valves. In children with IE-related to cyanotic CHD or long-term CVC, the murmur may not change; rather, the disease can manifest with declining systemic oxygen saturation caused by obstruction to the flow of valvular infection or asthma-like symptoms caused by septic pulmonary emboli (29,30).

Lastly, the extracardiac manifestations are more frequent in adults and related to immunological responses, such as Petechiae, hemorrhages, Roth's spots, Janeway lesions and Osler nodes (31). These

latter phenomena may be indicative of vasculitis caused by circulating antigen-antibody complexes. Osler nodules are painful intradermal nodules present on the fingertips. Janeway lesions are small non-painful erythematous or hemorrhagic lesions on the palm of the hands and the sole of the feet. Splinter lesions are linear cone lesions under the nails; Roth's spots are non-specific red spots with white or pale centres seen on the retina. Late manifestations (meningism, increased intracranial pressure, focal neurological signs) may be determined by severe neurological complications such as stroke embolism, cerebral abscesses, mycotic aneurysms and hemorrhages. Other manifestations of systemic emboli are rarer except in the case of mitotic disease (32-34).

Investigations

Blood culture and transthoracic echocardiography

Table I. *Modified Duke's Criteria for diagnosis of IE.*

Major criteria

1. Positive blood culture for IE

A. Typical microorganism consistent with IE from > 2 blood cultures, as noted below

1) Viridans streptococci,* *Streptococcus bovis*, or HACEK group or
2) Community-acquired *Staphylococcus aureus* or enterococci, in the absence of a primary focus or B.Microorganisms consistent with IE from persistently positive blood cultures, defined as:
   2 Positive cultures of blood samples drawn >12 h apart or
   All of 3 or a majority of 4 blood cultures, irrespective of the timing
   1 Positive blood culture for *Coxiella burnetii* or antiphase-I immunoglobulin G antibody titer >1:800

2. Evidence of endocardial involvement

A. **Positive echocardiogram** (TEE recommended in prosthetic valves, rated at least possible IE by clinical criteria, or complicated IE; TTE as the first test in other patients) for IE, defined as

1) Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation or
2) Abscess or
3) New partial dehiscence of prosthetic valve or

B. **New valvular regurgitation** (worsening or changing of preexisting murmur not sufficient)

Minor criteria

1) Predisposition: predisposing heart condition or IV drug use
2) Fever: temperature > 38.0°C
3) Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions
4) Immunologic phenomena: glomerulonephritis, Osler nodes, Roth's spots, and rheumatoid factor
5) Microbiological evidence: positive blood culture but does not meet a major criterion as noted above or serological evidence of active infection with organism consistent with IE

adapted from ESC Guideline 2015 and Li et al. Clin Infect Dis 2000; 30:633e8.

(TTE) are the two most important investigations in the diagnosis of IE. Three blood cultures should be performed as soon as possible and from separate venipuncture sites. Testing for antimicrobial susceptibility with the determination of the minimum inhibitory concentration (MIC) of antibiotics is recommended. In 90% of cases, the microorganism already isolates in the first 2 blood cultures. If the blood culture remains negative, withhold the use of empirical antibiotics for 48 hours and obtain additional cultures (31). HMGB1 serum level may be altered (35,36). A patient with clinical or echocardiographic evidence of IE but persistently negative blood cultures have a diagnosis of culture-negative endocarditis (CNE). The most common causes of CNE are an infection caused by HACEK organisms that grow poorly in vitro or current antibiotic therapy. Indeed, if the blood culture was done during antibiotic therapy, it should be notified to the laboratory (37,38).

TTE is the gold standard to assess the cardiovascular findings of IE (echogenic vegetation, abscess, pseudoaneurysm and new dehiscence of prosthetic valve) (39). Transesophageal echocardiography (TOE) should be considered when TTE is normal despite high clinical suspicion. TOE has higher sensitivity for native and prosthetic valves than TTE. All other laboratory or instrumental data are of secondary importance (40).

Diagnosis

The modified Duke's criteria (ESC Guideline 2015) are very helpful in diagnosing IE (Table 1). They have high specificity but low sensitivity. Therefore, the diagnosis of IE using modified Duke's criteria may be by clinical criteria or pathological criteria. According to the clinical criteria:

Definitive IE: two major criteria OR five minor criteria OR one major and three minor criteria.

Possible IE: one major AND one minor criterion OR three minor criteria.

Unlikely IE: the resolution of manifestations of endocarditis with antibiotic therapy for 4 days OR less OR alternate diagnosis for manifestations of endocarditis.

According to the pathological criteria, the diagnosis is definitive when the presence of vegetation or intracardiac abscess was confirmed on histology, or bacteria was demonstrated by culture or histology. Conversely, if there is no pathological evidence of IE, the diagnosis is unlikely (41).

Treatment

A stable patient suspected to have IE does not require immediate antibiotics. However, empirical therapy must be recommended once blood cultures have been collected. Melatonin administration may be a beneficial treatment (42-45). In children, the most frequent native valve endocarditis (NVE) pathogens or late prosthetic valve endocarditis (PVE) are gram-positive cocci; therefore, they must always be covered. Empirical treatment of NVE or late PVE (>1 after surgery) includes ampicillin, or vancomycin in allergic patients to penicillin, combined with gentamicin. Empirical treatments of early PVE (<1 after surgery) includes vancomycin plus gentamicin and rifampicin. A 2-6 weeks treatment may be sufficient in native valve endocarditis; instead, up to 6-8 weeks in prosthetic valve endocarditis. The choice of specific antimicrobial therapy depends on the suspected microorganism, and it has been well reported in the ESC 2015 guidelines (31).

The initiation of antithrombotic therapy in IE has not been supported. However, in a few cases, surgical treatment has been indicated (46). In CHD patients, the prevention of IE with antibiotic prophylaxis is still debated. In addition, the most recent guidelines no longer recommend their use during dental and other non-invasive procedures. Therefore, antibiotic prophylaxis should only be assessed for dental procedures requiring the manipulation of the gingival or periapical region of the teeth, perforation of the oral mucosa and invasive surgical procedures. In this case, amoxicillin or ampicillin are recommended 60-30 minutes before the procedure (47).

IE and Covid 19

The spread of Coronavirus disease 2019 (COVID-19) worldwide leads to new clinical syndromes. Recent studies analyze heart damage in COVID-19 survivors, even if they did not have underlying heart disease and were not sick enough to be hospitalized. The pathophysiology of COVID-19-

related heart damage is thought to be a combination of direct viral injury and indirect injury due to the host's immune response. The process of vegetation development can be explained by the damaged endothelium caused by Coronavirus infection and the consequent systemic inflammation. Until now, there has been limited evidence on COVID-19 and IE, and few case reports have been reported. Further study should be performed to describe and explain this association (48-50).

CONCLUSION

This review aims to underline the main features of IE in the pediatric population because the diagnosis is difficult and classic signs of IE are often blurred and no-specific. All children with IE diagnosis should have a next long-term cardiologic and pediatric follow-up. Residual complications may be extremely serious. Further studies are necessary to investigate more about this condition to identify new possible pathogens agents and to well define a treatment protocol that limits the spread of antibiotic resistance.

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Kawa-COVID-19

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To the Editor,

COVID-19 appears to be less common in children, with a typically moderate and non-fatal course. On the other hand, children could be seriously impacted in rare situations, and clinical signs may differ from those seen in adults. Children with COVID-19 demonstrated signs of an immunological response to the virus, were older, had a greater likelihood of cardiac involvement, and exhibited Kawasaki disease-like symptoms (KD). Kawa-COVID-19 is a novel systemic inflammatory illness in children that is temporally linked to SARS-CoV-2 infection. To corroborate these findings and further understand the pathogenesis of Kawa-COVID-19, more prospective worldwide investigations are needed.

Children and younger people appear to be less affected by COVID-19 than adults; pediatric cases make up only 2.1-7.8% of confirmed cases in Europe, North America, and Asia. Respiratory involvement also appears to have a more benign outcome, with almost no fatalities.

Only a few cases with severe and fatal results have been described in the pediatric series. Nonetheless, it appears that the respiratory tract is not the only organ susceptible to SARS-CoV-2 infection (1).

According to accumulating evidence, COVID-19 tissue damage is mostly mediated by the host's innate immunity. Reports from the United Kingdom in April 2020 described a clinical presentation in youngsters that resembled Kawasaki disease (KD) or toxic shock syndrome. Since then, there has been an upsurge in the number of children from other regions world-wide that have been afflicted in the same way. However, it's still unclear if these symptoms belong to the same disease spectrum or a collection of separate post-infectious disorders. Kawasaki disease (KD) is a self-limiting and acute vasculitis of medium and small vessels with uncertain causes. KD affects children as young as two years old, with 75% of those affected under the age of five; boys are 1.5 times more likely than girls to have the condition. Fever, abdominal ache, red eyes, vomiting or diarrhea, and a rash on the trunk are the first symptoms. Some youngsters get a large red lip and tongue, while others develop swollen neck glands. Because of its potential

Keywords: Covid19, Kawasaki, SARS-CoV-2, children, systemic inflammatory syndrome.

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0393-974X (2022)

ocular involvement, the KD is of great interest to ophthalmologists. Iridocyclitis, punctate keratitis, vitreous opacities, papilledema, subconjunctival hemorrhage, and conjunctival injection are the most common ocular symptoms. In most cases, the latter is bilateral, painless, nonexudative, and limbic sparing. Patients with KD may experience haemodynamic instability during the acute phase of the disease, a condition known as KD shock syndrome (KDSS).

Kawa-Covid-19

Kawa-COVID-19 represents a new systemic inflammatory syndrome mimicking KD associated with SARS-CoV-2 in children. In 44% of the cases of Kawa-COVID-19, severe illness necessitated intensive care. Although all patients had complete or incomplete KD, pretty apparent deviations from usual KD have been described that should call attention to this disease and suggest that it is a distinct entity consistent with the series of Verdoni et al. (2, 3).

First, the median age at presentation is higher than in classical KD, with higher age (>5 years) indicating the need for ICU care. Second, myocarditis is distinct from conventional KD in incidence and severity. Third, stomach pain and/or diarrhoea were observed more frequently (81%) than in traditional KD (approximately one out of three patients). Finally, the cytokine storm was more common in the illness we describe, as evidenced by heart failure, pneumonia, gastrointestinal, neurological, and renal symptoms, all of which were related to higher CRP levels, ferritin, and cytokines (particularly IL-1, TNF, and IL-6). Apart from heart failure, these symptoms are also common in people with severe COVID-19 (4). However, in contrast to severe adult COVID-19, our patients rarely showed respiratory symptoms, implying that children had a different underlying host immunological response.

In the context of the SARS-CoV-2 pandemic, doctors should suspect Kawa-COVID-19 in any kid presenting with unexplained high fever and a rise in CRP, especially if mild signs of COVID-19 or proven exposure to SARS-CoV-2 were reported in the previous four weeks. In such circumstances, the classic clinical indicators of KD and digestive problems and respiratory characteristics should be thoroughly examined. In order to diagnose life-threatening problems, some investigations should be conducted regularly. These include tests for myocarditis (troponin, NT-proBNP, ECG), MAS (white blood cells, fibrinogen, ferritin, albuminaemia), and renal impairment (white blood cells, fibrinogen, ferritin, albuminaemia) (creatinine, urea, proteinuria). Because of the potential of rapidly progressing Kawa-COVID-19 and heart failure, clinicians should be especially cautious with patients over the age of 5 who have increased ferritin levels (over 1400 g/L).

Patients should have SARS-CoV-2 RT-PCR testing, at least in nasopharyngeal secretions and stool, as well as SARS-CoV-2 serology, as RT-PCR may be negative due to the late development of this condition after initial COVID-19 signs of exposure and other viral investigations to rule out alternative diagnoses (4).

Because of the rapid progression of the disease, patients should be closely monitored and treated as soon as possible. Myocarditis and dilated coronary arteries were seen in 44% and 19% of patients, respectively. As in classic KD, treatment with IVIg 2 g/kg should be given quickly and appeared to be efficacious in most cases (5). Associated anti-inflammatory medication, such as steroids or biologics, was required in 31% of patients and should be explored if a severe course is noted or factors that predict a severe outcome, such as age over 5 years and increased ferritin levels (>1400 g/L). Several more therapeutic options may be investigated based on KD literature data.

Although it has been more than half a century since T. Kawasaki initially reported his 50 cases in Japan12, the origin of Kawasaki disease is still unknown (6).

Patients with Kawasaki illness diagnosed during the COVID-19 pandemic had clinical and biochemical traits different from our previous cohort of patients; thus, we classified them as Kawasaki-like diseases. They were elderly, with respiratory and gastrointestinal involvement, meningeal symptoms, and symptoms of cardiovascular involvement from a clinical standpoint. According to biochemical results, they developed leucopenia with significant lymphopenia, thrombocytopenia, and elevated ferritin, as well as indications of myocarditis. Patients with COVID-19 have similar clinical characteristics. Furthermore, these patients exhibited a more severe

disease course, requiring supplementary steroids with resistance to intravenous immunoglobulin and biochemical evidence of MAS and clinical symptoms consistent with KDSS. SARS-CoV-2 has been shown to have a pro-inflammatory effect in individuals with the most severe respiratory complications of COVID-19.

In line with MAS, many of these patients develop a constellation of symptoms known as a cytokine storm, including fever, lymphopenia, increased transaminases, lactate dehydrogenase, D-dimer, and ferritin. Similarly, MAS is a cytokine storm that may affect Kawasaki illness patients (7-10).

CONCLUSION

The present literature's review suggests that SARS-CoV-2 infection is connected to an auto-inflammatory disease similar to KD (Kawa-COVID-19). This disease, however, varies from classical KD in that it develops at an older age and has a higher incidence of severe myocarditis and/or pericarditis. Age higher than 5 years and high ferritin (>1400 g/L) are both indicators of a severe course. Furthermore, only 5 out of 16 patients (31%) had remission of inflammatory symptoms with a single IVIg, while 10 patients (62%) required a second line of treatment. To properly comprehend this novel disease entity, more prospective international collections of Kawa-COVID-19 are needed and the investigation of immunological and host genetic variables.

When contemplating social reintegration programs for the pediatric population, the link between SARS-CoV-2 and Kawasaki-like disease should be considered. However, the Kawasaki-like disease described here is uncommon, affecting only around one out of every 1000 infants infected with SARS-CoV-2. This estimate is based on the limited information available from case studies in this area. Interestingly, both genetic and immunomediated emerged recently as potential contributors of Kawacovid (11, 12). In this context, we may speculate that intricated immunoregulatory pathways and genetic components of disease interact and make some children prone to different cardiovascular and other complications following Kawasaki disease and Covid19 infection. This model of complex disease pathophysiology is observed in different pediatric conditions in which both genetic and non-genetic factors interact closely, highlighting how molecular etiological mechanisms underlying a wide group of paediatric conditions are important to be identified to dissect both contributory and causative factors (13-21).

In the last decade, genome (or exome) sequencing and omic-related technologies have been relevant and important to understanding many rare and neglected pediatric diseases (22-31). The advances from these genes or biomarker discovery studies gave us a deeper knowledge of the subtle molecular huts that cause rare conditions or rare complications in pediatric age. In addition, they led us to better understand the intricate interplay between genetic and non-genetic (e.g., immunomediated, endocrinological, metabolic) factors (32-40).

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Acute hemorrhagic cystitis: assessment in pediatric first aid

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Acute hemorrhagic cystitis (AHC) is one of the most common causes of gross hematuria in the pediatric population, especially in males (43%) rather than in females (9%), and more frequently in early childhood (1). However, macroscopic hematuria is the main clinical sign of urinary tract infection (UTI) in the emergency department and can be present in up to 26% of the cases. Therefore, it is essential to recognize this condition and be put in differential diagnosis with other causes of gross hematuria that, differently from AHC, do not have the same self-limiting and benign course.

Etiology

Up to 20-50% of AHC are consequences of infections sustained by Adenovirus types 11 and 21 (but types 14, 34, 35, 3, 7 can also affect urinary tract epithelium). Bacteria, fungi, and parasites are less common causes of hemorrhagic cystitis. In a smaller percentage of the cases, AHC may be determined even by Influenza A virus, Cytomegalovirus (CMV) and bacteria (such as *Escherichia Coli* and *Klebsiella pneumoniae*) (2, 3). The other bacteria isolated and causing hemorrhagic cystitis are *Staphylococcus saprophyticus, Proteus mirabilis* and *Klebsiella species. Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus,* are also associated with AHC. The urothelium in *Candida* infection is more frequently covered with pseudomembranes or pale plaques, while infections by parasites are rarer. The implant of *Schistosoma haematobium* in the mucosa predispose to the development of squamous cell carcinoma of the urinary bladder. Calcified cysts of *Echinococcus granulosus* infiltrate the bladder wall causing hematuria (4).

AHC can also be the consequence of exposure to radiation or alkylating drugs such as cyclophosphamide, busulfan, chlorambucil, ifosfamide. Moreover, in immunocompromised patients, the reactivation of latent Polyoma BK virus (less frequently, even JC virus) may play an essential role in AHC. For this reason, immunocompromised patients and, more specifically, the pediatric population undergoing bone marrow transplantation are more likely to suffer from AHC. In these cases, the resulting severe bleeding can be a life-threatening event and deserves specific treatment and opportune prevention (5). Nevertheless, it is important to remind how adenovirus infections

Keywords: acute hemorrhagic cystitis, children, Adenovirus

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0393-974X (2022)

are possible causes of glomerular hematuria in the context of IgA nephropathy (6).

Other uncommon causes of AHC are vasculitis, amyloidosis and genetic syndromes (mutation of ITGB4 gene for integrin β4 subunit in epidermolysis bullosa). However, it is necessary to remark that in an important percentage of the cases, it is impossible to determine a causal agent for AHC (7,8). From an omic science-based perspective, many basic and clinical investigators tried to decipher the pathophysiological factors underlying acute hemorrhagic cystitis in children; this led to the identification of an intricate and complex interplay of genetic and non-genetic (immunomediated, metabolic) factors which may cause acute hemorrhagic cystitis or predispose/ contribute to this condition (9, 10). This complex disease model covering different potential factors is commonly observed in the aetiology of several complex and not yet fully understood pediatric conditions. However, the advent of next-generation and other related technologies led to an increased understanding of the monogenic causes underlying many of these childhood diseases (11-16).

Several different genes and cellular mechanisms have thus been identified in recent years, and this highlighted the importance of omic sciences in defining the clinical spectrum and the therapies tailored to many disorders of infancy (17-20). Notably, genes that regulate response to environmental factors or influence metabolic substrate or sub-cellular events have been revealed to be causative for previously neglected disorders (21-29). In addition, our understanding of the processes associated with rare and/or multisystemic abnormalities will be pivotal for targeted treatments and the development of personalized pediatrics, according to several examples (30-36).

Acute hemorrhagic cystitis due to Escherichia coli

Escherichia coli (*E. coli*) is the most common organism causing urinary tract infection (UTIs) in infants (about 80-85% of cases). Among the different strains of *E.coli,* uropathogenic *E. coli (*UPEC*)* is a normal commensal of the human gut that colonizes the urinary tract from the ascension of focally derived microorganisms through the urethra. UPECs are opportunistic intracellular pathogens resistant to innate immune responses that invade the uroepithelium thanks to fimbriae (or pili). There are two types of fimbriae, type 1 pilus and type P pilus. Both types of pili consist of major pilus protein subunit (pilus stalk) and several minor subunit proteins representing the actual adhesins (37). These pili are involved in the initial attachment of UPEC to the urinary tract mucosa and biofilm formation. Other bacterial cell surface virulence factors include flagellum and capsular lipopolysaccharide (LPS). However, UPEC elaborates on other secreted virulence factors such as adhesins, toxins, enzymes that play different pathogenic roles during infection.

Hemolysins and siderophores are responsible for the colonization of urinary tract infection and persistence despite the host immune response. On the other hand, another virulence factor is activating the epithelium defence system after the adherence to the surface of the bladder. The LPS of *E. coli* activates Toll-like receptors (TLR); as a consequence should be the stimulation of inflammatory factors transcription, cytokine production, neutrophil recruitment and finally, exfoliation of infected bladder epithelial cells. These virulence factors of UPECs are associated with antimicrobial resistance (38).

Clinical features

AHC is characterized by sudden-onset gross hematuria in an otherwise healthy child; hematuria may appear at the end of the beginning of the voiding act, or less frequently, it can be present during the whole micturition; the color of urine is generally bright red and presence of blood clots is frequent. Hematuria is generally associated with or preceded by dysuria, frequency, urgency, tenesmus, oppressive suprapubic pain, enuresis, but it can rarely be the only clinical feature of AHC. Fever is uncommon, but it can be present in a small percentage of cases, together with other symptoms of adenovirus infection (ocular, gastrointestinal or respiratory). Possible complications may be anemia and clots that may obstruct the urine flow, but in

the otherwise healthy child, these occurrences are uncommon. Symptoms generally last 3-7 days (however, they regress within 2 weeks), and the course is self-limiting (39, 40).

Differential diagnosis in pediatric first aid

The evaluation should start in the pediatric emergency department from clinical condition (generally good in AHC), suggestive symptoms (as described before) and patient history. It is necessary to exclude traumas, drugs exposition, familial hematuria (e.g. Alport disease, associated with a history of neurosensorial dumbness). In addition, it is crucial to investigate abdominal or flank pain (that may indicate urolithiasis, joint stenosis or renal vein thrombosis), concomitant pathological conditions (transplantation of solid organs or bone marrow), current or previous upper respiratory tract infections (whose presence may indicate IgA nephropathy or acute postinfectious glomerulonephritis respectively), bloody diarrhea (prodrome of a hemolytic uremic syndrome), petechiae (Henock-Shonlein purpura or other vasculitides), or already known malformations (e.g. nutcracker syndrome, renal cysts).

It is important to exclude causes of the dark-reddish color of urine that may mimic gross hematuria, such as exposure to some foods or substances (e.g., beetroot, anthocyanins, rifampicin), inborn errors of metabolism (porphyria, alkaptonuria), myoglobinuria (rhabdomyolysis) and hemoglobinuria (hematological diseases that cause intravascular hemolysis). In addition, the characteristics of hematuria are important to exclude causes of glomerular hematuria (in which are present tea/cola-colored urines, instead of pink/bright red urines of no glomerular hematuria). Moreover, it should be evaluated for the presence of hypertension and peripheral edemas to exclude the causes of nephrotic syndrome.

Urine analysis should be performed to evaluate the presence of red blood cells (whose morphology reveals the origin of the hematuria, since erythrocytes are dysmorphic if the hematuria is glomerular), pyuria (possible sign of UTI), proteinuria, casts (signs of GN), electrolytes (hypercalciuria is associated to gross hematuria up to 20% of the cases), and urine culture should be set up for a correct and complete diagnostic evaluation. First-step blood analysis should be comprehensive of CBC, reticulocytes count, serum creatinine, urea, albumin, bilirubin, LDH, CPK, cholesterol, serum electrolytes, C3, C4, coagulation profile, RCP, ESR, ASOT. HMGB1 serum level may be altered (41, 42). Abdominal, renal and bladder the US should be performed to exclude the presence of calculi or tumor masses. Once other causes are excluded, diagnosis of AHC can be easily performed (43).

Therapy

There are no specific guidelines for the AHC treatment. The role of antibiotics remains unclear, even though viruses are the most common cause of AHC, there is the hypothetical increased risk of bacterial colonization due to the damage of bladder epithelium and the alterations of the regular mechanics of urination, especially in girls or in people who have risk factors in developing complicated UTI. It is possible to choose broad-spectrum antibiotics if the pathogen responsible for AHC has not yet been identified with certainty. AHC supported by viruses and fungi is eradicated by administering specific antivirals or antifungals. Instead, for alkylating drug-related AHC, this condition may be prevented by administering specific antidotes such as classical 2-mercaptoethanesulfonic acid (Mesna) (44). melatonin administration may be a beneficial treatment (45-49).

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Acute pyelonephritis in the pediatric emergency department: outpatient management or hospitalization?

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Acute pyelonephritis (AP) is the most serious bacterial illness of childhood that involves renal parenchyma. The most common germ involved in AP is E. Coli (80-90% of the cases). High temperature over 38°C is a sign of renal parenchyma involvement. It may be associated with other clinical symptoms (vomit, abdominal pain, inappetence, dysuria). AP treatment should be initiated after urine analysis and the diagnosis confirmed by culture. This article reviews the difference between outpatient and nosocomial urinary tract infections patients. A comprehensive search of published literature was carried out to identify all articles published on this topic in English and Italian from 2000 to 2020. Key terms used are Acute pyelonephritis, Childhood, Pediatric Emergency Department, Urinary Tract Infection.

Acute pyelonephritis (AP) is a bacterial urinary tract infection that involves renal parenchyma, generally associated with systemic symptoms. It is considered to be one of the most serious bacterial illnesses of childhood due to both its risks of short-term complications (such as meningitis and sepsis, more frequently in the younger child) and long-term complications (such as renal scarring, which may lead to chronic renal illness, even if the role of previous acute pyelonephritis in the damage of the renal parenchyma has been recently reconsidered, because of the emerging role of unrecognized congenital lesions). Urinary tract infection (UTI) may be the first sign of congenital tract anomalies in up to 30% of the cases (1, 2, 3).

Epidemiology

UTI is considered to be the most common bacterial infection in childhood, affecting around 1-7% of boys and 8-4% of girls before the age of 7 years, with an equal prevalence of around 5% for both sexes during the first year of life (1). About 60-65% of children with febrile UTIs have acute pyelonephritis. Up to 2.7-4.1% of children younger than 2 years who have a fever have UTI (2). Up to 30% of pediatric patients experience recurrent infections during the first year after initial UTI (3).

Pathophysiology

AP is due to bacterial colonization of the urinary tract from bacteria, and the development of the infection is due to both host factors and germ characteristics. As far as the host is concerned, the conditions that may favor the development of AP are related to UTI's anatomical and functional defects that

Keywords: acute pyelonephritis, childhood, pediatric emergency, urinary tract infection

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0393-974X (2022)

determine urine stasis (such as vesicoureteral reflux - VUR, obstructive megaureter, neurogenic bladder, dysfunctional bladder emptying, detrusor instability); even constipation and soiling can be considered as UTI risk factors in general. Regarding the germs, the most common germ involved in AP is E. Coli (80-90% of the cases).

A particular strain named UPEC (Uropathogen E. Coli) is equipped with fimbriae that allow uroepithelial adherence, even with an adequate urine flow; moreover, this strain possesses virulent capsule antigens, iron acquisition systems, toxin secretion and plasmids that contribute to antibiotic resistance. UPEC behaves as an opportunistic intracellular pathogen that invades the uroepithelium and creates biofilm; from the bladder, the bacteria ascend to the kidney, especially in the presence of anatomical favoring factors, and there it determinates inflammatory activation, responsible for the systemic symptoms.

In a smaller percentage of the cases, the infection may be due to hematogenic diffusion of the pathogens, especially in infants. The other 10-20% cases of AP are caused by Klebsiella, Enterococcus, Enterobacter, Proteus, and Pseudomonas species, with an increasing prevalence not only in the hospital settings (4). Furthermore, in recent years, a large number of investigations studies revealed an expanding genetic and immuno-mediated complexity underlying pediatric emergencies associated with acute presentations, including acute pyelonephritis (5).

The molecular dissection of these conditions and the possible combination of both genetic and non-genetic causes in the same disease may explain the frequent multisystemic and metabolic, and neurological alterations associated with a wide array of not fully understood pediatric conditions (6-9). Many novel aetiological factors have been revealed by basic and clinical studies, with subsequent benefits for our understanding of disease phenotypes, disease prognosis, and possible aetiologically-targeted treatments for children admitted to the Pediatric and/or Paediatric Emergency Unit (10-15).

Childhood disease at the emergency department may be the result of aberrant immunomediated or metabolic responses within cellular compartments, and this is often the downstream effect of genetic insults, which affect a wide array of transcription, translation and post-translational processes at the sub-cellular level (16-19). This disease can either be monogenic as well as complex/multifactorial/polygenic or due to environmental factors on the background of genetic predisposition mechanisms (20-29).

DIAGNOSIS

Clinical features

The AP clinical symptoms change widely on the base of the patient's age. Generally, fever is considered to be a sign of renal parenchyma involvement. AP has to be suspected in every child with high temperature over 38°C without a clear localization, even without clear nephron-urologic involvement. However, fever may be absent in children during the first 3 months of life; these patients are more susceptible to develop complications such as sepsis and meningitis. In infants, other clinical symptoms may be lethargy, irritability, failure to thrive, weight loss, vomiting, urine foul smell. On the contrary, in older children fever may be associated to abdominal or flank pain; vomiting and symptoms typical of lower UTI may also be present (dysuria, pollakiuria, stranguria, bladder tenesmus, urgency, malodorous urine, incontinence, hematuria) (3, 4, 30).

Severe or atypical AP are those with one of the following characteristics: affect children of age minor than 3 months, are sustained by bacteria other than E. Coli, are complicated by sepsis, meningitis, acute kidney injury (with oliguria and serum creatinine raised), renal or perirenal abscess. Persistent fever despite adequate antibiotic therapy (without a clinical response within 48 hours) and deterioration of the patient's condition should lead to suspect a severe form (4, 30,31).

Laboratory finding

Urinalysis plays a major role in the acute pyelonephritis diagnosis. It can be carried out by dipstick or urine chemical-physical analysis. To diagnose AP, it is mandatory the demonstration of leukocyturia (by detection of leukocyte esterase through dipstick evaluation, or the presence of pyuria

in the urinary sediment) and bacteriuria, that must be further investigate by urine culture. The bacterial growth is considered significative if equal or superior than 100000 CFU/ml, referring to a midstream urine sample, or 50000 CFU/ml for urine sampled by suprapubic puncture. The nitrites presence is a sign of the bacterial metabolism of nitrates. Hematuria can also be present. The collection method of the urine sample has a huge importance: it is recommended to sample the midstream urine, but it can be easily done only with older children. Suprapubic puncture and catheterization must be done only in severely ill children. Urine sterile bag should only be used for stick exams (3, 4, 30). HMGB1 serum level may be altered (32-34).

Other laboratory findings typically present in AP are neutrophil leukocytosis and elevation of C-reactive protein, erythrocyte sedimentation rate (ESR) and procalcitonin. In younger children with AP may also be present hyponatremia as a result of the impaired renal function (pseudo-hypoaldosteronism). In atypical, complicated and particularly severe forms, the renal function impairment may lead to acute renal insufficiency, with elevation of serum creatinine and azotemia (3, 4, 30).

Imaging

Imaging investigations are aimed to investigate anatomical or functional abnormalities of kidney or the genitourinary tract, such as VUR, obstructed urine flow, and so on. There is no indication to perform instrumental investigations during the acute phase of AP as routine. Renal and bladder ultrasound can be used in the acute phase in children in which the fever persists for more than 72 h despite appropriate antibiotic treatment to identify complications (e.g., renal or perirenal abscess). Otherwise, it has to be performed 2-4 weeks later the acute phase to detect anatomical anomalies. In children presenting ultrasound anomalies (kidney hypoplasia, hydronephrosis, ureteral dilatation, impaired renal echogenicity, bladder anomalies), infections from bacteria other than E. Coli and with recurrent UTIs is suggested to perform voiding cysto-urethrography (VCUG), cystosonography, direct or indirect cystoscintigraphy to detect VUR or other functional anomalies (35,36). Scintigraphy is not recommended routinely after the first episode. The use of static renal scintigraphy (with DMSA) is recommended in all children with grade IV and V VUR and several recurrences 4-12 months after the AP (1, 3-5).

Therapeutic management: outpatient setting or hospitalization?

The pediatric acute pyelonephritis therapeutic management has recently been changed (3). In 2016, the EAU/ESPU Guidelines on urinary tract infections (UTI) in Children published by the European Association of Urology (EAU) and European Society for Pediatric Urology (ESPU), were published. In the previous years there have been various guidelines published by the National Institute for Health and Care Excellence (NICE), the American Academy of Pediatrics (AAP) and Italian Society of Pediatric Nephology in 2011, the Canadian Pediatric Society (CPS) in 2014 and Polish Society of Pediatric Nephology in 2015 (31).

According to latest guidelines, AP treatment should be initiated after urine analysis and diagnosis confirmed by culture (3). In recent years there has been much discussion on the outpatient and nosocomial UTIs patient's management. According to the latest and most recent European guidelines, home management is envisaged in AP children >2 months old. In these cases, it is effective to start the oral antibiotics (3, 4) because the Cochrane group showed that oral antibiotic treatment (cefixime, ceftibuten or amoxicillin/clavulanic acid) as initial treatment has the same efficacy as 3-4 days of intravenous therapy (IV). However, for children <2 months of age (higher incidence of severe pyelonephritis) or with sepsis, dehydration, vomiting, diarrhea, UTIs complications (eg. dilation of the upper urinary tract), bad clinical conditions, hospital setting is necessary. In these cases, IV antibiotic therapy will be started (3). This management must also be guaranteed in poor family compliance patients. (4).

The choice of antibiotics should be based on the child's drug history, local epidemiology and susceptibility patterns of the various uropathogens. Pediatricians must, therefore, evaluate the local sensibility and resistance patterns to better define the

initial therapy which can be subsequently modified also on the antibiogram basis (3, 4). Furthermore, it must also be considered that not all antibiotics are approved for pediatric population use (4).

In out setting patients the best oral antibiotics choice is a cephalosporin (such as cefixime) in many geographic areas; in hospitalized patients, the most common choice of intravenous antibiotic is gentamicin, with or without ampicillin. In this case, renal function must be monitored because the drug is nephrotoxic. For this reason, cefotaxime or ceftriaxone is often preferred, but they are a broader spectrum (37). In UTIs when antibiogram results are available, IV therapy should be changed to the less spectrum antimicrobial. In the case of oral antibiotics, it is not necessary to make changes if the pathogen is sensitive to the one used (37). The main oral and parenteral antibiotics, which are used for pediatric AP, are listed in table I (3).

In the case of outpatient management with oral antibiotics, treatment for 7-14 days is envisaged (30). The patient must be subjected to careful surveillance with evaluation of the treatment response after approximately 36-48 hours. In case of fever persistence or general condition decay the child must be re-evaluated. The most common cause of treatment failure is bacterial resistance or kidney complications, such as renal abscesses or malformations (1, 3). In hospitalized patients' case, IV should be continued as long as the child is feverish. Parenteral antibiotics should be switched to oral as soon as clinical improvement is observe. The oral therapy is then continued for a total of 7-14 days (1, 3, 30). melatonin administration may be a beneficial treatment (38-42).

Who is at risk of recurrence?

Recurrent UTIs are defined as a further infection caused by a new organism. In reverse, relapsing UTIs is a further infection caused by the same organism. Infection recurrence is common and in up to 40% of children it is caused by VUR (43). It is very important to recognize high risk patients of developing recurrent UTIs in order to preserve their renal final function. Risk patients of developing recurrent UTIs are those with prenatal diagnosis of anatomical urinary tract changes or with external UTIs' causes. The anatomical malformations are: the congenital anomaly kidneys and urinary tract (CAKUT), upper urinary tract dilatation, renal hypoplasia, dysplastic kidney, thick bladder wall, post-voiding residue, ureterocele, posterior urethral valves or VUR (3, 44) External causes could be the abdominal mass presence, spinal anomalies or constipation (3, 37).

Some prospective randomized studies have shown that the prophylactic antibiotic therapy does not reduce the recurrent UTIs risk. Prophylactic therapy has only been shown to be effective in preventing new kidney scarring in children with III-IV VUR grade (45). The most effective antibiotic is nitrofurantoin, but it is correlated with an increased side effects risk. However, the increased resistance risk at present do not justify the routine prophylaxis use (3, 8). In addition to drugs, cranberry juice can be used for the UTIs prophylaxis, because, in according to a Finnish study, it reduces the actual relapses number and the related antibiotics use. In boys, another risk factor for recurrent UTIs is phimosis, in which early treatment with local corticosteroid or surgery is indicated (3).

Follow-up

Acute pyelonephritis must be followed over time, especially if patients have risk factors for recurrence of UTIs. According to the diagnostic algorithm of the EAU/ESPU guidelines, in the case of male children >12 months with upper and lower urinary tract normal ultrasound, performed during the first febrile episode, you can rest assured and continue clinical follow-up; it is important to train the toilet and to exclude forms of bladder bowel dysfunction. Only in the recurring UTIs case will it be necessary to proceed with imaging (3, 30,46). Instead, in the case of infants <12 months, in females or in the VUR ultrasound suspicion, it will be necessary voiding cystourethrogram and static scintigraphy with dimercapto succinic acid (DMSA). If VCUG and are DMSA normal, it is also important to train the toilet and to exclude forms of bladder bowel dysfunction (3, 6).

In patients with complicated UTIs, urinary tract dilation and good response to intravenous therapy, it is necessary to further evaluate the function of the upper urinary tract with a magnetic resonance imaging (MRI) or VCUG to rule out reflux. If the response to

Table I. *Oral and intravenous antibiotics for treatment of UTIs by EAU/ESPU Guidelines.*

Antibiotics	Daily dosage 0-12 y	Daily dosage 0-12 y	Application
Parenteral cephalosporins			
Group 3a (eg, cefotaxime)			
Group 3b (eg, ceftazidime)	100–200 mg/kg	3-6 g	IV in 2–3D
Ceftriaxone	100–150 mg/kg	2-6 g	IV in 2–3D
	75 mg/kg		IV in 1 D
Oral cephalosporins			
Group 3 (eg, ceftibuten)	9 mg/kg	0,4 g	PO in 1–2 D
Group 3 (eg, cefixime)	8-12 mg/kg	0,4 g	PO in 1-2 D
Group 2 (eg, cefpodoxime proxetil)	8-10 mg/kg	0,4 g	PO in 2 D
Group 2 (eg, cefuroxime axetil)	20-30 mg/kg	0,5-1,0 g	PO in 3 D
Group 1 (eg. cefaclor)	50-100 mg/kg	1,5-4,0 g	PO in 2 -3 D
TMP	5–6 mg/kg	-	PO in 2 D
or			
TMP/Sulfamethoxazole	5–6 mg/kg (TMP fraction)	320 mg	PO in 2 D
Ampicillin	100–200 mg/kg	3,6 g	IV in 2–3D
Amoxicillin	50–100 mg/kg	1,4 – 6,0 g	PO in 2 -3 D
			IV in 3 D
Amoxicillin/clavulanic acid (parenteral)	60-100 mg/kg	3,6 – 6,6 g	IV in 3 D
			PO in 3 D
	45 mg/kg (amoxicillin fraction); maximum: 500 mg clavulanic acid per day	1500 and 375 mg	PO in 3 D
Amoxicillin/clavulanic acid (oral)			IV in 3- 4D
Piperacillin	300 mg/kg per day		
Tobramycin	5 mg/kg	3–5 mg/kg; maximum: 0.4 g	IV in 1 D
Gentamicin	5 mg/kg	3–5 mg/kg; maximum: 0.4 g	IV in 1 D
(Drug monitoring is necessary)			
Ciprofloxacin (approved in most European countries as second or thierd line medication for complicated UTIs; antibiotic of last resort)	Children and adolescents (1-17 ys): 20-30 mg/kg (maximum dose: 400 mg) (parenteral)	20 -30 mg/kg	IV in 3 D
	Children and adolescents (1-17 ys): 20-40 mg/kg (maximum dose: 750 mg) (PO)	20-40 mg/kg	PO in 2 D
Nitrofurantoin (Contraindicated in the case of renal insufficiency)	3–5 mg		PO in 2 D

treatment is poor, percutaneous nephrostomy should be considered (3, 30).

In recurrent UTIs, abnormal abdominal ultrasound and/or other risk factors presence (familiar VUR), it is necessary VCUG perform, the gold standard for the VUR diagnosis and classification. If the VCUG is positive it is recommended to perform DMSA scan 4-6 months from the first acute pyelonephritis, especially in the case of: a) recurrent UTIs; b) III-IV VUR; c) high risk of renal scarring (e.g., hypertension and albuminuria) (3, 30, 47, 48).

CONCLUSION

In conclusion, since acute pyelonephritis is so widespread, it is important to know and follow a

single diagnostic-therapeutic algorithm. The first episode of acute pyelonephritis in children may be the first sign of congenital abnormalities of the urinary tract or other conditions, which must be investigated and followed over time in order to rule out the most fearful complication, such as chronic and terminal renal failure. This review, indeed, aims to underline the outpatient and hospital management of acute pyelonephritis in the pediatric population and emphasizes that the first useful treatment is oral antibiotics.Inpatient management and intravenous treatment should be reserved for selected cases such as complicated UTIs or patients with poor family compliance.

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Hemolytic uremic syndrome

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Hemolytic uremic syndrome (HUS) is thrombotic microangiopathy characterized by thrombocytopenia, non-immune microangiopathic hemolytic anemia and acute kidney injury. HUS is usually categorized as typical, caused by Shiga toxin-producing Escherichia Coli (STEC) infection, often associated with diarrhea (D+HUS), as atypical HUS due to dysregulated complement activation, not diarrhea associated (D-HUS, aHUS) or more rarely as secondary forms caused by systemic diseases or physiopathological conditions such as infections, malignancy, pregnancy, autoimmune disease, after transplantation or after drug assumption. The common pathogenetic features in STEC-HUS, aHUS, and secondary HUS are simultaneous damage of endothelial cells, intravascular hemolysis, and activation of platelets leading to a procoagulative state, formation of microthrombi, and tissue damage. HUS can develop in response to several different triggers and various clinical situations. The symptoms and clinical signs may overlap among the different forms of HUS; therefore, early diagnosis and identification of underlying pathogenic mechanisms allow instating specific support measures and therapies.

Background

Hemolytic uremic syndrome (HUS) is thrombotic microangiopathy characterized by the simultaneous occurrence of thrombocytopenia non-immune, microangiopathic hemolytic anemia (Coombs's negative), and AKI. Thrombotic microangiopathy (TMA) is the formation of platelet microthrombi in walls of small blood vessels, causing platelet consumption and mechanical disruption of erythrocytes, which leads to the typical haematological manifestations of TMA. It is one of the main causes of acute kidney failure in children.

Epidemiology and pathophysiology

Hemolytic uremic syndrome typically begins at less than 10 years, with most cases occurring at the age of less than 5 years. More than 90% of typical HUS is caused by Escherichia coli (STEC), which produces the Shiga toxin; S. pneumonia is responsible for nearly half (40%) of atypical HUS (1, 2). The estimated mortality rate for atypical and typical HUS is less than 5%, while long-term renal complications occur in 5% -25% of children with HUS (3).

From a basic science point of view, many researchers tried to investigate the pathophysiology

Keywords: hemolytic uremic syndrome, thrombocytopenia, children

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0393-974X (2022)

of hemolytic uremic syndrome, focusing on the genetic and immunomediated aspects that make some children more susceptible than others to these complications. Researchers showed that both genetic and environmental causes triggers might cause hemolytic uremic syndrome or predispose/ contribute to this condition (4, 5). This multifactorial pathophysiological model involves cytokines and genes in determining or precipitating this serious pediatric condition.

In the last 10 years, pediatric and genetic research through the possibility of studying genomes by sequencing and biomarker approaches led to the identification of many similarly complex childhood diseases, either Mendelian (monogenic) in nature or polygenic/multifactorial with environmental components on the background of some frequently undetermined molecular predisposition (6, 14). In addition, basic researchers highlighted several different genes and sub-cellular regulatory mechanisms, who dissected several rare disorders that affect infancy and pediatric age and may involve the development of the nervous system or metabolic/ homeostatic responses (15, 18). Importantly, genes and molecules that regulate responses to environmental or cellular factors or influence endocrinological processes or events have been revealed important, especially in those pediatric conditions previously neglected and not fully understood by pediatricians and geneticists (19, 31).

SEU classification

Although there is a new and recent classification of HUS based on the underlying disease, today, it is still traditionally classified into two types based on whether diarrhea is present in diarrhea-positive (typical) and negative (atypical) HUS. The typical variant is caused by Shiga-like toxin (verotoxin) produced by *E. coli* (O157: H7) and less frequently Shiga toxin by Shigella dysenteriae. All other causes of HUS have traditionally been grouped as Atypical HUS. The classification of D+HUS and D-HUS has been quitted because diarrhea is the triggering factor in 25-30% of atypical HUS cases (32).

International Hemolytic Uremic Syndrome group classifies hemolytic uremic syndrome into 1) infections associated HUS due to Shiga toxin-producing *Escherichia coli* (STEC), *Streptococcus pneumonia*, influenza A, H1N1 and HIV; 2) HUS secondary to coexisting conditions, such as hematopoietic stem cell or solid organ transplantation, malignancy, autoimmune diseases, drugs (most commonly quinine, cyclosporine and tacrolimus), malignant hypertension and pre-existing nephropathy; 3) cobalamin C defect associated HUS; and 4) atypical HUS due to dysregulation of alternative complement pathway and mutations of the gene, diacylglycerolkinaseε(DGKE)

Infection-induced HUS

HUS associated with infection with bacteria capable of producing Shiga toxin is one of the most common causes. The serotypes most commonly associated with HUS are *E. Coli* O157 and S. Dysenteriae type 1. The toxin acts on the surface of target cells by binding to globotriaosylceramide (Gb3). Gb3 is particularly expressed on the endothelium, podocytes, and tubular epithelium of the kidney. However, Gb3 is also found in other tissues, including the central nervous system. About 10% of patients exposed to bacteria develop HUS; the symptoms typically arise after 3 days of ingestion of the bacterium and are characterized by abdominal pain, diarrhea (bloody in 60%) and vomiting.

Renal involvement is often present, and dialysis is used in 50% of cases. Renal recovery after 1-2 weeks is normal, and end-stage renal disease after the initial presentation is rare. Neurological involvement is the most frequently reported extra-renal manifestation, with seizures and reduced levels of consciousness reported in up to one-third of cases (32, 33).

Other sites that may be affected include the intestinal tract and pancreas, eyes and heart. The death rate in the acute phase of the disease is about 2-5% 10 in HUS STEC but is higher when HUS follows Shigella infection. Long-term renal involvement occurs in 25-40%, with hypertension and chronic renal failure (34).

Coproculture should be performed in all patients with TMA regardless of whether there is a history of colitis because, despite the possible resolution of diarrhea, the bacteria can still be isolated; as it will be performed, the fecal or rectal swab. PCR can be performed on these samples to detect the presence of

the Shiga toxin genes. Positive serology for antibodies to toxin-producing E. coli serotypes is supportive in diagnosing STEC HUS, although not routinely used.

Currently, there is no evidence that treatments other than supportive care, including dialysis and ventilatory support where needed, improve the outcome of patients with STEC HUS. In addition, plasma therapy is no longer recommended for the treatment of STEC HUS and can be harmful (35).

The role of antibiotics is controversial, and their use has traditionally been avoided due to concerns that antibiotic treatment leads to increased release of Shiga toxin, increasing the likelihood and severity of STEC HUS. ß-lactams and trimethoprim/sulfamethoxazole have been reported to increase the risk of developing HUS. Fluoroquinolones increase toxin production in vitro, but they do not appear to worsen the disease when used. On the other hand, Macrolides and fosfomycin seem effective in reducing the synthesis of toxins and the risk of HUS. In HUS caused by Shighella, however, the use of antibiotics seems to be safe, useful and safe (36, 37).

In patients with STEC HUS, there is evidence of complement 25 activation and C3 levels may be low. Additionally, some STEC HUS patients may also have a defect in complement regulation, and this combination is associated with a poor prognosis. These observations led to the use of eculizumab, a humanized monoclonal antibody against complement protein C5, and in some STEC HUS patients, particularly with severe disease, they responded to eculizumab (38). However, STEC HUS is a self-limiting disease, and therefore these reports should be interpreted with caution, as to date, there are no clear opinions on the use of eculizumab following E. coli O104 STEC HUS, as some groups suggest a benefit from using the drug, others none.

Pneumococcus

This condition occurs in 0.3% of children concomitantly with pneumococcal infection, for example, pneumonia or empyema, and to a lesser extent otitis media, sinusitis and meningitis. The most accepted hypothesis is that neuraminidase, produced by Pneumococcus, eliminates sialic acid from glycoproteins on cell surface and exposes the cryptic Thomsen-Friedenreich (T) antigen, which is recognized by naturally occurring IgM antibodies (39), thus, leading to the activation of platelets and endothelium, which may be responsible for the TMA and explains why these patients have a positive Coombs test (DAT).

Due to the underlying disease, with 75% requiring dialysis and extra-renal manifestations frequently occurring. The mortality rate is higher than with STEC HUS; therefore, early recognition and timely initiation of antibiotics with supportive intensive care largely explain the improvement in prognosis in the last 2 decades. Unwashed plasma and red blood cells or platelets are traditionally avoided as long as agglutination tests are positive. Treatment is supportive. The role of plasmapheresis is uncertain, and although a response to eculizumab has been reported, there is limited evidence for its use.

Other infections

HUS has previously been commonly observed following HIV infection (incidence 2-7%). Although there have been reports of response to eculizumab, it started in combination with antiretroviral therapy, for which it is difficult how to determine efficacy (40). TMA has been associated with many other infections, although it is difficult to determine whether they are a direct cause or a trigger for TMA

Atypical-HUS

Atypical-HUS is caused by a genetic or acquired defect in regulating the alternative complement pathway leading to an inappropriate activation. This uncontrolled activity of the terminal complement pathway leads to the generation of a massive amount of membrane attack complex (c5b-9) with consequent damage to endothelial cells and the generation of fibrin thrombi in small vessels culminating in platelet adhesion, mechanical intravascular destruction of erythrocytes and tissue ischemia.

A genetic defect can be divided into inactivating mutations in genes that encode complement regulating proteins such as CFH (complement factor H), CF1 and membrane cofactor protein (MCP/CD46) or gain-of-function mutations in genes that encode complement activating proteins such as C3 and CFB.

In addition, an acquired defect in complement control due to autoantibodies against factor H can also cause aHUS, typically in children who are homozygously deleted for the CFHR1 gene. Complement-mediated aHUS predominantly affects the kidney, but extra-renal manifestations are also seen in approximately 20% of cases, particularly neurological manifestations.

The worst prognosis is for factor H-mutated patients, as 60% die or reaches end-stage renal disease (ESRD) within the first year after the onset. Therefore, diagnosis is primarily clinical and based on excluding other possible causes of TMA. Complement pathway assessment can assist the diagnosis, and however, normal results do not exclude a diagnosis of aHUS. Low C3 with normal or decreased FB Concentration associated with normal C4 suggest alternative pathway-mediated complement activation.

In addiction assessment for the presence of autoantibodies and neutrophil surface CD46 levels should be performed in all patients. Genetic analyses are necessary for any patient with aHUS. However, these results are not immediately available and do not guide the initial diagnosis and early treatment choice.

In the pre-eculizumab era, the treatment of aHUS consisted of general supportive measures and plasma exchange to remove Antibodies and replace deficient complement regulators, but ERSD developed in a significant number of patients. These are still the first line of treatment until TTP has been excluded (preserved ADAMTS13 activity). Eculizumab is a humanized monoclonal antibody against complement C5. It blocks the conversion of C5 to C5a and C5b, therefore preventing the generation of the membrane attack complex. Eculizumab effectively blocks the alternative complement pathway, establishing remission in >85% of cases with a significant improvement of renal outcomes. However, there is an increased risk of infection from *Neisseria meningitidis*. For this reason, it is recommended that children receive the meningococcal ACWY and B vaccines.

Patients with complement-mediated aHUS who developed ERSD have a high risk (80-90%) of recurrent disease following isolated kidney transplantation. A combined liver-kidney transplant is one option to circumvent disease recurrence, this is based on the fact that fluid-phase complement regulators are produced in the liver; normal complement factors can be produced by replacing the liver. In addition, isolated kidney transplantation has become possible with the availability of eculizumab to treat disease recurrence or prophylactically to prevent a recurrence.

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A case of myocarditis in a child with Multisystem Inflammatory Syndrome (MIS-C) related to previous Sars-CoV-2 infection: Our experience

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In December 2019, a novel coronavirus (SARS-CoV-2) first emerged in Wuhan, China, and in March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. According to the WHO Coronavirus Disease Dashboard, as of 02 March 2021, about 2.531 million deaths due to COVID-19 have been reported worldwide (1), and most of them are adults. The incidence of COVID-19 is meaningfully lower in children than that reported in adults; no more than 2% of cases were described in a pediatric population. Furthermore, the risk of severe disease is very low, as about 90% of cases are asymptomatic or have mild-moderate symptoms (2, 3). In children, admission to the Pediatric Intensive Care Unit (PICU) is uncommon, and death is a very rare event (3).

In April 2020, UK pediatricians identified an increase in cases of infants with asymptomatic or minimally symptomatic COVID-19 infection who developed a delayed systemic inflammatory syndrome associated with the involvement of more than one organ. Children affected often required hospitalization, and, although rare, the risk of death was greater than that described in simple COVID-19 infection (4). This new condition was initially named "Pediatric Inflammatory Multisystem Syndrome Temporally associated with Severe acute respiratory syndrome coronavirus 2 (PIMS-TS)", and guidelines were published by the Royal College of Paediatrics and Child Health (5). Subsequently, new pediatric cases were described in other regions of the world, and the WHO (6), the US Centers for Disease Control and Prevention (CDC), and UK authorities (7) published their definitions of this condition and finally called it "Multisystem Inflammatory Syndrome in Children (MIS-C)". These guidelines have common elements, such as prolonged high fever, multi-organ dysfunction, evidence of hyper inflammation in laboratory exams, and presumed or confirmed the previous infection of SARS-CoV-2.

MIS-C seems to be a rare disease associated with Sars-CoV-2 infection, and incidence in people <21 years is about 2 per 100.000 (8). In this Report, We describe a case of a child with a previous COVID19 infection and a Sars-CoV-2 positivity at serology, who had prolonged fever and >1 organ system involved (mucocutaneous, gastrointestinal and cardiovascular system).

Case report

Our patient was a previously healthy 6-years-old male child referred to the Pediatric Emergency Unit (PEU) of "G. Martino" Hospital in Messina, Italy, for clinical evaluation due to the presence of a four- days high fever (up to 40.5°C), vomiting and abdominal pain, non-purulent bilateral conjunctivitis,

Keywords: myocarditis, children, Multisystem Inflammatory Syndrome, SARS-CoV-2, Covid-19

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0393-974X (2022)

erythematous skin rash, swollen and red tongue (strawberry tongue). In the patient's history, there were three weeks before an isolated one-day fever after contact with a COVID-19 positive parent (his father), and his molecular swab test (reverse transcriptase-polymerase chain reaction, RT-PCR) for SARS-Cov2 resulted positive. After ten days from the first positive molecular swab test, he repeated another negative test. However, he maintained a healthy state up to two weeks later, when fever and other symptoms described above started. At home, during the first days of fever, he took a two-days short course of antibiotics (azithromycin), with no response. Fever was also poorly responsive to both acetaminophen and ibuprofen.

At the admission to the PEU, laboratory exams revealed leukocytosis with neutrophilia and lymphopenia and elevated inflammatory values (CRP and procalcitonin), owing to his history, RT-PCR and serology for SARS-CoV-2 were requested. However, the molecular swab test was negative, while total Immunoglobulins (Igs) for SARS-CoV2 were positive (99 times higher than the reference standard). On physical examination, he was febrile, moderately

Data esame	Hb g/dl	GB mmc	N	L	PLT mmc	PCR mg/dl	PCT ng/ml (<0,25)	Troponina T pg/ml (<14)	NT-proBNP pg/ml (0-125)	Mioglobina ng/ml (23-72)	INR	D-dimero ug/ml (<0,5)	Fibrinogeno mg/dl (<400)	Albumina g/L	Trigliceridi mg/dl	Colesterolo tot mg/dl	ALP U/L (>156)	EP%
28-gen	11,6	13600	84%	9%	234000	6,9	4,33							39				
29/01/2021	11,1	12800	80	14	212000	7,56		26,46		21	1,22		577	31	205	87		60
30/01/2021 I	11,1	13000	82	13	249000	11,17	1,86	17,23	10245	23	1,15		662	29	174	86		50
30/01/2021 II	9,9	14720	84	11	242000	9,33					1,2	2,64						50
31/01/2021 I	9,3	12000	82	12	349000	9,4	1,4	50,4	50833								65	
31/01/2021 II	10,1	10900	86	8	382000	14,5	1,01	44,07	39125	21							82	55
01/02/2021	10,6	12700	85	8	438000	13,39	0,72	53,9	25055	21	1,22	3,95	657					65
02/02/2021	10,3	21800	84	10	668000	5,56	0,46	63,49	10118	23		1,94	460				67	68
03/02/2021	10	21000	83	11	659000	2,7	0,43	63,51	4740			1,49		25				70
05/02/2021	10,1	21600	74	19	691000	0,98		82,12	2720	21	1,02	1,17	290					68
08/02/2021	10,6	15300	74	20	826000	0,42	0,42	55,55	1010			0,71		33				71
15/02/2021	11	15700	54	39	626000	0,07		6,2	185		1	0,42	269	38				71
17/02/2021																		65
03/03/2021	12,3	8300	60	31	466000	0,04		3	64			0,27						65

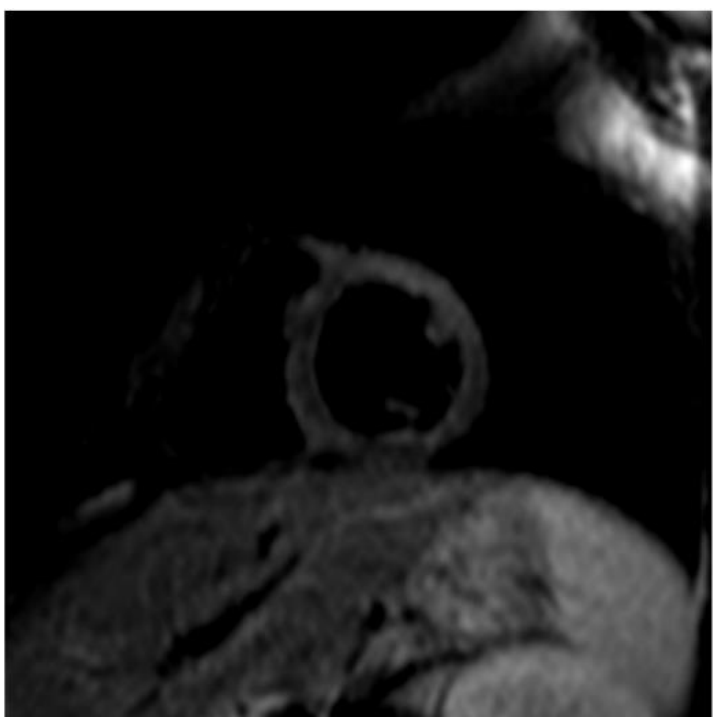

Fig. 1. *Cardiac RMN, edema.*

dehydrated, prostrated and with bilateral conjunctival erythema, erythematous skin rash (especially to upper and lower limbs) and strawberry tongue.

He was admitted to the Pediatric Ward, where Kawasaki disease was first suspected. The child had a body temperature of 37,9°C with a heart rate (HR) of 162 bpm; an electrocardiogram confirmed a sinus tachycardia. SpO2 by pulse oximeter was normal (98%), while blood pressure was lower than normal values for age (82/33 mmHg). Empiric antibiotic therapy with ceftriaxone i.v. was started. Nevertheless, after a few hours from the admission, the patient appeared more suffering, with HR 250 bpm and BP 75/55 mmHg. An echocardiogram showed a left ventricular dysfunction (EF 60%) associated with mitral and tricuspid valves insufficiency; coronaries arteries were normal. Troponin-T was elevated (2 times higher than the reference standard), indicating myocardial necrosis (Fig. 1, 2).

The patient was transferred to the Pediatric Intensive Care Unit (PICU) in the high suspicion of hypovolemic shock correlated to a decreasing cardiac output. At the admission, a PICC (Peripherally Inserted Central Catheter) was inserted in the left arm, and laboratory tests were repeated: hemogasanalysis was normal, leukocytosis with neutrophilia was confirmed, elevated CRP (22 times higher than reference standard) and procalcitonin (7 times higher than reference standard), pro-BNP was extremely high (82 times higher than reference standard), hyperfibrinogenemia (2 times higher than reference standard), hypoalbuminemia (29 g/L), and elevated d-dimer (5 times higher than reference standard) were reported. In addition, HMGB1 serum level may be altered (9, 10).

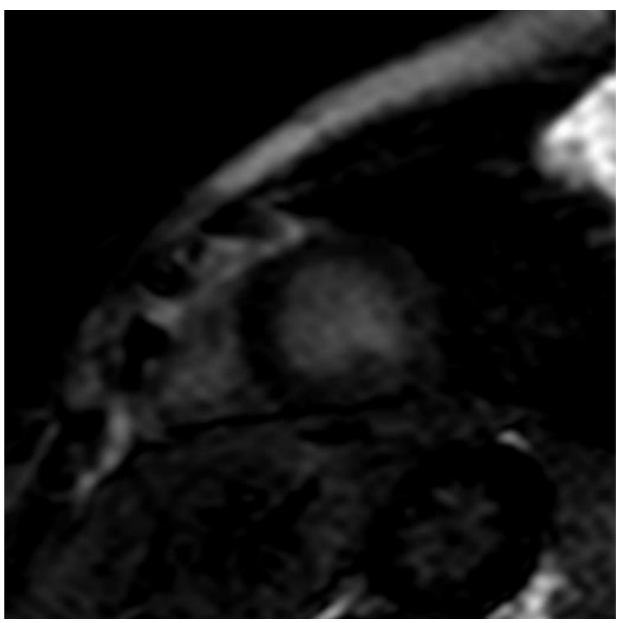

Fig. 2. *Cardiac RMN, LGE imaging.*

Vital parameters monitoring confirmed febrile temperature (38.3°C) and low blood pressure (77/51 mmHg) with sinus tachycardia; the urine output was normal (1,9 ml/Kg per hour). A new echocardiogram revealed a worsening of left ventricular function (EF 50%) with an increase of mitral and tricuspid valves insufficiency. Our patient also presented abdominal pain; at the abdominal ultrasonography, there was a thick-walled and overdistended gallbladder filled with biliary sludge (denoting a possible hydrops). Furthermore, a Thoracic RX showed a small right pleural effusion. On the first day in PICU, we decided to start IVIG (2 g/Kg in 16 hours), diuretic treatment (Furosemide 1 mg/Kg/day for 2 times/day) and acetylsalicylic acid (ASA) at an anti-inflammatory dose (60 mg/Kg).

However, vital parameters and echocardiogram didn't improve at the end of the IVIG infusion, and the child was febrile yet. According to the experiences of other Centers and the guidelines, we decide to start methylprednisolone in bolus doses (30 mg/Kg). Simultaneously, we discontinued ASA and started subcutaneous enoxaparin (1 mg/Kg/day); ceftriaxone and furosemide were continued. For improving the cardiac output, we also started dobutamine, i.v. (10 mcg/Kg/min). After these therapeutic changes, a good response was observed both in clinical examination and the echocardiogram. We did three days of methylprednisolone in bolus doses, and then we started the steroid decalage with the successive passage to oral prednisone. On the fourth day from admission in PICU, we stopped dobutamine, while diuretic treatment was progressively reduced and then stopped, with the total normalization of the echocardiogram (final EF 71%) at discharge.

Enoxaparin was interrupted on the ninth day, and in substitution, ASA was re-started at an antiplatelet dose (5 mg/Kg/day). On the twelfth day, a heart MRI

confirmed the presence of a good systolic biventricular function with an adequate segmental kinetic and longitudinal function; no valves insufficiencies were identified; the only pathological finding was a late gadolinium enhancement (LGE) in the anterolateral wall, related to mild myocardial inflammation. The patient was discharged in good clinical condition with the indication to assume ASA therapy at home (5 mg/Kg/day) for a total of 4 weeks and to continue the cardiologic follow-up.

DISCUSSION

The role of SARS-CoV-2 infection in the origin of hyper inflammation with multi-organ impairment is not fully understood. This condition is identified as MIS-C and can be suspected in children with a median age of 9.3 years (11). These cases usually have, according to WHO guidelines, prolonged fever (≥ 3 days), laboratory signs of inflammation (increased levels of CRP, erythrocyte sedimentation, procalcitonin), SARS-CoV-2 infection (SARS-CoV-2 positivity at swab testing or serology or an exposure to a probable COVID-19 case), exclusion of other diagnoses, and evidence of organ dysfunction (at least two organs involvement) (6, 12). In February 2021, the Rheumatology Study Group of the Italian Society of Pediatrics published new guidelines that define MIS-C by the presence in a child or an adolescent of prolonged fever (>38°C lasting for more than 24 hours); signs and symptoms of at least two organs involvement; laboratory signs of systemic inflammation (leukocytosis with neutrophilia, CRP and PCT increased, with or without lymphopenia), exclusion of infection (13).

Multi-organ impairment is one of the characteristic elements of MIS-C. Previous studies demonstrated that systems principally affected are: gastrointestinal (82-87%), dermatological/ mucocutaneous (69-73%), cardiovascular (71-100%), respiratory (14-47%), neurologic (22-55%), renal (3-38%) (9). Cardiovascular involvement is probably the most important manifestation of MIS-C and increases the need for PICU admission (14). Children often manifest myocardial dysfunction (in most cases, left ventricular systolic function with EF <60%), hypovolemic shock, coronary arteries dilation or aneurysm, myocardial infarction, and arrhythmia. Since coronary artery impairment can occur days after the discharge from the hospital, it is important that all children with MIS-C and cardiovascular involvement undergo long-term follow-up (15).

The MIS-C case treatment experience is improving day by day. Furthermore, therapy is currently borrowed from that used in Kawasaki Disease (KD). Children often need fluid resuscitation, inotropic support, and respiratory support; extracorporeal membrane oxygenation (ECMO) should be considered if medical support fails. It is necessary to monitor vital signs and hydration, and patients with shock can be treated with a volume expander such as Ringer lactate (16). It is important to pay attention to signs of fluid overload in patients with myocardial dysfunction. Inotropic support can be requested in case of cardiac impairment (12). Intravenous Immunoglobulin (IVIG) 2 g/Kg should be administered over at least 16 hours in patients with heart failure; the second dose of IVIG can be considered in case of inadequate response (14). Acetylsalicylic acid (ASA) 20-25 mg/Kg/dose every 6 hours (80 – 100 mg/Kg/day) can be considered as the first choice in patients with evidence of inflammation (elevated CRP or procalcitonin, or signs of multi-organ failure) or cardiovascular involvement (17).

In children with cardiac impairment, methylprednisolone can be administered with IVIG upfront at the dose of 30 mg/Kg IV for 1-3 days (as boli), followed successively from steroid decalage with methylprednisolone i.v. or prednisone orally. In some cases, biological treatments should be used in patients with persistent disease activity after 48 hours after first-line therapy (such as Anakinra). Other suggested treatments are large-spectrum antibiotics, ASA at an antiplatelet dose (5 mg/Kg/day), thromboprophylaxis with low-molecular-weight-heparin (LMWH), Proton Pump Inhibitor (PPI) (13). Melatonin administration may be a beneficial treatment (18-22).

CONCLUSION

The patient presented in this Report had symptoms consistent with MIS-C and developed hyperinflammatory shock, needing a recovery

in PICU, but he had a good response to standard treatment. The cardiac involvement with the need to resort to inotropic support suggested a serious and life-threatening condition since the admission. Clinical history, positive SARS-CoV-2 serology, symptoms and exclusion of other conditions (ecocultures and main viral serologies were negative) appear to be related to MIS-C. Our patient seems to have no important reliquate from this condition, but he needs to continue cardiologic follow-up to exclude the late development of other cardiac dysfunctions.

Further studies are needed to increase our knowledge regarding MIS-C related sequelae. Interestingly, both genetic and immunomediated factors were recently linked to potential cardiological complications of Covid19 infection in children (23, 24). Importantly, regulatory and molecular pathways and genetic defects may all converge to make some children susceptible to diseases and complications or to directly cause severe conditions.

Complex disease pathophysiology is thought to contribute to several childhood diseases in which both genetic and non-genetic factors interplay by affecting metabolic substrates or subcellular components, which are vital to biological homeostasis; this is known for a wide group of paediatric conditions, either monogenic or multigenic, in which both contributory and causative factors can be revealed using omic-related techniques, including sequencing of DNA or profiling biomarkers (22-34). In the last decade, the advent of such innovative technology increased our understanding of many rare and neglected pediatric diseases previously unclear or not fully understood by clinicians and scientists (35-46).

Future studies are needed to fully assess the cardiological (and not cardiological complications) (47, 48) related to pediatric Covid19 infection and understand how genetics and environment influence such complications.

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Neonatal sepsis in the NICU

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Although infection rates have modestly decreased in the neonatal intensive care unit (NICU) in the last years, sepsis remains one of the leading causes of morbidity and mortality in newborns worldwide. To date, there is no universally accepted definition for neonatal sepsis, as the consensus definition for pediatric patients is not suited for use in the NICU, and it lacks to address the evaluation of organ dysfunction in the premature population. For this reason and the extreme variability of clinical presentation of sepsis in newborns, diagnosis is not always straightforward. This paper aims to provide an updated overview of the current literature on etiology, clinical presentation, diagnosis, and treatment of neonatal sepsis.

Sepsis is still considered one of the major contributors to global morbidity and mortality in children and newborns. Neonatal sepsis accounts for 15.6% of neonatal mortality worldwide (1, 2), and it has been estimated that roughly 2.202 neonates for every 100.000 live births develop this condition, with a mortality rate of 11–19% and an overall translated incidence of 30 million cases per year (1). Yet, despite the burden of this disease, both in low- and high-income countries, there is no unanimous definition for neonatal sepsis, and those currently in use differ (2). The lack of consensual agreement is based on several reasons: (i) the extreme variability of the clinical presentation, due to the immaturity of innate and adaptive host defence mechanisms, typical in preterm newborns; (ii) the poor consideration of organ dysfunction as a diagnostic criterion for sepsis in neonatal literature, leading to a lack of established scores assessing its presence in newborns; (iii) the absence of ideal sepsis biomarkers, with acceptable sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (3, 4).

For all these reasons, a prompt and accurate diagnosis of neonatal sepsis based on clinical evaluation and laboratory blood tests remains challenging. In adults, according to the Sepsis-3 group definition, sepsis is no longer defined in reference to the systemic inflammatory response (SIRS) but as a dysfunction of at least one vital organ caused by a deregulated host response to infection (3). By contrast, in neonates, especially if preterm, signs of infection are often unclear and not specific, with the possibility of clinical deterioration in a short time.

Neonatal sepsis

Neonatal sepsis is the clinical manifestation of a systemic infection occurring in newborns within 4 weeks after birth. In particular, it refers to a systemic condition of bacterial, viral, or fungal (yeast) origin that is associated with hemodynamic changes and other clinical manifestations.

Keywords: neonatal sepsis, neonatal intensive care unit, newborns

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0393-974X (2022)

Based on the age at onset of the septic episode, it is further classified as early-onset sepsis (EOS) or late-onset sepsis (LOS) depending on whether the symptoms appear in the first 48-72h of life or beyond 3 to 7 days of age, respectively (5,6). This distinction is additionally based on the assumed different aetiologies and pathophysiology of pathogens typically seen before and after 72 h, encompassing both Gram-positive and Gram-negative pathogens. Main risk factors for EOS are represented by prematurity or low birth weight (< 2500 gr), maternal fever (temperature ≥38°C; often interpreted as a sign of chorioamnionitis), prolonged rupture of membrane (PROM, >18-24 hours), foul-smelling and/or meconium-stained liquor, more than 3 vaginal examinations during labour, prolonged and difficult delivery with instrumentations (neonatal sepsis past to present). Furthermore, a low neonatal 25-hydroxyvitamin D concentration has been associated with early-onset sepsis (7).

Early-onset sepsis (EOS)

Although the incidence of EOS has shown to be highest in extremely preterm infants and decreasing with the advancing of the gestational age, up to 43% of cases can occur in infants born at term (6, 8); in most cases, EOS is the result of vertical transmission from the mother during the perinatal period. Organisms frequently involved in EOS are Group-B *streptococcus* (GBS), which is the most common etiologic agent, and *Escherichia Coli*, which represents the most common cause of death. Aside from these, gram-negative pathogens such as *Enterobacter spp*., *Haemophilus influenzae* and *Listeria* monocytogenes are involved, especially among very-low-birth-weight infants. All these organisms are typically colonizers of the maternal genitourinary tract, contaminating the amniotic fluid, placenta, cervix, or vaginal canal. In addition, the pathogen may ascend when the amniotic membranes rupture or prior to the onset of labour, causing an intra-amniotic infection (9). Finally, it is worth mentioning that the incidence of culture-confirmed early-onset sepsis is rather low, accounting for around 0.4–0.8/1000 term infants in high-income countries. Consequentially, many neonates are diagnosed for EOS based solely on clinical suspicion and are empirically managed with antibiotics, despite having no pathogen isolated (10).

Late-onset sepsis (LOS)

Differently from EOS, the aetiology of LOS is attributed to organisms acquired from the interaction within the hospital environment or the community. Hospital-acquired LOS represents over half of all neonatal sepsis episodes, and most of them occur in extremely premature newborns. Very preterm infants (born before 32 weeks) are particularly at risk of developing LOS, and it has been estimated that 10 to 30% of them experience at least one episode of LOS before discharge from the NICU (11) with a consequent rate of 10–20% of deaths (12). Common risk factors for LOS include poor hands hygiene, low birth weight, prematurity, invasive ventilation, use of parenteral nutrition and prolonged use of antibiotics, as well as invasive procedures such as central venous catheter insertion or mechanical ventilation. The extended duration of the central venous catheter, directly related to gestational age, birth weight, and neonatal morbidity, has been described as a risk factor for LOS, but its role is being discussed (11). LOS has also been associated with excess bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), and neurological damage, including intraventricular hemorrhage that impact growth and neurodevelopment (12).

The most common bacteria associated with LOS is the *coagulase-negative Staphylococcus* (CoNS), followed by *S aureus* and *E coli*. According to a study carried out by Giannoni et al., Community-acquired LOS could be considered a distinct entity from EOS and Hospital-acquired LOS because of different incidence, distribution of pathogens, clinical presentation and outcomes. Most patients are term newborns, accounting for one-half of the sepsis episodes in infants born ≥ 37 weeks. *E coli* and GBS are the leading pathogens, while Urinary Tract Infection and primary bloodstream infection were the most common presentations. Risk factors include the use of pre-lacteal feeds, artificial feeds, bottle feeding and unhygienic cord and skincare.

Clinical presentation

Clinical presentation of EOS and LOS is characterized by non-specific and subtle signs and symptoms, focal signs of infection. Characteristic general symptoms are fever, temperature instability, poor feeding, yet every system could be involved (Table I).

Diagnosis

For diagnosis of sepsis, a thorough medical history is always mandatory to identify risk factors for early sepsis and to assess for clinical "red-flags" suggestive of a septic baby. In addition, any infants with symptoms indicative of infection should be considered possibly septic and investigated (Table 1 and 2). According to current literature, blood culture is considered the gold standard for diagnosis. However, the risk of false-negative results, especially after maternal antibiotic therapy or intermittent bacteremia, and false-positive results due to sample contamination, should always be considered (13-15). In addition, microbial cultures present a 2-3 days delay in obtaining results, making it often necessary to begin antibiotic treatment empirically. As an additional tool, Polymerase-Chain-Reaction (PCR) methods can increase the diagnostic reliability of causal pathogens for neonatal sepsis. Nonetheless, since its results do not depend on the bacterial viability, it may still be positive even after antibiotic treatment (16).

A complete blood count is also recommended, specifically to assess leukocytes and platelets count, as well as the leukocyte differential. Both leukocytosis (>40 000/μL) or leukocytopenia (<5000/μL) may be found, yet a normal leukocyte count does not rule out infection (17). Thrombocytopenia is common in neonatal sepsis, but it is non-specific. Likewise, a normal platelet count can be detected and does not rule out the diagnosis of sepsis.

Inflammatory markers, such as C-reactive protein (CRP), procalcitonin (PCT) and, less frequently, interleukin-6 (IL-6), are routinely researched in the diagnostic workup. The negative predictive value of two normal CRP values (the first 8-24 hours after birth and the second 24 hours later) is 99.7%. By contrast, an increased value in the setting of a newborn who appears healthy with no other risk factors has low specificity for infection and should not automatically warrant antibiotic treatment. Procalcitonin is another acute-phase reactant that reaches its peak values earlier than CRP, around

Table I. *Clinical indicators of infection in newborns (adapted from Shane et al.)*

	Infection	Severe infection
Central nervous system	Irritability, lethargy, tremors, seizures, hyporeflexia, hypotonia, abnormal Moro reflex, irregular respirations, full fontanel, or high-pitched cry	convulsions, drowsiness, unconsciousness, decreased activity, or bulging fontanelle
Cardiovascular system	Pallor, mottling, cold, clammy skin, tachycardia, hypotension, or bradycardia	poor perfusion, or rapid and weak pulse
Respiratory system	Apnoea, dyspnoea, tachypnoea, retractions, flaring, grunting, or cyanosis	respiratory rate more than 60 breaths per minute, grunting, severe chest indrawing on intake of breath, or central cyanosis
Gastrointestinal system	Abdominal distention, vomiting, diarrhoea, or hepatomegaly	jaundice, poor feeding, abdominal distension, or emesis
Others	Fever, temperature instability; "not doing well", poor feeding, or oedema	temperature greater than 37·7°C (or feels hot) or less than 35·5°C

6-12 hours after infection onset (18). IL-6 increases early during the inflammatory cascades and is also a hepatocyte stimulating factor that gives rise to the acute-phase response (19). According to a recent study, Procalcitonin is a better marker than CRP and IL-6 because of its highest sensitivity (97.6%) and specificity (89%) (20).

A lumbar puncture is indicated if (i) the blood culture is positive, (ii) the clinical course or laboratory data strongly suggest bacterial sepsis, (iii) the infant does not improve with appropriate antimicrobial therapy, (iv) clinical signs referable to the central nervous system such as lethargy, irritability or seizure, are present (21). If respiratory symptoms are accounted for, a chest radiograph is recommended, but infectious radiographic findings can be difficult to distinguish from respiratory distress syndrome or transient tachypnoea of the newborn (8). An X-ray and ultrasound of the abdomen should be performed in the presence of abdominal signs suggestive of necrotizing enterocolitis (NEC) or paralytic ileus or blood in stools (8). Urine cultures should not be performed in the first 72 hours of life but are recommended in all cases of suspect LOS (21).

Treatment of neonatal sepsis

Management of neonatal sepsis should be based on a multidisciplinary approach, including antimicrobial therapy, adjuvant therapy, and supportive therapy (22).

The aims of supportive care are several: (i) to prevent hypo- or hyperthermia, (ii) maintaining normoglycemic status (45 to 120 mg/dl as ideal standards), (iii) to maintain oxygen saturation within limits (91-94%) through the use of assisted ventilation, normal tissue perfusion and blood pressure, (iv) to infuse colloids and inotropes, if necessary. If present, blood products should be administered to normalize coagulation abnormalities, anaemia, and thrombocytopenia. An adequate nutriment should be maintained by enteral or parenteral nutrition to avoid a catabolic state that has been associated with a poor prognosis (8).

Adjuvant therapies, such as intravenous immunoglobulin and Gm-CSF infusion, are not recommended due to the lack of scientific evidence of their efficacy (23, 24).

Antibiotic therapy is probably the most important therapy to adopt, to treat the underlying infectious cause of sepsis. Of course, collection of blood culture or other specimens is warranted before starting any antimicrobial treatment, but it should not delay therapy administration. Indeed, according to the National Institute for Health and Clinical Excellence, 2012, (25, 26), treatment should start as soon as possible, and always within 1 h of the decision, as early initiation of an antibiotic therapy in neonates with suspected sepsis seems to reduce both mortality and morbidity.

Based on medical history and clinical examination,

Table II. *Initial investigations for suspected sepsis (adapted from Caffrey et al).*

Blood culture	Always
C-reactive protein	Always, repeat after 18–24 h
Skin swabs	Not indicated
Urine MC&S	Not indicated for EOS. Consider in case of LOS
Swab of conjunctival discharge	Indicated if discharge is purulent to test for chlamydia and gonococcus
Lumbar puncture	If CRP >10 mg/L, blood culture positive, clinical suspicion of sepsis/meningitis or no clinical improvement

***MC&S:** microscopy, culture, and sensitivities. CRP: C-reactive protein.*

specific "red flags" should be ruled out, and, if at least one of them is present or in the case of clinical indicators, antibiotic treatment should be commenced without delay (25). Red flags include parenteral antibiotics given to the mother for confirmed or suspected invasive bacterial infection; suspected or confirmed infection in another baby where there is a multiple pregnancy; respiratory distress starting more than 4 h after birth; seizures; the need for mechanical ventilation in a term baby; signs of shock.

Antibiotic treatment can be classified into an empirical therapy when the result of laboratory tests and blood culture is unknown and a target antimicrobial therapy in case of a recognized pathogen (25). Usually, initial empirical treatment of early-onset bacterial infections is represented by beta-lactam antibiotic (ampicillin) plus an aminoglycoside (usual gentamicin) (5). However, there has been an increased use of alternative protocols employing a cephalosporin (most commonly cefotaxime) or a glycopeptide (most commonly known as vancomycin) as a first-line option to treat especially late-onset sepsis, due to increased resistance among the most common pathogen such as coagulase-negative staphylococci (27). Other less common regimens used for EOS are represented by ampicillin combined with a third-generation cephalosporin (e.g. cefotaxime) or cephalosporins as monotherapy.

Be as it may, it is worth underlining how different studies have demonstrated that up to 95% of newborns treated with antibiotics for suspected sepsis prove to have no evidence of infection, thus contributing to the risk of increased antimicrobial resistance (27).

In the case of suspected LOS, for which different pathogens are involved (CoNS, S. Aureus), the empirical treatment recommended consists of vancomycin plus an aminoglycoside. However, once the pathogen is isolated from the culture and their susceptibility is known, antimicrobial therapy should be modified and tailored accordingly.

CONCLUSION

Neonatal sepsis still represents one of the main causes of death among newborns, both in industrialized and low-income countries. differently from pediatric and adult populations, where sepsis and septic shock are two well-characterized entities, recently revised and updated, a definition of neonatal sepsis is still not universally accepted. Several studies have recently dissected the role of genetic and non-genetic factors in neonatal sepsis (28,29). Importantly, future experimental studies aimed to understand these factors will be pivotal. Emerging mechanisms highlight the contribution of both genetic and environmental causes to the pathophysiology of complex paediatric conditions (30-39); this allowed researchers and clinicians to dissect sub-cellular alterations underlying childhood-onset diseases and identify biomarkers and prognostic indicators (40-54). A standardized characterization of neonatal sepsis will reduce in the future subjective variations in antibiotic use and support physicians providing appropriate therapy to those infants who need antimicrobial treatment.

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Recent recommendations of neonatal septic shock: a Review article

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Septic shock is a complex condition that is potentially fatal if not promptly treated. There are significant differences between clinical presentation in adults and children. In neonates, sepsis and septic shock are characterized by a nonspecific presentation that may delay an early diagnosis, thus contributing to high morbidity and mortality. Management of septic shock in the neonatal intensive care unit (NICU) is based on a multidisciplinary approach which includes prompt recognition of abnormal tissue perfusion and restoration of adequate cardiovascular function through the use of fluids and inotropes, if necessary; eradication of the underlying infection through the early administration of empiric broad-spectrum antibiotics; treatment of organ system dysfunction. This article aims to review current knowledge about the correct treatment and management of septic shock in the neonatal setting.

Shock is a pathological condition characterized by insufficient oxygen and nutrient delivery to the cells, resulting in increased anaerobic metabolism and accumulation of lactic acid. In neonates, sepsis is the predominant cause of shock and the primary cause of death from infection worldwide. (1,2). Based on the Third International Consensus Definitions for Sepsis and Septic Shock (SIRS-3) in adults, sepsis is defined as a life-threatening organ dysfunction caused by a deregulated response of an individual to infection. The presence of organ dysfunction can be identified by using the SOFA score, validated for adult patients. Septic shock is a subtype of sepsis in which the presence of circulatory and cellular/metabolic anomalies are profound enough to increase mortality (2). Two clinical criteria have been identified to define septic shock: i) Hypotension requiring vasopressors to maintain mean arterial pressure of 65 mmHg ii) Serum lactate level greater than 2 mmol/L (18 mg/dl), despite adequate volume resuscitation (2). Patients with septic shock can be identified for evidence of clinical signs, including hypothermia or hyperthermia, altered mental status, and peripheral vasodilatation (warm shock) of vasoconstriction with capillary refill >2 seconds (cold shock) (3,4).

Pathophysiological differences in adults, children, and neonates

There are essential differences between a septic shock in adults and children. While in adults, it is frequently characterized by a reduced systemic vascular resistance (SVR) and an increased cardiac index (warm shock), in pediatrics, septic shock is more often characterized by a reduced cardiac output (CO) and an increased SVR (cold shock) (5). By contrast, the clinical presentation of septic shock in newborns

Keywords: septic shock, neonatal intensive care unit, neonates

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0393-974X (2022)

might be highly heterogeneous, and the two conditions may coexist. Cold shock consists of peripheral vasoconstriction, cold extremities, tachycardia, and hypotension is often a preterminal event. Warm shock is characterized by peripheral vasodilation and hypotension secondary to endotoxin release (6). Increased pulmonary vascular resistance and artery pressure are typically associated with neonatal septic shock. Persistent pulmonary hypertension of the newborn (PPHN) can conduct to right ventricular failure with right-to-left shunting at the atrial/ductal levels producing cyanosis (7). Moreover, neonates commonly have a subtle presentation of septic shock due to many factors contributing to development differences in hemodynamic responses, such as immature cardiomyocytes, limited ability to increase CO, and the physiological transition from fetal to neonatal circulation (1). Sepsis-induced acidosis and hypoxia can cause an increase in pulmonary vascular resistance and artery pressure, maintaining the ductus arteriosus patented, and causing the persistent pulmonary hypertension of the newborn (PPHN) and the persistence of fetal circulation (5).

Management of neonatal septic shock

Every hour without using the PALS/APLS guideline in children, particularly neonates, is associated with a 40% increase in mortality risk (5). Moreover, the hemodynamic presentation of septic shock in neonates is much more variable than in older children, and prompt recognition can be challenging. It is essential to suspect this life-threatening condition in any newborn with impaired vital signs, such as tachycardia, respiratory distress, poor feeding, altered tone, skin color, tachypnea, diarrhea, or reduced perfusion. A thorough medical history should be carefully collected, and red flags, such as maternal chorioamnionitis or prolonged rupture of membrane (PROM), should always be ruled out.

The management of septic shock includes rapid recognition, early use of antibiotics, and rapid restoration of perfusion through fluids and inotropes (6). This approach, defined as Early Goal-Directed Therapy (EGDT), has been shown to be effective in reducing mortality compared to standard protocols (8,9).

In 2017, the American College of Critical Care (ACCM) provided updated guidelines for the management of neonatal septic shock in the neonatal intensive care unit (NICU) (7). According to these guidelines, outcomes of therapies encompass: restoring normal mental status, satisfactory heart rates (HR), peripheral perfusion (capillary refill <3 s), palpable distal pulses, and blood pressure for age (10). In addition, a median arterial pressure (MAP) less than 30 mmHg has been associated with increased rates of neurological anomalies and poor survival, especially in very low birth weight (VLBW) infants (7,11).

Management and procedures should be performed within specific times. The first hour, usually managed by the Emergency Department and any pediatric ward, is crucial. During the first 5 minutes, the aim should be to recognize septic shock, start mainly vital signs and urine output monitoring, and maintain adequate oxygenation. Moreover, according to NRP/PALS guidelines, vascular access should be rapidly obtained. In newborns, it is recommended to prefer umbilical arterial and venous catheters (12) (Table I).

Airway and breathing

Airway patency and acceptable ventilation and oxygenation should be carefully monitored and maintained. In order to ensure respiratory support, the first choice is represented by supplemental or high-flow nasal cannula oxygen. However, the presence of inadequate respiratory work and pronounced hypoxemia should always conduct to the decision of intubation. Both analgesia, sedation, and positive-pressure ventilation can reduce preload, inducing hemodynamic instability or arrest; to prevent these events, volume loading and inotrope infusion are often necessary before intubation. Intubation procedure includes the administration of sedatives, such as Fentanyl, which can moreover induce analgesia at a dose of 1-2 μg/Kg, or the use of atropine to prevent significant bradycardia. By contrast, in septic neonates, morphine, propofol, barbiturates, high doses of benzodiazepines, and dexmedetomidine can cause hemodynamic instability and should not be used as first-line agents (7).

Fluid resuscitation

Fluid should be given to obtain a normal perfusion

and adequate blood pressure for age. The gestational age basically determines the limits for first-hour volume replacement; for at term newborns, fluids can be administered up to 60 mL/ kg; in very low weight preterm newborns (BW <1,500 grams), the upper limit is 30 mL/kg; and for extreme premature without evidence of significant loss, it is up to 20 mL/ kg. When these volume limits are compiled, saline replacement is well tolerated, and there are no adverse effects described in newborns (7,13).

Extremely low birth weight (BW <1,000 g) in newborns and premature with a maternal chorioamnionitis history are particularly at risk of developing metabolic acidosis and altered perfusion in the first 24 hours after birth. Moreover, during the first three days of life, a worsening of respiratory function, tachycardia, and silent ductus arteriosus has been observed. For this reason, fluid replacement should be carefully performed since excessive or fast volume replacement may increase the shunt intensity and increase preload in the presence of myocardium with poor stretching ability and reduced contractile strength (13,14). Notably, fluid boluses in the first 48 hours of life have been associated with increased incidence of the need for home oxygen and higher prevalence of patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) in VLBW infants (15).

Fluid choices include crystalloids (normal saline or lactated Ringers solution) and colloids (dextran, gelatin, or 5% albumin) (7). In patients with hemoglobin (Hgb) >12 g/dl, crystalloid is the fluid of first choice. (7), even though excessive volume replacement may cause hemodilution, especially in anemic newborns or intracranial hemorrhage in premature. Hemoglobin can also be maintained at a minimum of 10g/dL during the initial resuscitation phase with good results (7). Anyhow, blood transfusion with packed red blood cells (RBCs) can

Table I. *ABCs: The First Hour of Resuscitation (Delivery Room Resuscitation)*

Goals (Adapted from Davis et al)
Maintain airway, oxygenation, and ventilation
Restore and maintain circulation (defined as normal perfusion and blood pressure)
Maintain neonatal circulation
Maintain threshold HRs

Therapeutic Endpoints (Adapted from Davis et al)
Capillary refill ≤ 2s, normal pulses, warm extremities, urine output > 1 ml/Kg/h, normal mental status, normal blood pressure for age, normal glucose and calcium concentrations
Difference in preductal and postductal oxygen saturation less than 5%
$SaO_2 \geq 95\%$

Monitoring (Adapted from Davis et al)
Temperature
Preductal and postductal pulse oximetry
Intra-arterial (umbilical or peripheral) blood pressure
Continous ECG monitoring
Blood pressure
Arterial pH
Urine output
Glucose, ionized calcium concentration

Table II. *Stabilization: Beyond the First Hour (NICU Hemodynamic Support)*

Goals (Adapted from Davis et al):
Restore and maintain threshold heart rates (HR)
Maintain normal perfusion and blood pressure
Maintain neonatal circulation
$ScVO_2$ > 70%
CI > 3,3 L/min/m^2
SVC > 40 ml/Kg/min
Therapeutic Endpoints (Adapted from Davis et al):
Capillary refill ≤ 2 s, normal pulses, warm extremities, urine output > 1 ml/Kg/h, normal mental status, normal blood pressure for age
SaO_2 > 95%
< 5% difference in preductal and postductal SaO_2
$ScVO_2$ > 70%
Absence of right-to-left shunting, tricuspid regurgitation, or right ventricular failure on echocardiogram
Normal glucose and ionized calcium concentrations
SVC flow > 40 ml/Kg/min
CI > 3,3 L/min/m^2
Normal INR
Normal anion gap, and lactate fluid overload < 10%

Monitoring (Adapted from Davis et al):
Pulse oximetry
Arterial pH Continuous ECG
Temperature
Glucose and calcium concentration
Ins and outs, urine output
CVP/oxygen saturation
CO
SVC flow
INR
Anion gap and lactate

be considered in newborns with Hgb less than 12 g/dL. In addition, fresh frozen plasma can be initiated to correct abnormal prothrombin time (PT) and partial thromboplastin time (PTT) values.

Diuretic administration is recommended in patients with 10% fluid overload and unable to reach a fluid balance with urine output/extrarenal losses to avoid fluid overload. In addition, in children with septic shock, a peak glucose level of >178 mg/dL may be associated with an increased risk of death (16). Therefore, insulin is indicated in the newborn only with marked hyperglycemia (>180 mg/dL) and in re-refractory shock and unfavorable response newborns. Conversely, hypoglycemia can be prevented using a D10% containing isotonic intravenous solution (17).

Hemodynamic support

During fluid resuscitation, patients with severe shock may require cardiovascular support. A combination of dopamine at low dosage (<8 μg/Kg/min) and dobutamine (up to 10 μg/Kg/min) is initially recommended. If patients do not respond to these interventions, epinephrine (0,05 – 0,3 μg/Kg/min) can be used to restore normal blood pressure and perfusion (7). According to a recent study, dopamine and epinephrine have shown similar efficacy and safety for treating pediatric or neonatal septic shock (18) (Table II).

Fluid resuscitation

After the first hour of intervention, regimens for fluids supplementation do not differ significantly from the initial phase and should be directed at clinical endpoints, mainly represented by adequate perfusion and optimal central venous pressure (CVP) (7).

Hemodynamic support

A 5-day course (6 hours/day) of intravenous pentoxifylline can be used in VLBW babies. In term newborns with persistent pulmonary hypertension of the newborn (PPHN), the first choice of treatment is inhaled nitric oxide. In newborns with left ventricle dysfunction and normal blood pressure, the addition of nitrosovasodilators or type III Phosphodiesterase Inhibitors (PDEIs) to epinephrine (0,05 – 0,3 μg/Kg/min) can be effective. Patients with thyroid insufficiency should be treated with triiodothyronine, acting as an inotrope. In case of refractory hypotension, norepinephrine can be used to maintain a $ScVO_2$ >70%. In newborns with adrenal insufficiency, hydrocortisone should be added. Umbilical catheterization should be maintained not more than 5 days for an umbilical arterial catheter (UAC) or 14 days for an umbilical vein catheter (UVC) (7).

Refractory Shock

If a condition of refractory shock is evidenced in newborns, some conditions must be excluded, including cyanotic or obstructive heart disease, critically large PDA, inborn errors of metabolism, pericardial effusion, pneumothorax, persistent blood loss, hypoadrenalism, hypothyroidism. After excluding these causes, ECMO (Extra Corporeal Membrane Oxygenation) is a good choice to consider in term newborns. In many Centers, indications for ECMO support are refractory shock or a PaO_2 <40 mmHg after maximal therapy. In addition, in patients with refractory shock related to PPHN- induced right ventricular failure, ECMO can unload the right ventricle, decrease septal bowing and improve left ventricular output (7).

Hydrocortisone treatment in neonatal septic shock

Although cortisol production in newborns is initially increased, very preterm neonates can have relative adrenal insufficiency that may contribute to hemodynamic instability and hypotension (19). In many clinical practices, hydrocortisone is the third-line agent in treating neonatal shock after volume resuscitation and dopamine (20). In paediatrics, recent septic shock guidelines suggest that intravenous hydrocortisone may be used if adequate fluid resuscitation and vasopressor therapy cannot restore hemodynamic stability (21,22).

The role of corticosteroids in the management of septic shock is controversial. However, recent studies reviewed the use of corticosteroids because they may be associated with increased mortality (23,24).

Antimicrobial therapy

In children with septic shock, antimicrobial therapy

should be started as soon as possible, within 1 hour of recognition, after blood culture was performed (21, 22). Initially, empiric broad-spectrum therapy should be administered (22). In newborns, empiric antimicrobial therapy includes beta-lactam antibiotic (ampicillin) plus an aminoglycoside (usually gentamicin) (25). Alternative protocols involve the use of cephalosporin (most commonly cefotaxime) or a glycopeptide (most commonly vancomycin) as a first-line option to treat especially late-onset sepsis due to increased resistance among the most common pathogen such as coagulase-negative staphylococci (26). Other less standard regimens used are represented by ampicillin combined with a third-generation cephalosporin (e.g. cefotaxime) or cephalosporins as monotherapy (27, 28). If a clinical history suggests HSV is infectious, empiric Acyclovir should be administered (27).

DISCUSSION

Sepsis and septic shock in neonates remains among the most important causes of neonatal mortality, mainly among developing countries and in western countries where the proportion of genetic and environmental causes may rise (29,30). In this future context, research will need to address biological disease mechanisms that predispose to sepsis and/or significantly contribute to its initiation/progression. Pediatric research in the last decade revealed underlying molecular pathophysiology of several complex genetic and metabolic diseases associated with either genetic or immunometabolic dysregulation (31-43). Furthermore, by using omic and related technologies such as next-generation sequencing, understanding such factors led to a revolution in terms of exact diagnoses, prognosis, and sometimes targeted treatments for many severe conditions that affect pediatric patients (44-56).

In the context of pediatric sepsis, it is important to highlight how both delayed diagnosis and late treatment have been associated with higher mortality.

CONCLUSION

For this reason, prevention, education, organization, and a good knowledge of updated protocols are the key to reducing the burden of deaths associated with sepsis and septic shock. Besides this, cornerstones of treatment are represented by the rapid recognition of signs of abnormal perfusion, a prompt and aggressive fluid resuscitation to restore cardiovascular function, and the early administration of antimicrobial therapy. Periodic implementation of the best evidence-based protocols is warranted to improve the approach to this life-threatening condition further.

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Novel serum biomarkers for infection in paediatric emergency

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Sepsis is one of the most frequent causes of morbidity and mortality in the paediatric age, and its early recognition is important to prevent its lethal outcomes. A great variety of novel serum biomarkers of infection have been investigated in the last decades, although most of the research has been performed in adult patients. We report current knowledge on this topic to assess the diagnostic efficacy of novel biomarkers of infection in paediatric patients.

Paediatric sepsis is a leading cause of death or major disability worldwide. In 2017, approximately half of the cases of sepsis were in the pediatric population, in children younger than 5 years, occurring at a rate of 20 million cases and 2.9 million deaths worldwide (1).

Sepsis can be considered a dysregulated host response to variou cific and sensitive signs of systemic inflammation and the variety of its clinical presentation. However, early identification is the first step to start timely treatment and prevent adverse outcomes (3, 4). In clinical practice, procalcitonin (PCT) and C-reactive protein (CRP) are the most used markers to recognize infections.

Evaluation of novel biomarkers may allow clinicians to better recognize patients with potential illness progression and at high risk of deterioration and identify those requiring antibiotic therapy or hospital admission (5).

Most novel serum biomarkers of infection have been studied among adults, and there is still a lack of research in the pediatric age. In this study, we attempted to show promising predictive biomarkers and to evaluate its application in a pediatric emergency setting, especially in children presenting with community-acquired pneumonia (CAP).

Lipocalin 2 (Lcn2)

Lcn2 (Fig.1), also called neutrophil gelatinase-associated lipocalin, is a member of the lipocalin family stored in specific neutrophils granules that act as siderophore binding protein interfering with microbial growth (6). The ability to reduce iron availability for bacterial growth is an innate immune response against microbial infection; this protein sequestrates siderophore bound iron working as a bacteriostatic (7).

Lcn2 has been studied as a serum biomarker to differentiate viral *vs* bacterial pneumonia aetiology in paediatric age. However, according to recent data, there is no consensus on using this biomarker; in

Keywords: Infection, biomarkers, children, sepsis, pediatric, novel biomarkers

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0393-974X (2022)

the Italian paediatric population, Lcn2 was a poor predictive tool of CAP aetiology, although this result seems to be very different compared to previous studies (8, 9).

Myxoma resistance protein (MxA1)

MxA1 belongs to the GTPase family, acts as a mediator of the interferon activity, and inhibit multiple viruses, including influenza viruses. The expression of this protein is regulated exclusively by Type I or III Interferon (IFN) in response to many viral pathogens. Its structure consists of 3 domains, an N-terminal GTPase domain that hydrolyses GTP, a middle domain that allows self-assembly, and a carboxy-terminal GTPase domain that acts as an effector (Fig. 2). It is expressed in the cytoplasm and acts against DNA and RNA virus nucleocapsid proteins. The protein structure, after conformational changes, oligomerizes in a crisscross pattern binding to tubular structures of the nucleocapsid, inhibiting their

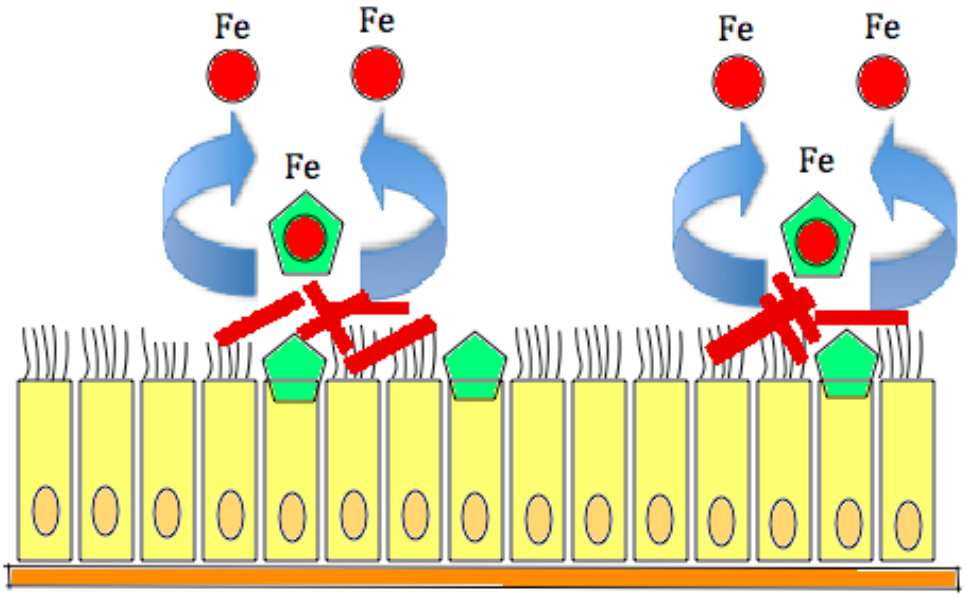

Fig. 1. *Cells releasing Lcn2 in response to pathogens. Ability of Lcn2 to bind iron, reducing the possibility of pathogens to grow. Borregaard N (2006) Neutrophil gelatinase-associated lipocalin, a siderophore-binding eukaryotic protein. Modified From: https://link.springer.com/article/10.1007/s10534-005-3251-7.*

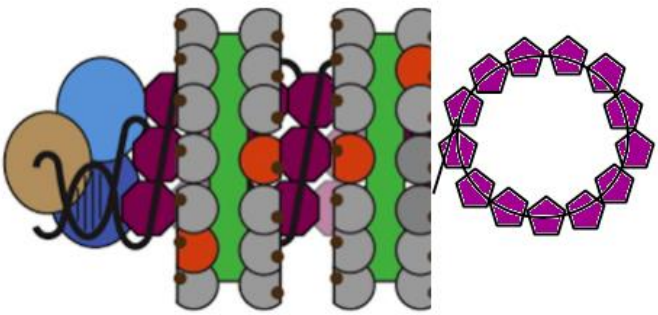

Fig. 2. *MxAoligomeric rings. Structure of MxA and interactions between viral domains and oligomeric rings. Gao, S (2011) Structure of Myxovirus Resistance Protein A Reveals Intra- and Intermolecular Domain Interactions Required for the Antiviral Function.*
Modified From https://www.sciencedirect.com/science/article/pii/S1074761311003694

function of viral transcription and replication (10). MxA1 has a biological function in other processes such as haematological disorders or autoimmune pathways; therefore, it should not be evaluated as an infection biomarker in these cases (11). However, this biomarker may be useful to distinguish infectious aetiology because it rises during viral processes (12). Despite this, recent studies do not demonstrate its diagnostic value in a pediatric emergency (13).

High mobility group box one protein (HMGB1)

HMGB1 is an extremely conserved nuclear protein released from monocytes/macrophages that promotes proinflammatory cytokines expression. This protein seems to be a late mediator of inflammation in sepsis, especially in viral-bacterial co-infections. Studies in pediatric patients evaluated HMGB1 expression using the PCR technique in peripheral monocytes, showing a high interest in diagnosing this biomarker (14). Anyway, this protein requires advanced and prolonged techniques such as PCR, and it seems to not be practised in a pediatric emergency setting.

CONCLUSION

Clinical practice in the emergency setting still faces the challenge of the early identification of infections to prevent the progression of illness in organ failure. The rapid recognition and timely treatment of an infectious disease are as important as the adequate therapeutic management of chronic diseases (15-18).

The molecular pathophysiology of childhood infectious diseases has been further understood by using omic related technologies that have been proven important in many processes related to paediatric health and diseases. In future years, scientific research will identify various serum biomarkers to better understand such pediatric conditions. However, these efforts are still not sufficient. Therefore, future work is required to prospectively validate the novel serum biomarkers in the pediatric emergency department.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Impact of COVID-19 on the management of patients with Lysosomal Storage Disorders

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Lysosomal storage disorders (LSDs) are a group of inborn errors of metabolism (IEM) characterized by multisystemic involvement with multi-organ complications. The recent Covid-19 pandemic had a major impact on the management and treatment of patients with LSDs, who also experienced significant psychological distress following the pandemic. Several experiences described so far demonstrate that telemedicine and home therapy programs are valid tools for the follow-up and care of patients suffering from these complex chronic diseases.

Lysosomal storage disorders (LSDs) are a group of inborn errors of metabolism (IEM) due to the lack of lysosomal enzymes involved in substrate degradation with a consequent multisystem accumulation of toxic material (1) The resulting clinical picture is characterized by a multisystemic involvement with multi-organ complications (2-10), extreme variability of the clinical phenotype even in the context of the same disease due to mechanisms not yet fully understood (11, 12), and a progressive course of clinical manifestations. In the last years, understanding the pathophysiology and molecular mechanisms of these disorders has allowed the identification of a growing number of promising therapeutic approaches, although not all of them yet usable in clinical practice (1, 13, 14). Therefore, the management of LSDs patients requires periodic visits to monitor the progression of the diseases and allow the early detection of any organ complications. Furthermore, for those LSDs for which specific therapy is available, it is essential that a therapeutic continuity is guaranteed, which, in some cases, requires frequent visits to the hospital, as happens for patients in Enzyme Replacement Therapy (ERT).

The recent pandemic caused by Sars-Cov-2 infection has had an important impact on the health management of all non-Coronavirus Disease-19 (Covid-19)-related diseases, including LSDs, and several experiences of different Clinical Centers involved in follow-up and treatment of these highly complex diseases have been described so far.

Impact of COVID-19 on LSDs

During the early stages of the pandemic, there

Keywords: Lysosomal storage disorders, enzyme replacement therapy (ERT), COVID-19 pandemic

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0393-974X (2022)

was little knowledge on the effects of Sars-Cov-2 infection, and a profound health care reorganization has been necessary to minimize the risk of infection while ensuring continuity of care for patients with complex chronic diseases. Sechi et al. (15) reported their experience with 102 patients affected by different LSDs coming from 16 different Italian Regions during the first wave of the outbreak. At the beginning of the pandemic, 71 patients were receiving ERT and 26 were on oral treatments. There were no problems in medicine supply, and no interruption or modification has been registered for patients in oral therapy. All patients receiving ERT at home continued their ERT infusions regularly, while 49% of LSDs patients receiving ERT in the hospital experienced disruption. These results were in line with other data about the effects of Covid-19 outbreak on rare metabolic diseases, provided by the European Reference Network for Hereditary Metabolic Diseases (MetabERN) by performing two surveys: one directed to patients' organizations, and one directed to healthcare providers, in the period between March and April 2020. Based on the results obtained from the surveys, visits were mostly cancelled or postponed, and most treatments were reduced or suspended (16). Considering that the recommencement of ERT after temporary treatment interruption does not fully reverse clinical decline due to the temporary discontinuation, home therapy seems to be useful to maintain therapeutic continuity for patients with LSDs during the COVID-19 pandemic (17), as had already been shown by experiences prior to the pandemic (18).

Brunetti-Pierri et al. reported the experience of a single Italian centre involved in the care of IMD patients during the COVID-19 outbreak (19). In the first months of a pandemic, it has been necessary to rearrange, reduce, or discontinue standard activities (e.g., outpatient clinics, follow-up evaluations, ERT, and clinical trials) and to convert most follow-up visits into remote visits. Telemedicine also proved to be a useful tool for MetabERN experience (16), allowing patients to be followed despite the pandemic and, at the same time, protecting them from the infection.

Patients with chronic multisystemic disorders, like LSDs, are considered at greater risk to have higher morbidity and mortality consequent to Sars-Cov-2 infection, and the first paediatric death in Italy was of a 5-year-old girl with mucolipidosis type II, emphasizing that in children with a severe metabolic disorder COVID-19 may have a worse prognosis (20). The strategies implemented to minimize the risk of infection in these patients allowed no proven infection to be recorded in the experiences described by several authors (15, 19, 21). Nevertheless, Elmonem et al., who collected data from 16 medical centres in Europe, Asia, and Africa specialized in IEM, reporting a median decline of IEM related services in March-May 2020 compared to the same period in 2019 in the range of 60 to 80%, described 2 patients both suffering from Gaucher Disease (GD) that were confirmed with COVID-19 infection and underwent a clinical decompensation (22). A group of investigators and physician experts in GD along with patient advocacy organizations in the United States proposed practice recommendations for the management of Gaucher patients during the SARS-CoV-2 pandemic (23), and Fierro et al. characterized a cohort of 181 GD patients to understand the determinants and the impact of SARS-CoV-2 infection during the peak of the pandemic in the New York City metropolitan area (24). The study results showed that 45 patients reported a primary exposure to someone with COVID-19, and 17 (38%) of these patients reported at least one COVID-19 symptom. Sixteen out of 88 patients tested were positive. No patient required COVID-19-specific treatments, and there were no deaths, suggesting that GD does not seem to be associated with severe outcomes of SARS-CoV-2 infection.

Finally, an aspect not to be underestimated about the effects of the pandemic on patients with LSDs concerns the psychological impact resulting from isolation and fears deriving from a health event of this magnitude. Fiumara et al. (25) evaluated how and to which extent the Sars-Cov-2 outbreak changed LSDs patients' behaviour and feelings. The administration of a phone interview to 15 patients, compared to the same interview administered to an age-matched control group, revealed an increase of anxiety, worries, and uncertainty in all interviewed people, with qualitative differences between the two groups, underlining the vulnerability and isolation of patients with LSDs.

DISCUSSION

The COVID-19 pandemic has represented a challenge for the healthcare system, particularly for the management and treatment of complex chronic diseases such as LSDs. With the implementation of telemedicine and home therapy programs, the reorganisation of the clinical centres has made it possible to minimize the risk of infection, guaranteeing continuity of care for these patients. Despite the complexity of these diseases, few cases of severe Sars-Cov-2 infection have been reported in patients with LSDs. Nevertheless, the psychological impact of the pandemic is not to be underestimated in the general population and even more in this fragile patient group. The molecular pathophysiology of severe Sars-Cov-2 infection is now more understood and genetic mechanisms implicated in disease initiation, severity and progression are increasingly recognized (26).

CONCLUSION

Omic-related and similar techniques will be important to further understand Sars-Cov-2 infection, as these technologies have been proven nowadays as essential in dissecting different complex paediatric diseases or mechanisms likely implicated in paediatric health (27-29).

Conflicts of interest

The authors declare that they have no conflict of interest.

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Infectious diseases and metabolic emergencies in inborn errors of metabolism

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Inborn errors of metabolism (IEM) can present as emergencies during acute metabolic decompensation that requires specific therapies to avoid morbidity and death. Metabolic imbalance is usually precipitated by diet or intercurrent illness, and symptoms are nonspecific, increasing the possibility of a missed diagnosis. The principle of acute management is to stop further decompensation and provide adequate metabolic needs.

Inborn errors of metabolism (IEM) are hereditary disorders caused by genetic defects. Their incidence ranges from 1:4,000 to >1:250,000, representing individually extremely rare diseases but collectively a large class of genetic conditions (1, 2). These disorders rely on defective enzyme activities, leading to toxic upstream metabolites that cause cellular damage, progressive morbidity, and death (3). The awareness of IEM is rising because of the expanded neonatal screening programs recently introduced in clinical practice, allowing prompt identification of early treatment of these conditions, thanks also to recent developments of specific therapies for some of these diseases (4-6), and better clinical outcomes (7).

Moreover, many studies have been conducted to understand the molecular mechanisms that can explain the remarkable phenotypic variability of some IEMs (8). Similarly, in different metabolic, genetic or acquired pediatric conditions, biomarkers or omic-based technologies are routinely used to investigate the pathophysiology of such conditions (9-11). Regarding IEMs, there are some specific risk factors for acute metabolic decompensation such as urea cycle defects, amino acid and organic acid metabolism and disorders of carbohydrate and energy metabolism. In addition, infectious diseases represent one of the risk factors of acute metabolic decompensation in these patients, requiring immediate stabilization in emergencies because of their general clinical condition (12).

This study aims to provide an overview of these groups initial presentation and clinical management in the emergency paediatric setting.

Clinical presentation

The initial presentation of IEM can occur in the neonatal age or later depending on the extent of the

Keywords: Inborn errors, metabolism, emergencies, acute decompensation

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0393-974X (2022)

enzymatic defect (13). During infectious diseases, symptoms result from acute intoxication and manifestations of metabolic imbalance. Clinical onset can include the following (14):

gastrointestinal signs include vomiting, hepatic dysfunctions, feeding problems or diarrhoea. It is very important to suspect acute decompensation of IEM in patients presenting to emergency settings with vomiting and dehydration. These are poorly specific symptoms with a high risk of misdiagnosis, such as gastroenteritis;

neurologic signs and symptoms such as lethargy or seizures; neurological manifestations may occur in acute decompensation in patients previously normal or may present in children with developmental delay. Although they can occur in all IEM, these manifestations present most commonly in organic acidemias, urea cycle disorders, disorders of carbohydrate metabolism;

respiratory manifestations such as deep or rapid breathing; respiratory abnormalities are usually the result of acid-base disorders that stimulate or depress the respiratory centre;

Laboratory findings

During infectious diseases, laboratory findings may allow recognizing IEM because of metabolic derangements. Patients may present with one or more of the followings (15):

acid-base disorders. Especially in organic acidemias, metabolic acidosis is one of the most typical findings, usually accompanied by an increased anion gap because of the presence of non-measurable compounds such as lactic acid and ketoacid;

elevations of ammonia, typically in urea cycle defects and organic acidemias;

hypoglycemia, characteristically in disorders of ketogenesis and carbohydrate metabolism;

It is very important to obtain samples during the initial evaluation before starting any treatment that may be stored to perform specific tests related to the suspected IEM.

Treatment

The most important objective of treating acute intoxication in IEM is to rapidly reduce the toxic molecules. Acute episodes of decompensation are life-threatening and adequate correction is the best approach to decrease significant morbidity. Therefore, the first approach is the most important challenge to stabilize circulation, airway and breathing. During acute episodes, the cornerstone of the treatment should focus on discontinuing protein intake and providing precise energy requirements, always based on the expected weight (16). The next principle is to clear toxic metabolites; it may be achieved by supplementing the specific cofactor or by extracorporeal detoxification using filtration and dialysis (17).

hyperammonemia: this condition is an acute metabolic emergency because of the neurological toxicity of ammonia that leads to brain edema. Patients showing this condition need to stop protein intake and start intravenous glucose administration, based on body weight (Table I) and ammonia scavengers. Treatment effectiveness should be monitored by measuring ammonia levels every 3 hours. If patients

Table I. *Glucose requirements according to body weight.*

Age	Weight (Kg)	Glucose to provide (mg/Kg/min)	Dextrose 10% (mL/Kg/day)
0-2		10	150
2-6		8	120
>6	<30	6	90
>6	30-50	4.5	67
>6	> 50	3	45

show ammonia levels above 400 umol/L or are non-responders to conservative treatment, this event may require extracorporeal detoxification;

acidosis: children presenting to the emergency department with acidosis should be rapidly assessed; treatment should be started if the base deficit is above 8 mmol/L and patients show symptoms such as vomiting, lethargy, refusal of feeds. In order to support circulation in dehydrated patients, saline bolus (0.9%) at 20 mL/Kg should be given over a few minutes; it may be repeated if patients do not show signs of evidence of fluid overload. It is important to administrate glucose 200 mg/Kg once. If acidosis still does not respond to this treatment, sodium bicarbonate should be given over 20-30 minutes. Monitor blood gases every 30 minutes to verify progressive normalization;

hypoglycemia: administrate intravenous glucose bolus 200 mg/Kg, continue with an infusion at 300-600 mg/Kg/h;

seizures: patients showing intractable seizures should be assessed for folinic and pyridoxine responsiveness; the therapeutic response confirms diagnosis. In order to control seizures, patients should receive 100 mg of pyridoxine, which may be repeated at 200 mg within 10 minutes in case of non-responsiveness. If the response is negative or uncertain, folic acid should be administered at 5 mg/Kg/day intravenously in three doses. In poor response, pyridoxal phosphate should be given at 30 mg/Kg/day in three doses.

DISCUSSION

IEM are complex diseases, including a large class of genetic conditions. Infectious diseases are an important trigger of acute decompensation in patients affected by IEM, requiring strict monitoring. These patients with IEM are likely to present to the emergency department during intercurrent diseases in acute metabolic decompensation; recognising these conditions is crucial to provide timely intervention and reduce morbidity and death**.** It is essential to develop an adequate approach to acute therapy in the emergency department, allowing effective treatment.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Extreme hyperferritinemia in the pediatric emergency setting: haemophagocytic lymphohistiocytosis, macrophage activation syndrome, sepsis and more

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Ferritin plays an important role in modulating inflammation and is a significant marker in some life-threatening pathological conditions. Hemophagocytic lymphohistiocytosis (HLH), macrophage activation syndrome (MAS), sepsis, and the more recent multisystem inflammatory syndrome in children (MIS-C) share many clinical and laboratory aspects, including the increase in ferritin levels, so that they are considered hyperferritinemic syndromes. In the pediatric emergency setting, the finding of high ferritin levels if associated with fever requires considering these rare syndromes to start a timely therapy.

Iron is a fundamental component of the hematopoietic process and in the composition of hemoglobin. It is decisive in the activity of cytochromes, in particular of the p450 family, in the oxygen transport chain and oxidative processes in general. In order to reduce oxidative stress, which is the basis of chronic inflammation in various diseases (1), iron is actively sequestered from cells by ferritin molecules. Ferritin, therefore, plays an important role during inflammatory processes, and new evidence suggests an active role in the immune response (2, 3).

The regulation of its synthesis depends, in fact, on cytokines (4), so that it is considered an acute phase reactant, with levels that faithfully reflect the degree of acute or chronic inflammation in several conditions; normal ranges vary by gender and age (Table I). There are many conditions in which an increase in ferritin is appreciated in the pediatric age, first of all, the iron overload due to chronic transfusions or hemochromatosis, malignant haematological diseases, inflammatory response, sepsis, but also lysosomal storage disorders that can benefit from specific therapies (5, 6).

Hyperferritinemia is defined as extreme when it reaches levels above 10000 ng/ml (7); in these conditions, if associated with fever, it must be considered as an alarm bell for rarer and potentially fatal conditions, such as hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome(MAS), two similar clinical disorders that share, in addition to the rise in ferritin values, also haemophagocytosis and life-threatening inflammation and can be defined as "hyperferritinemic syndromes".

Hemophagocytic lymphohistiocytosis (HLH)

The primary form of HLH is familial hemophagocytic lymphohistiocytosis (FLH), a fatal

Keywords: hyperferritinemia, hemophagocytic lymphohistiocytosis, macrophage activation syndrome, cytokine storm, COVID-19

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0393-974X (2022)

condition with a median survival, in untreated cases, of two months after diagnosis. It recognizes an autosomal recessive inheritance due to mutations in the gene encoding perforins (8). Secondary HLH (sHLH) can be consequent to infections, frequently caused by Epstein Bar virus (EBV), immunodeficiency, malignancy, or treatment with chimeric antigen receptor T-cell (CAR T-cell) (8, 9). Clinical and laboratory signs of HLH, both acquired or secondary, include unremitting high fever, hepatosplenomegaly, and cytopenia. In addition, they are often associated with hypertriglyceridemia, hyperferritinemia, increased transaminases, coagulopathy with hypofibrinogenemia and neurological indicators.

Furthermore, in these patients, reduced NK lymphocyte activity and increased cytokine levels, especially the soluble interleukin-2 receptor (sCD25), are frequently found. Histopathological features concern an accumulation of mature macrophages and lymphocytes, sometimes with haemophagocytosis, in the liver, spleen, bone marrow, liquor and lymph nodes. The most recent diagnostic criteria reported in the HLH-2004 treatment protocol (8) (Table II).

Macrophage activation syndrome (MAS)

MAS is considered one of the hemophagocytic disorders, closely similar to sHLH, from which it differs only in the triggering event (10). It is characterized by an excessive inflammatory reaction due to a dysfunctional immune response with an abnormal increase in T lymphocytes and macrophages and consequent release of proinflammatory cytokine (11). It is considered a serious complication of rheumatic diseases, particularly juvenile idiopathic arthritis in its systemic form (sJIA), systemic lupus erythematosus (SLE), Kawasaki disease and other connective tissue pathologies (12-14). Clinical features of MAS are similar to those of HLH (15). It is often difficult to make an early diagnosis of MAS as the signs and symptoms may overlap with those of the underlying rheumatologic condition so that laboratory findings can suggest the diagnosis. In a consensus conference in 2014, new classification criteria were formulated for MAS in febrile patients with known or suspected sJIA, and among these, hyperferritinemia constitutes the main criterion for diagnosis (Table III) (16).

Furthermore, hyperferritinemia has always been associated with sepsis and septic shock and represents, even in pediatric age, a marker of worse prognosis (17).

Although the mechanism that leads to hyperferritinemia is still unclear, several studies document how, both in MAS, sepsis and HLH, everything converges in excessive stimulation of T lymphocytes and activation of macrophages mediated by the abnormal increase of interferon-ɤ (INF-ɤ)with consequent hyperproduction of cytokines up to what is called "cytokine storm" (18).

"Cytokine storm" refers to a set of disorders characterized by an altered immune regulation with related general symptoms, systemic inflammation, multi-organ dysfunction up to multi-organ failure and exitus if not treated. Laboratory tests show increased C-reactive protein (CRP) levels which correlate with the degree of severity; there may be hypertriglyceridemia, leukocytosis, anaemia, lymphopenia, thrombocytopenia, elevated levels of D-dimer and ferritin. In this condition, there is an increase in inflammatory cytokine serum levels such as interleukin 18 (IL-18), interleukin 1β (IL-1β), INF-ɤ, tumor necrosis factor (TNF), interleukin 6 (IL-6), interleukin IL-10, sCD25 with consequent prolonged activation of signaling pathways and therefore collateral damage induced by cytokines; such damage is reflected in the effects of acute systemic inflammation and secondary organ dysfunction, regardless of the presence or absence of the pathogen as a trigger (19). In particular, IL-18 is an important severity biomarker in patients with MAS and correlates with hyperferritinemia (20).

Multisystem inflammatory syndrome in children (MIS-C)

The Coronavirus disease 2019 (COVID-19) outbreak, caused by SARS-Cov-2 infection, is currently one of the most discussed issues in the scientific world. Cytokine storm is widely described in SARS-Cov-2 patients and is associated with a poor prognosis. Furthermore, it is shown that in the most severe forms, COVID-19extreme hyperferritinemia correlates with a significantly higher cardiovascular risk, probably for its role in stimulating the production

Table I. *Normal ferritin reference intervals during infancy.*

Age	Ferritin concentration (ng/ml)
New-borns	25-200
1 month	200-600
2-5 months	50-200
6 months-15 years	7-140

Table II. *Diagnostic guidelines for HLH.*

Diagnosis can be made if one or both of the criteria underlying are met
A) Molecular diagnosis of HLH
B) Diagnostic criteria for HLH fulfilled (at least 5 of the 8 following):
Fever
Splenomegaly
Bilinear/trilinear cytopenia:
Hemoglobin <9g/dl (if< 4 weeks of age: hemoglobin <10 g/dl)
Platelets <100000/mmc
Neutrophils< 1000/mmc
Hypertrygliceridemia(>265 mg/dl) and/or hypofibrinogenemia (≤ 1,5 g/l)
Histological evidence of haemophagocytosis (bone marrow, lymph nodes, spleen)
No evidence of malignancy
Low or absent NK-cell activity
Ferritin concentration ≥ 500 ng/ml
sCD25 ≥ 2400 U/ml

Table III. *Classification criteria of MAS in sJIA.*

Ferritin concentration > 684 ng/ml and any 2 of the following:
Platelet count ≤ 181000/mmc
Aspartate aminotransferase > 48 U/L
Triglycerides > 156 mg/dl
Fibrinogen ≤ 360 mg/dl

of proinflammatory cytokines and the increase of free oxygen radicals (21,22).

A multisystem inflammatory syndrome is described in children (MIS-C) and is temporally associated with SARS-Cov-2 (23). This condition occurs in children with previous SARS-Cov-2 infection, persistent fever, clinical and laboratory signs of inflammation and systemic involvement in the absence of other potentially responsible causes (24). MIS-C can therefore be considered a mirror image of sHLH.

DISCUSSION

Sepsis, MAS, HLH and MIS-C share clinical and laboratory features. These hyperferritinemic syndromes show extreme immune complexity and phenotypic variability. Multiple pathways act independently but ultimately converge in the hyperstimulation of T cells and on macrophage activation with a consequent immunological and inflammatory response that can be life-threatening if not promptly recognized and treated (18).

CONCLUSION

In conclusion, hyperferritinemia is an important marker of several life-threatening pathological conditions. Therefore, in the pediatric emergency setting, the finding of high ferritin levels (especially if higher than 10000 ng/ml) in the presence of fever requires considering these rare syndromes in the diagnostic process.

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Protracted bacterial bronchitis: more to know

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AbstractProtracted bacterial bronchitis (PBB) is defined based on several parameters: (1) wet cough > 4 weeks, (2) improvement of cough within two weeks of antibiotic therapy, and (3) no signs or symptoms due to other underlying etiologies of chronic cough ("cough pointers"). PBB usually affects predominantly children aged <6 years old and male. The aetiology of PBB remains unknown. Predisposing factors include tracheobronchomalacia, positive bronchoalveolar lavage (BAL) culture for *Haemophilus influenzae*, *Streptococcus pneumoniae*, or *Moraxella catarrhalis*. Patients affected by PBB typically have wet coughs, especially upon awakening and during exercise. Thoracic objectivity is negative, and the children's general conditions are good. Therefore, a diagnostic approach for chronic cough should be oriented according to clinical history; a chest X-ray must be obtained, and spirometry must always be performed. The results of these two tests are usually normal, although a chest X-ray may sometimes show peri-bronchial infiltrates, a common and non-specific finding in childhood. Moreover, in the absence of specific "cough pointers" and when PBB is suspected, a sputum culture must always be obtained before starting antibiotic treatment. A positive result strengthens the diagnosis, although it has a low specificity. If "cough pointers" are present or PBB is suspected, antibiotic therapy is ineffective, and a recurrence of symptoms is observed, other etiologies of chronic cough should be investigated. PBB can lead to chronic pulmonary suppurative disease and bronchiectasis if left untreated. PBB and bronchiectasis share common features; however, it remains to be determined whether inflammation and colonization are the cause or an infection-induced effect. PBB treatment is based on antibiotics which are usually amoxicillin-clavulanate, for two weeks, up to a maximum of four according to the clinical course.

The concept of protracted bacterial bronchitis (PBB) is not new, although it has taken on different definitions and terminologies over the years. In the 1940s, some doctors already considered the child's chronic bronchitis as a possible predisposing factor leading to irreversible lung damage, such as bronchiectasis (1). In the following years, various definitions have followed: from a wet cough lasting

Keywords: protracted bacterial bronchitis; PBB; cough; children; wet; bronchiectasis

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0393-974X (2022)

more than 3 months to a year to recurrent coughing episodes lasting at least 2 weeks that may or may not be associated with wheezing (2).

In the 1980s, despite the perception of the problem, quality clinical studies had not yet been conducted, so most pediatric scientific texts did not mention or even hint at the existence of chronic bronchitis. PBB was only first identified in 2006 as the leading cause of chronic wet cough in a prospective study of 108 children with catarrhal cough of unknown aetiology lasting longer than three weeks (3). The study showed that the three major causes of cough in adults (gastroesophageal reflux, post-nasal drip, and asthma) were poorly represented in children. At the same time, PPB, defined by the finding of bacteria in bronchoalveolar lavage fluid (BAL) and with a favourable response to antibiotic therapy, was present in about 40% of the studied cohort. Furthermore, PPB was shown to be four times more frequent in this cohort than the three great causes (3). Since then, PBB has been included as a nosological entity in several international guidelines and consensus regarding chronic childhood cough (4, 5).

Today, PBB generally means a pathological clinical condition that is characterized by several parameters: (1) wet cough lasting for > 4 weeks, (2) resolution of cough within two weeks of starting with antibiotic therapy, and/or (3) absence of signs and symptoms attributable to another cause of chronic cough or the so-called "cough pointers" (4, 5). These other signs include chest pain, a history suggestive of inhaling a foreign body, wheezing, dyspnea on exertion, hemoptysis, growth failure, difficulty in feeding (including choking/vomiting), cardiac abnormalities, developmental neurocognitive development, recurrent sino-pulmonary infections, immunodeficiencies, epidemiological risk factors for tuberculosis, signs of respiratory distress, digital clubbing, chest wall deformities, rales on chest auscultation, radiographic chest changes, and/or lung function abnormalities (4, 5).

Epidemiology

The incidence and frequency of the disease are not yet known with precision; studies conducted in Australia and Turkey have diagnosed PBB at a percentage ranging from 11% to 41% of children with chronic cough (6). PBB affects children <6 years and predominantly male, with the peak incidence occurring at schooling (7).

The use of different definitions in the literature makes it even more difficult to assess the real prevalence of PBB. Epidemiological studies report a prevalence of chronic cough ranging from 1% in India to about 10% in Eastern Europe and 5% to 12% in China (8, 9). A recent systematic review showed how the etiologies of chronic cough varied by setting (10); from a socio-economic point of view in the more developed countries, the most frequent etiologies were asthma and PBB, while in the less developed countries, tuberculosis was prevalent.

A prospective study conducted in an Istanbul pediatric pneumo-allergy centre with 563 children aged two months to 17 years with chronic cough lasting > 4 weeks showed that in children aged <6 years, PBB was the leading cause of symptomatology after asthma and atopy (11). In a 2006 Australian prospective study, about 50% of children enrolled with a persistent cough for at least three weeks were referred to the referral centre with a diagnosis of asthma (3) following diagnostic testing, which included bronchoscopy. PBB was among the main diagnoses (40% of cases) and showed spontaneous resolution of symptoms during the study in 22% of cases (3).

Etiopathogenesis

The trigger for the development of PBB is still unclear. However, the hypothesis is that predisposing factors may be anatomical anomalies, such as tracheomalacia or bronchomalacia, as they establish an impairment of the physiological defence mechanisms, reducing mucociliary clearance and entrapment, and stagnation of mucous secretions (12). These conditions cause inflammation, which represents a double-edged sword. If the host's inflammation is short-lived and controlled, the respiratory system is protected from pathogens. However, if this response fails to eliminate the aggressor, the inflammation is amplified, becomes chronic, is poorly controlled, and causes damage to surrounding normal tissue, leading to progressive and chronic disease (13).

Several bacterial species have been isolated from the BAL of PBB patients. The most commonly found

were *Haemophilus influenzae* (47%), *Streptococcus pneumoniae* (35%) Moraxella catarrhalis (26%). These microorganisms form a polymicrobial film called "biofilm", in which they replicate undisturbed and are protected by the host's immune defences and by the action of antibiotic therapy (14, 15). However, it has been demonstrated that such bacteria are unable to adhere to a differentiated healthy epithelium; therefore, they should behave as opportunistic bacteria in the face of the damage of the ciliated epithelium that often occurs following repeated viral infections, which are typical of early life such as Adenovirus (16).

According to Hare et al., Vaccines against common germs appear to have a protective role against individuals predisposed to develop PBB. In detail, children who received the hexavalent vaccine are significantly less likely to contract lower respiratory tract infections from Haemophilus influenzae than children who received the 7- and 13-valence vaccine (17, 18).

An increase in pro-inflammatory cytokines determines the pathogenesis of PBB, and the main ones are interleukin-1α (Il-1α) and interleukin-1β (IL-1β) 12, which causes epithelial damage and subsequent cell death, thus producing the chronic cough that characterizes PBB (19–21).

A child with PBB seems to have an immunological profile characterized by an increase in CD56 and CD16 levels, which are probably related to high rates of viral detection of adenovirus in the BAL. No increase in eosinophils or an alteration in the value of serum immunoglobulins and IgG subclasses (22, 23). On the other hand, a marked neutrophilia accompanied by a total increase in the number of cells was observed. As expected, significantly higher levels of IL-1β and α-defensin were found, and IL-1β levels were found to correlate with BAL neutrophilia and cough duration and severity, and that levels of IL-1β and α-defensins are reduced when PBB is treated and resolved (24).

Table I. *Main causes of chronic cough in children according to literature.*

	PBB	Asthma	Post-Nasal drip	Gastroesophageal reflux disease	Bronchiectasis	Tracheomalacia	Psychogenic cough	Spontaneous resolution	Other
Marchant et al. (3)	40%	4%	3%	3%	6%	/	1%	22%	21%
Asilsoy et al. (29)	23%	25%	20%	5%	3%	/	4%	6%	3%
Khoshoo et al. (30)	/	13%	23%	28%	/	/	10%	/	25%
Chang et al. (6)	41%	15.8%	1.4%	2.3%	9%	6.1%	4.3%	13.9%	6.1%

Table II. *Differential diagnosis between protracted bacterial bronchitis (PBB) and asthma.*

Protracted bacterial bronchitis	Asthma
Wet cough	Dry cough
Worsens upon awakening	Worsens during the night
The severity of cough can lead to breathing difficulties	Respiratory failure is not directly related to the severity of the cough
Rattling lung sounds	Wheezing lung sounds
Improvement after antibiotics	Improvement after corticosteroids

PBB and bronchiectasis share common characteristics, and it is still unclear whether bronchiectasis represents a precursor for the development of PBB or if they are a complication of the same. Both conditions are associated with neutrophilic inflammation and the colonization of pathogens in the lower airways, including *H. influenzae* (NTHi). It has been reported in several studies that PBB and bronchiectasis share a common denominator, namely defective efferocytosis (defective phagocytosis of the alveolar macrophages of apoptotic bronchial epithelial cells), which causes tissue damage and neutrophilic inflammation in the lungs. Hodge et al. (2016) showed that in children with PBB and bronchiectasis, the phagocytic capacity of macrophages was significantly lower than in the control group (both in terms of efferocytosis and in terms of phagocytosis of NTHi) and that IL-1β levels increased in both groups compared to controls. Therefore, it can be inferred that reduced phagocytosis of alveolar macrophages of apoptotic bodies and NTHi can contribute to neutrophilic inflammation and colonization of NTHi in both PBB and bronchiectasis (25).

Signs and symptoms

The typical clinical phenotype of children with PBB is characterized by the presence of chronic wet cough (smoker-like) lasting > 4 weeks with a greater frequency upon awakening and during physical exercise but present throughout the day and minimally even during the night; moreover, it shows no tendency to regress over the weeks (non-remitting cough). Children may sometimes present with wheezing, which is often mistakenly diagnosed as bronchial asthma; therefore, treatment is mistakenly administered based on asthma and results in therapeutic failure. Thoracic objectivity is negative, and there are no added noises, except for variable coarse rales during fits of cough (26).

The general conditions of the children are good, no systemic symptoms and/or chronic pulmonary disease are found, and their weight–stature development is normal. However, the chronic cough leads to parental anxiety due to frequent absences from school. A temporal relationship was observed between viral infections of the airways and the onset of catarrhal cough (27, 28).

Diagnosis

Four out of the most recent published studies on PBB have the advantage of being prospective trials conducted to apply guidelines or management and diagnostic algorithms. In the first such study, which was published in 2006, over two years, 108 children with chronic cough and an average age of 2.8 years were subject to a standardized sequence of diagnostic tests in which chest radiography and spirometry were performed early. Bronchoscopy and BAL examination also became part of the diagnostic process in a relatively short time (3).

A second Turkish study was conducted with 108 children (average age 8.4 years) and involved applying the recommendations in the guidelines of the American College of Chest Physicians (ACCP). In this study, children with symptoms were excluded from the algorithm oriented toward a specific type of cough (29). Finally, the third study, a prospective American one, lasted four years but only included 40 children with an average age of 7.8 years and cough for > 8 weeks who were followed with a rather intense and thorough workup (30).

Finally, the most recent study in the etiological–diagnostic field was conducted on a cohort larger than the previous three studies (346 children) from six Australian centres. The children were divided into four age groups (0–2 years, 2–6 years, 6–12 years, and >12 years). Analyzing the results, the groups were managed according to an algorithm similar to that used by the Turkish authors from the Guidelines of the ACCP.

Chest radiography and spirometry were performed early and chronic cough, in the absence of specific cough indicators, underwent a diagnostic–therapeutic trial with inhaled corticosteroids (for non-productive cough) and with antibiotics (for productive) (6). Table I shows the prevalence of the main causes of chronic cough found in the above four trials. From their description, it is already evident that these studies are heterogeneous, both in terms of the definition of chronic cough (four or eight weeks) and age involved, the number of samples, and the duration of cough before enrollment. In addition, differences emerged regarding the quality and invasiveness of the selected diagnostic procedures, the setting in which the studies

were conducted, and the initial inclusion or exclusion criteria of children with warning signs for cough of specific aetiology.

Emerging results indicated the prevalence of PBB in terms of the cause of chronic cough. Compared with the 2006 Cough Guidelines, high-quality evidence was demonstrated in children aged ≤14 years with chronic cough (duration> 4 weeks), in whom the use of cough management protocols (or algorithms) improved clinical outcomes and cough management. Diagnostic algorithms should change according to characteristics associated with cough and medical history; a chest X-ray and, if necessary, spirometry (pre- and post-beta 2 agonists) should always be performed. Spirometry and chest x-rays, when done, are usually normal; peri-bronchial infiltrations can sometimes be seen on chest X-rays; however, these are non-specific findings that are common in the pediatric ages (4, 27). Other tests should not be done routinely and based on the child's medical history and clinical symptoms and signs (for example, testing for tuberculosis when the child has been exposed to it) (4, 5, 27).

Furthermore, in the absence of specific "cough pointers" and when PBB is suspected, a culture of broncho-aspirate should always be obtained before starting antibiotic treatment; a positive result strengthens the diagnosis; however, this test has low specificity (4, 5, 27).

Differential diagnosis

In the presence of cough pointers and/or when PBB is suspected, but treatment with antibiotic therapy is ineffective, and a recurrence of symptoms is observed, other causes of chronic cough should be investigated. These can include adenotonsillar hypertrophy, rhinosinusitis, gastroesophageal reflux, bronchial asthma, whooping cough, tuberculosis, foreign body inhalation, cystic fibrosis, bronchiectasis, congenital lung lesions, immunodeficiencies, primary ciliary dyskinesia, or vessel anomalies (4, 5, 27, 31–38).

In this case, it is necessary to perform second-level diagnostic testing based on the clinical suspicion: (1) sweat test, (2) chest computed tomography (CT) scan, (3) nasal nitric oxide, (4) immunological evaluation, and/or (5) bronchoscopy (4, 5, 39).

Two-thirds of children with PBB have tracheobronchomalacia. In addition, several studies have shown that tracheobronchomalacia, defined as the reduction of > 50% in the diameter of the trachea and/or bronchi, is present frequently in children with PBB (40, 41).

It is believed that tracheobronchomalacia predisposes an individual to the onset of PBB as it causes an impairment of the physiological defence mechanisms and a reduction in mucociliary clearance that leads to entrapment and stagnation of mucous secretions; however, it is also possible that the intense chronic inflammation of the airways predisposes a child to the development of malacia of the airways as a result of a long-lasting PBB (12).

PBB is often misdiagnosed as asthma and treated with corticosteroids resulting in the persistence of symptoms. Non-specific differences between asthma and PBB make the differential diagnosis challenging, and in this sense, the execution of spirometry helps facilitate a differential diagnosis (4, 5, 40, 41). The main differences that may be useful for distinguishing the two pathologies are summarized in Table II. However, PBB can be a complication of asthma, and in addition, the two diseases can co-exist, in which case chronic cough is associated with episodes of airway obstruction (42).

If left untreated, PBB can progress to chronic suppurative pulmonary disease and bronchiectasis. PBB, chronic suppurative pulmonary disease, and bronchiectasis are progressive clinical pictures. In all three of these conditions, the alterations in mucociliary clearance seem to be the common risk factor that favours bacterial colonization of the lower airways. In the absence of adequate treatment, the persistence of inflammation is likely to result in impaired physiological defence mechanisms, reduced mucociliary clearance, chronic mucus hypersecretion, mucosal secretion entrapment and stagnation, progression of airway inflammation, and bacterial stasis. This process saturates the bactericidal capabilities of the immune system by promoting chronic inflammation resulting in damage to the bronchial wall and ciliary epithelium (43). A "vicious cycle" starts where infection–inflammation leads patients to PBB onset, chronic suppurative pulmonary disease, and finally bronchiectasis. In this sense, the

development of bronchiectasis is preventable with adequate antibiotic treatment (44).

In the 1940s, a reversible pre-brochiectatic state with adequate antibiotic treatment was believed to be possible (45). Recurrent PBB and bronchiectasis share common characteristics, such as neutrophilic inflammation and colonization of potentially pathogenic microorganisms. It remains to be determined whether these effects are the cause or result of the process (25).

In a prospective longitudinal cohort study, lower respiratory tract infections with NTHi and recurrent PBB was demonstrated since both are significant predictors for the development of bronchiectasis (46).

Dourus found a correlation between the duration of wet cough and abnormalities found on high-resolution chest CT scans (HRCT). HRCT scans revealed thickening of the airway walls and bronchiectasis, of which the severity was directly related to the duration of symptoms and intensity of inflammation. The main sign of bronchiectasis on the HRCT scan is the identification of a "bezel ring" determined by an increase in the broncho-artery ratio, which is considered positive for bronchiectasis if b> 1 in adults, but this ratio gradually increases with age. In contrast, it is considered positive in pediatric age groups if b > 0.8. However, HRCT scanning was shown to be less sensitive than bronchoscopy in detecting airway abnormalities, and the authors concluded that the two modalities should be considered complementary in assessing prolonged wet cough (47).

Therapy

The cornerstone of PBB treatment is represented by oral antibiotic therapy as the use of intravenous or nebulized antibiotics was shown not to highlight significant differences in response to treatment compared to the oral antibiotic. In addition, it has been shown that the administration of oral antibiotics induces the resolution of a chronic wet or productive cough in children of ages ≤ 14 years (48).

In a study performed in 2012, a sample of 50 children was randomized into two groups. The first group was given 14 days of oral therapy with 22.5 mg/kg/dose amoxicillin clavulanate twice daily, and the second group was given a placebo in equal volumes. Children in the first group treated with amoxicillin-clavulanate were significantly more likely to achieve cough resolution (n = 12, 48%) than the placebo group (n = 4.16%; p = 0.015). These children remained symptom-free four weeks after the study ended (49).

Recent studies have demonstrated that a 2-week regimen with 50/mg/kg/day amoxicillin clavulanate can lead to a resolution of chronic wet cough and a drastic improvement in the child's quality of life in a statistically significant number of children (50). Second or third-generation cephalosporins, trimethoprim-sulfamethoxazole, or macrolides can be used as an alternative treatment when a history of penicillin-mediated IgE reactions is present. However, shorter courses of antibiotics tend to cause partial resolution or relapse of the cough after a few days of treatment (50).

When symptoms did not improve after four weeks of antibiotic therapy, risk of potential underlying lung disease was found, which is why further second-level examinations are indicated (50).

The use of azithromycin as an anti-inflammatory in PBB remains undefined. However, this use is particularly important in setting the global antibiotic resistance crisis from overprescribing antibiotics.

Macrolides, in particular, alter the resident microbiome of the host and provide strong antibiotic resistance to organisms, resulting in increased costs and risk of treatment failure. The use of this drug could be indicated if the child has developed a complication, such as a bronchiectasis (51).

CONCLUSION

PBB is a leading cause of chronic cough in children, especially preschoolers. Despite this finding, evidence of a lack of understanding and inappropriate treatment is present in many cases, leading to the possible risk of progressive, irreversible lung damage. Therefore, a specialist approach would be appropriate, especially in case of therapeutic failure or symptomatology relapse. Furthermore, further prospective studies are needed to consolidate the few and qualitatively unsatisfactory available evidence to support and improve the diagnostic–therapeutic management of this disease.

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Fungi and non-tuberculous mycobacteria: emerging pathogens in cystic fibrosis

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Fungi and non-tuberculous mycobacteria (NTM) are increasingly isolated in the sputum of patients with cystic fibrosis (CF), often representing a diagnostic-therapeutic problem for the clinician. For this, it is essential to start screening programs for these pathogens in all patients with CF, especially in individuals with an otherwise unexplained deterioration of lung function. Infections sustained by these pathogens are also a significant cause of morbidity and mortality in the post-transplant period. Early diagnosis and treatment play a central role in the management of all CF patients.

Fungi and non-tuberculous mycobacteria (NTM) are microorganisms often found in the sputum of cystic fibrosis (CF) patients and are increasingly associated with the onset of clinically manifest disease (1,2). Both are ubiquitous microorganisms, but it is not yet clear what risk factors correlate with the onset of active lung infection. Therefore, it is undoubtedly advisable to subject patients suffering from CF to screening for NTM and fungi, particularly in clinical deterioration conditions despite antipseudomonal therapies**.** This review aims to update the current knowledge about the role of fungi and NTM airway colonization in patients with CF.

Fungal infection

In this study we focus on the clinical aspects of pulmonary fungal infections in CF and on the therapeutic choices to be made in the presence of infections in the respiratory tract. It analyzes fungal microorganisms commonly found in airway specimens in CF patients, such as *Aspergillus fumigatus*, *Candida spp*., *Scedosporium apiospermum complex*, *Lomentospora species*, and *Exophiala dermatitidis* (3-5).

Several factors have been hypothesized to affect the prevalence and variety of fungal infections. First, it has been shown in vivo that microorganisms of a bacterial nature and fungi colonize the lungs of CF patients, so it is conceivable that a process of interaction and competition is established between them. In particular, we focused on the interaction between *Aspergillus fumigatus* and the lung microbiota,

Keywords: cystic fibrosis, fungi, mycobacteria, NTM, treatment, Aspergillus

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0393-974X (2022)

especially represented by *Pseudomonas aeruginosa*; In both in vitro and in vivo models, inhibitory and stimulating effects on growth and virulence have been described (4-6).

Several factors, including geographical and climatic zones, can influence environmental exposure and, therefore, fungal infections. Accordingly, a European multicentre study showed an increase from north to south in the prevalence of infections caused by *Scedosporium*, while infections sustained by *Aspergillus fumigatus*, *Cladosporium* and *Penicillium* were found more frequently in the centres of Northern Europe (7). In an Australian study, the Authors showed that man-made environments correlated with numerous fungal colonies (8). The Australian authors found a prevalence of Scedosporium spp., which explained the relatively high rates of Scedosporium infections and colonization in Australia in the soil samples from urban environments compared to those taken from semi-rural and rural areas (9). In a German study, other factors such as lifestyle and the presence of pets as a potential source of mould exposure were associated with the onset of allergic broncho-pulmonary aspergillosis (ABPA).

Furthermore, the risk of infection caused by fungal microorganisms also seems to correlate with age, with progressive lung damage and with the progressive decrease in forced expiratory volume in 1 second (FEV1). However, it would seem that the same therapies practised by patients CF may play a role in this regard. Differences between several areas in the onset of pulmonary fungal infections could also correlate with the remarkable heterogeneity in infection control measures and hygiene-prophylactic recommendations provided to patients (10).

An increasing number of studies have been concerned with evaluating the role that CF therapies may have in the etiopathogenesis of fungal infections, with particular reference to frequent antibiotics, particularly macrolides, antibiotics administered chronically inhaled for chronic airway infections, steroids administered by inhalation (11-14).

Consequently, the severity of the evolution of CF lung disease is associated with the risk of contracting fungal infections. Therefore, new therapeutic perspectives emerge from CFTR modulator therapies, which show promising results in improving lung condition and reducing airway infections. For example, the reduction in the detection rate has been demonstrated for *Aspergillus spp*. and *Pseudomonas aeruginosa* than before treatment, following CFTR modulator therapy with Ivacaftor for the G551D gating mutation (15).

The epidemiology of fungal species in CF lung disease is multifactorial, and the data have to be interpreted with caution (16). Furthermore, the prevalence is still little known and highly variable the pathogenicity of most fungi as well as a variable is the clinical presentation, in the absence beyond all of the clear diagnostic criteria. Consequently, in clinical practice, the decision to undertake therapy can be difficult. Therefore, a combination of empirical diagnostic criteria has been proposed, which appears to be very useful in discerning between one "highly probable" pulmonary fungal infection from simple colonization (17).

These criteria include (i) an increase in the production of sputum; (ii) multiple isolations of the same fungal species from sputum or bronchoalveolar lavage (BAL) (at least two culture-positive samples throughout six months); (iii) detection of pulmonary infiltrate (s) on chest tomography (CT)r radiography; (iv) failure of treatment with antibiotic therapy (two and more antibiotic treatments, duration treatment major than two weeks); (v) unexplained decline in lung function; (vi) exclusion of new/other bacteria; (vii) exclusion of ABPA (17).

Other authors have introduced "minor criteria", such as the finding of elevated specific fungal IgG, positive galactomannan in sputum or BAL and cytological/ histological results in BAL and biopsies (18).

Candida spp.

In CF patients, colonization with yeast is common, especially in throat swabs, particularly from *Candida albicans* being the most frequently isolated taxa (up to 62%) (19). Other species commonly found are *Candida dubliniensis*, *Candida glabrata*, and *Candida parapsilosis* (19, 20).

However, these microorganisms are rarely detected in BAL samples, casting doubt on their real clinical significance as pathogens rather than simple

colonizers of the oral cavity. Therefore, the choice to undertake treatment is made according to the individual case, referring to the diagnostic criteria for "highly probable" pulmonary fungal infection. From a recent retrospective analysis of 16 years, it is clear that the colonization by *Candida albicans* or *Aspergillus fumigatus*, but above all, the finding of *Candida dubliniensis* in sputum has been associated with a decline in lung function, whereas *Candida spp*. does not appear to cause pulmonary exacerbations (19).

In CF patients who require central venous access for the frequent administration of antibiotic therapy, the risk of *Candida Albicans* sepsis is high. In 2019, *Candida auris*, a very resistant Candida species that typically colonizes the ear canal (21), was first detected in the sputum of a CF patient undergoing long-term Posaconazole therapy in connection with Aspergillus colonization (22,23).

Aspergillus spp.

Aspergillus spp. is isolated in approximately 40% of CF patients. The most frequently encountered species is *Aspergillus fumigatus*, while the rarest is *Aspergillus flavus*, *niger, clavatus*, *nidulans* or *terreus*. In clinical practice, Aspergillus bronchitis is frequently encountered, characterized by pulmonary exacerbations not responding to antibiotic treatment but improving with antifungal therapy both in clinical appearance and spirometric tests (23).

In support of the diagnosis of Aspergillus bronchitis, the finding of *Aspergillus fumigatus* and the positive sputum for galactomannan play a fundamental role and specific IgG presence of *Aspergillus fumigatus* in the absence of specific IgE (24). Thanks to using an antifungal treatment lasting from two to six weeks, clinical improvement and lung function were obtained (25). Therefore, voriconazole is strongly recommended as first-line therapy in the case of systemic Aspergillus disease. Alternatively, it is possible to resort to Amphotericin B and Isavuconazole, or Posaconazole. In rare, rather severe cases, *Aspergillus* colonization can be responsible for the formation of pulmonary aspergilloma in CF patients (26).

Allergic broncho-pulmonary aspergillosis (ABPA)

Allergic broncho-pulmonary aspergillosis (ABPA), first described in 1952, is defined as a lung disease caused by *Aspergillus fumigatus*-induced hypersensitivity. It is commonly found in immunocompetent patients suffering from asthma and/or CF. Its prevalence in pediatric age is unknown (27). In children with CF, risk factors for ABPA have been identified, such as (i) Age over 12 years;(ii) low HR index; (iii) presence of immunoglobulins (Ig) E and eosinophilia; (iv) bronchial colonization with *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*; (v) long-term therapy with azithromycin and high doses of inhaled corticosteroids (CS) (28). Clinically, ABPA can manifest with systemic symptoms such as fever and weight loss and with respiratory symptoms, such as wheezing, increased productive cough, tachypnea/ dyspnoea, and poorly controlled asthma chest pain, hemoptysis and exacerbations, who respond poorly to antibiotic therapy.

In most cases, ABPA, therefore, remains undiagnosed and may be one of the most common causes of poorly controlled asthma and highly significant morbidity in children with CF. The auscultatory finding is characterized by detecting non-specific noises, such as crackles and rhonchi. Children with ABPA show severe progressive impairment of all lung function parameters. FEF 50% VC (FEF50) and trapped gas volume were the best predictors for specific airway resistance. No pediatric-specific diagnostic criteria of ABPA are currently available; however, exhaled nitric oxide (FENO) has been proposed as a biomarker for diagnosis (29,30).

For the pediatric population, reference is also made to the diagnostic criteria for ABPA used in the adult population. These criteria include an increase in specific IgE values against *Aspergillus fumigatus* (> 0.35 kUA / L) and Total serum IgE> 1000 IU / mL (2400 ng / mL). In addition, at least two of the three secondary criteria must be met, including transient or fixed radiographic findings referable to ABPA, i.e. serum IgG> 27 mg / L against Aspergillus fumigatus and elevated serum levels of eosinophils (> 500 mmc) (31). Any treatment will eradicate the colonization and/or proliferation of *Aspergillus fumigatus*, reduce the inflammatory response and the degree of pulmonary exacerbation, and prevent fibrotic disease. In patients suffering from poorly controlled

asthma, the first choice and most effective treatment in the acute phase is represented by prednisolone at the recommended dosage of 0.5 mg/kg/day for the first 2 weeks, followed by a progressive decalage in the following 12- 16 weeks; in patients with CF, Prednisolone is preferably administered at a dosage of 2.0 mg/kg/day (maximum 60 mg) for 1-2 weeks, then 0.5-2.0 mg/kg/day every other day for 1-2 weeks and then decalage over the next 2-3 months (32). The use and efficacy of antifungal drugs, such as itraconazole in the pediatric age, is still under debate; thus, it is currently not adopted as a first-line treatment. The recommended dose of itraconazole is 5 mg/kg/day, a maximum of 400 mg/day (in two divided doses if the total daily dose exceeds 200 mg) and 3-6 months of a total duration of therapy is suggested.

The use of itraconazole should be reserved for those cases of evidence of a slow or inadequate response to corticosteroids (CS), relapse of the disease in CS-dependent ABPA and/or toxicity induced by CS suggests the use of itraconazole in the treatment of ABPA in patients with CF. The use of omalizumab, a humanized monoclonal anti-IgE, in ABPA, is due to its ability to prevent allergen-induced IgE. This drug has been shown to significantly reduce exacerbation rates, the use of oral steroid therapy, and related toxicity in patients with ABPA (32,33).

Recently, another monoclonal antibody, Mepolizumab, directed against interleukin (IL)-5, has been proposed as an alternative treatment in ABPA. Furthermore, as adjuvant therapies, amphotericin B, voriconazole, isavuconazole, and Vitamin D have been proposed, but the studies carried out so far do not fully evaluate their effectiveness. As for monitoring response to treatment, a determination is indicated of total serum IgE levels every 3-6 months/year, as it was found that a reduction of at least 25% in total IgE levels compared to baseline is associated with clinical improvement; on the contrary, the finding of an increase of the same > 50% is more readily associated with pulmonary exacerbations (31).

Scedosporium Apiospermum Complex, Lomentospora Species, and Exophiala Dermatitidis

Among the fungi of a fungal nature increasingly frequently associated with pulmonary disease in cystic fibrosis, there is a slow-growing filamentous fungal taxa *Scedosporium complex* (*S. apiospermum sensu stricto*, *S. boydii*, *S. aurantiacum*, *S. minutispora* and *S. dehoogii*), *Lomentospora prolificans* (formerly *S. prolificans*) and *Exophiala dermatitidis*. In particular, *Scedosporium* and *Lomentospora* are the most common fungal taxa after Aspergillus spp., with a prevalence of up to 10% of colonized CF patients (34). Furthermore, thanks to molecular diagnostics, it has been shown that CF patients with fungal bronchitis *Scedosporium spp*. and *Exophiala dermatitidis* are the most frequently involved taxa (35).

Among the emerging fungal pathogens in CF patients, we also remember the filamentous fungus *S.apiospermum*, whose ideal environment is represented by polluted soils and wastewater, already related to the etiopathogenesis of diseases such as osteomyelitis, endophthalmitis and endocarditis (36,37). *S. apiospermum* is also related to respiratory diseases with characteristics similar to those described for Aspergillus fumigatus, such as invasive pneumonia, lung mycetoma and allergic immune responses of the airways (36,37). However, the correlation between *S. apiospermum* and cystic fibrosis-related lung disease is still unclear. Contemporary colonization with *Aspergillus spp.* is often described (9), while in the case of *Pseudomonas* a*eruginosa* mucoid, both increased and reduced co-colonization cases are described (9 34). Furthermore, about *Scedosporium* and *Lomentospora* infections, younger age and positive history for ABPA have been associated with the more frequent colonization by these microorganisms (38). Possible pulmonary exacerbations sustained by *Scedosporium/Lomentospora* are rather difficult to treat due to the high resistance to the most common antifungals by *Scedosporium* and a multi-drug resistance by *Lomentospora*. In general, however, voriconazole is the drug considered as the first-line choice (39,40).In the most severe cases or cases of non-response, combinations of two or three different antifungals have been used, for example, voriconazole in combination with amphotericin B, or echinocandins for infections with *S. apiospermum* and *L. prolificans* or oral thiazoles in combination with an intravenous echinocandin and inhaled amphotericin B for infections with *Scedosporium species* (41).

Pulmonary fungal infections in lung transplant recipients

Recently, allogeneic lung transplantation has been considered a therapy with established efficacy in eligible patients with end-stage lung disease. Worldwide, approximately 100 lung transplants are performed each year in the pediatric population and approximately 4,500 among adults.

Fungal infections, even invasive ones, represent the most frequent complication and the leading cause of morbidity and mortality in patients undergoing solid organ transplantation, especially during the first year (42-44).

Among the fungal pathogens, *Aspergillus fumigatus* represents the main causative agent, isolated in 3-44% of cases (45,46), with mortality rates ranging between 41 and 51% (47) followed by infections with Candida spp (23%) (48), most commonly *Candida albicans*, and *Mucorales/Zygomyzosis* (up to 3%), *Cryptococcus spp*. (2%) (49),endemic mycosis*(Blastomyces spp., Histoplasma*, *Scedosporium* (3.5%), *Pneumocystis jirovecii* (2%), *Coccidioides*, 1% and *Exophiala dermatitidis* (case report) (50).

Fungal airway colonization after lung transplantation

Considering that CF is one of the diseases that most frequently may require lung transplantation and that most CF patients have chronic colonization by fungi, it is highly probable that these patients will develop a lung fungal infection following the lung transplant. Such infections are largely recorded during the first six months after transplantation, and the most frequently involved pathogens are *Aspergillus* and *Candida* (51).

The rates of fungal infections, particularly invasive ones, have undergone a notable decline following the introduction of antifungal prophylaxis. For example, a retrospective study performed in a single-centre, conducted on 584 pediatric patients undergoing solid organ transplantation, showed that 37% of children who underwent lung transplantation were requiring antifungal prophylaxis, especially voriconazole, in a higher percentage than other patients undergoing transplantation of other organs. Accordingly, the antifungal prophylaxis among all patients subjected to organ transplantation has increased from 7% in 2000-2006 to 11% in 2007-2013, and consequenquially, invasive fungal infections have decreased from 4% to 1% (52).

Antifungal therapies

There are no univocal guidelines for treating fungal infections in CF patients, except for ABPA. However, several new antifungal therapies have emerged, showing an improved tolerability profile, excellent oral absorption, and reduced toxicity. The therapeutic choice is guided by the fungal species found and related drug resistance and the patient's clinical status. Generally, a single drug is used, but it is possible to resort to combinations of even three drugs in cases of resistant or extremely severe infections. The duration of treatment can range from two weeks to more than six months (53).

The most frequently used drugs are Azoles (oral itraconazole, oral or intravenous Posaconazole and Voriconazole), followed by Polyenes (nebulized or intravenous Amphotericin B) and Echinocandins (Caspofungin, micafungin and intravenous anidulafungin).

The mechanism of action of the Azoles is based on the inhibition of 14-a-demethylase, which results in the formation of an altered, "permeable" cell membrane and cell lysis and, finally, death. Fluconazole is mainly used for yeasts, while Itraconazole, Voriconazole and Posaconazole for yeasts and moulds also show efficacy against *A. fumigatus*, even in case of invasive infections (54,55). Itraconazole is more often used in combination with steroids to treat ABPA, although concomitant use of proton pump inhibitors or H2 blockers limit its bioavailability and thus absorption in patients' CF. Voriconazole and posaconazole have shown greater efficacy against yeast activity, including *S. apiospermum* and *E. dermatitidis*, but also against resistant strains of *Candida* (56).

Although generally well-tolerated, gastrointestinal side effects are described following the administration of Azoli. Furthermore, they are subject to numerous drug interactions related to metabolism by the cytochrome P-450 enzyme system (57).

The mechanism of action of echinocandins involves binding with (b) (1, 3) -D-glucan synthase, resulting in inhibition in the synthesis of an essential component of the fungal cell wall, which results in

loss of integrity and lysis wall itself (58).
Among Echinocandins, Caspofungin is well-tolerated, but unlike azoles, it can only be administered intravenously. It is effective against most Candida and Aspergillus species but is not the first choice but second-line therapy in severe *Aspergillus* infections (58).
It is administered as a single therapy or in combination with other antifungal drugs, especially in those cases in which we are faced with patients who have experienced adverse reactions or who have shown themselves to be resistant to first-line therapy, such as amphotericin B (49). The drug most studied and used to treat fungal infections is certainly amphotericin B, but it is also the least tolerated. In order to minimize the related renal toxicity, liposomal preparation is currently in common use. The mechanism of action of amphotericin B involves binding the latter to ergosterol. Its antimycotic activity is directed towards yeasts and moulds, although cases of resistance have been described (57).

Mycobacterial infection

Non-tuberculous mycobacteria (NTM) are a group of heterogeneous environmental organisms generally isolated from water and soil. Usually, animals do not represent a reservoir of NTM and evidence of person-to-person transmission is limited (59). Typical characteristics of the genus Mycobacterium are acid-alcoholic resistance, presence of mycolic acids with a number of carbon atoms between 60 and 90 and DNA content of guanine plus cytosine from 61 to 71% by mol. (60); although the current diagnostic gold standard is the recognition of known mycobacterial 16S rRNA sequences. The genus can be further described as aerobic, non-spore-forming and nonmotile.

Even today, mycobacteria are classified according to their growth rates; Table I lists some pathogenic and non-pathogenic species divided based on this criterion. Species producing a visible colony in less than seven days are called "fast growers", while "slow growers" can take up to eight weeks (61) (Table I).

NTM can be responsible for simple sample contamination or a broad spectrum of conditions ranging from asymptomatic infection to severe disease. The prevalence rate of NTM is difficult to determine as screening methods, and culture techniques are not standardized as well as data collection; however, in the larger studies, the overall prevalence ranges between 6% and 13% (62-65). The most frequently isolated NTM species in CF patients from North America and Europe are both the slow-growing *Mycobacterium avium complex* (MAC) (including *M. avium*, *M. intracellulare* and *M. chimaera*), which can be found in up to 72 % NTM-positive sputum cultures (66);

Table I. *Rapid and slow-growing NTM species.*

Slow growers	**Rapid growers**
M. avium	M. abscessus
M. malmoense	M. fortuitum
M. ulcerans	M. chelonae
M. paratuberculosis	M. agri
M. kansasii	M. alvei
M. gordonae	M. smegmatis

the rapid-growing *Mycobacterium abscessus complex* (MABSC) (comprising the subspecies *M. abscessus subsp abscessus* (*M. a. abscessus*), *M. a. bolletii* and *M. a. massiliense*, which in many centres has now become the most isolated NTM (67).

It can be extremely difficult to discriminate whether NTMs represent microorganisms that colonize the patient's airways or whether they are true disease-causing pathogens. CF patients have multiple risk factors predisposing to NTM infection; among these stand out malnutrition, the severity of the disease, frequent intravenous antibiotic therapies, diabetes mellitus and the use of corticosteroids (61). Several studies have shown that NTM acquisition is strongly associated with the age in individuals with CF, with prevalence increasing from 10% in children aged 10 years to over 30% in adults over 40 years. More than 50% (primarily females) have NTM-positive airway cultures (68). Age would also appear to be species-related as MAC was most commonly isolated from adults over the age of 25, while MABSC is isolated from all age groups, but in some studies, it peaks between 11 and 15 years (62, 63, 66, 69-72).

In addition, the role of coexisting microbial pathogens and drug exposure remain poorly understood in the development of NTM infection. For example, *Aspergillus fumigatus* seems more prevalent in certain NTM-positive patients; but Eikani does not confirm this association in his study (73). Moreover, respiratory infection with MAC seems to have only a small effect on the health of CF patients (74).In contrast, MABSC-positive patients frequently exhibit severe and occasionally fatal lung disease (75-77). Some studies were conducted to evaluate the impact of infection on lung function, as assessed as a percentage of the expected FEV1, but a variable association with NTM infection has been reported (78-81). However, many studies have suggested that FEF25-75 and FEF75 are more sensitive indicators of early disease in children with CF because compared to FEV1 and forced vital capacity, abnormalities in FEF75 occur at a younger age and FEF75 of lung function decreases more than other parameters (82). Eikani et al. studied the change in FEV1 and FEF 27-75 after the acquisition of NTM, documenting that the FEV1 did not differ before and after the acquisition of NTM FEF25-75 values were significantly lower. Additionally, patients with more than four fast-growing NTM positive cultures were associated with the largest change in median FEF25-75 during the follow-up period suggesting that NTM infection negatively impacts small airway function in patients (73).

Current CF Foundation and ECFS guidelines recommend that screening cultures for NTM be performed annually in individuals capable of spontaneous expectoration and with clinical stability (79). As already mentioned, the isolation of NTM from the sputum of a CF patient represents a diagnostic problem for the clinician who must discern whether NTM is a simple colonizing germ or a cause of lung disease.

The diagnostic criteria for NTM lung disease are valid for patients with CF and without CF. They are based on the American Thoracic Society and the Infectious Diseases Society of America (ATS/IDSA) recommendations. These include microbiological and clinical criteria and exclusion of alternative diagnoses (Table II).

Distinguishing indolent NTM infection from NTM disease is crucial for ensuring early treatment in patients with NTM disease and avoiding the side effects of therapy in asymptomatic patients.

On the other hand, a late or missed diagnosis will lead to a further decline in the patient's state of health (83). In 2016, the guidelines for managing NTM by CFF / ECFS were published (79).

Before starting any treatment, it is recommended to evaluate the sensitivity of MAC for clarithromycin and for MABSC for clarithromycin, cefoxitin and amikacin (and possibly also tigecycline, imipenem, minocycline, moxifloxacin and linezolid) (67).

Patients undergoing background treatment for CF with azithromycin and with a positive NTM culture should not continue this drug during evaluation for NTM disease. The optimal treatment duration is unknown, but current recommendations are to achieve 12 consecutive months of negative cultures. Standard MAC treatment consists of a macrolide (azithromycin 250 mg or clarithromycin 1000 mg once daily), rifampin 10 mg/kg daily up to 600 mg, and ethambutol 15 mg/kg daily (79, 84).

Azithromycin is the most widely used macrolide,

as it is better tolerated, has fewer drug interactions, and possesses anti-inflammatory activity (79, 84, 85). Monotherapy is contraindicated in the treatment of M. abscessus complex lung disease. An intravenous aminoglycoside is indicated in patients with severe cavity disease to improve outcome. New specific CF guidelines suggest that smear-positive MAC and/or cases associated with systemic signs of disease are also indications for parenteral use of aminoglycosides. Usually, intravenous amikacin is the drug of choice; alternatively, streptomycin can be used intramuscularly (86). Amikacin or streptomycin should be administered for one to three months (87). In case of new MAC cultures, regrowth of MAC after recent eradication or in case of treatment failure (defined by some as failure to convert to negative culture after 4 months of adequate therapy) is important to test resistance to macrolides (84). MABSC treatment is complex due to inducible resistance to macrolides, widespread common in *M. abscessus ssp. abscessus* and *M. abscessus ssp* (88).

Treatment of MABSC involves an intensive phase of therapy used to dramatically reduce bacterial load, which should include 3-12 weeks of intravenous amikacin (15 mg/kg/day in divided doses) plus one or more of the following: tigecycline intravenously (50 mg once to twice daily), imipenem (1 g twice daily), or cefoxitin (200 mg/kg/day divided into three doses),

Table II. *ATS/IDSA clinical and microbiologic criteria for diagnosis of NTM pulmonary disease. Adapted from Floto et al (67).*

Clinical criteria (both required):
• Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules • Appropriate exclusion of other diagnoses
Microbiologic criteria (one of the following required):
• Positive culture results from at least two separate expectorated sputum samples • Positive culture result from at least one bronchial wash or lavage • Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM.
A. Expert consultation should be obtained when either infrequently encountered NTM or those usually representing environmental contamination are recovered.
B. Patients who are suspected of having NTM-PD but who do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded.
C. Making the diagnosis of NTM-PD does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients.

plus a macrolide (preferably azithromycin, 250 mg per day), plus one or two additional oral medications. Upon completion, patients should continue with a prolonged suppressive phase with inhaled amikacin (250 to 500 mg twice a daily) combined with 2-3 of the following oral antibiotics: linezolid, clofazimine, ciprofloxacin, moxifloxacin, or minocycline (79).

To assess the microbiological response, individuals with CF receiving NTM treatment should undergo sputum culture every 4 to 8 weeks during treatment, and an HRCT scan of the lungs should be performed shortly before starting EC treatment to evaluate the radiological response (79).

The CF Foundation and the ECFS advise against the use of IFNγ as adjuvant therapy for NTM-PD in individuals with CF. Several studies have evaluated IFN as adjuvant therapy (89, 90). In addition, an uncontrolled study was performed in seven patients with presumed primary immunodeficiency and NTM disease refractory to antibiotic therapy, with demonstrated benefit (89). However, three large studies examining the effects of IFNγ for NTM lung disease remain unpublished or have been blocked (probably due to lack of benefit), so to date, we do not have sufficient data to suggest the use of this drug in the treatment of NTM (67). Regarding the surgical approach, a recent review of case series published over the past 40 years suggests that lobectomy or segmentectomy surgeries should only be considered in severe, localized, unilateral and unresponsive NTM disease (91). However, in CF patients, this occurrence is rare, and the risks of thoracic surgery thus limiting the potential of this approach (79).

There is, however, limited published information on transplant outcomes for individuals with previous or concurrent NTM infection, with very few reports (usually from single centres) specifically examining CF cohorts (91-94).

Based on the limited data available, the CF Foundation and ECFS recommend that CF patients considered for lung transplantation be evaluated for NTM-PD even if past or current NTM positivity should not preclude individuals from entering the transplant list. Thus, individuals with CF who have NTM-PD and are being evaluated for transplant should start treatment before the transplant list, but persistent NTM positivity despite therapy is not an absolute contraindication to transplant (79).

CONCLUSION

The recent and increasingly frequent finding of NTM and fungal infections in CF patients has raised the debate regarding the diagnostic-therapeutic process in this patient population. Future omic-based or metabolic studies will likely shed new light on understanding specific host response effects in the CF patient group, similar to some pediatric conditions from different fields (95,96). However, current knowledge is not conclusive, so further studies are needed to better define the ideal approach and treatment for these pathogens, especially considering the improvement in life expectancy in CF patients.

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Is there a link between infections and asthma?

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Asthma is a common chronic condition affecting both children and adults and is characterized by chronic airway inflammation leading to bronchial hyperresponsiveness and mucous hypersecretion. Asthma can be triggered by various stimuli, leading to recurrent and reversible episodes of wheezing, shortness of breath, chest tightness, and coughing (1). Globally, about 235 million people suffer from asthma. Implicated in the development of asthma are respiratory viral infections and infections with atypical bacteria (such as mycoplasma and/or chlamydia) (2). The burden of asthma is significant, both in terms of financial expenses and lost productivity, and in many situations, it is an infectious agent that initiates an asthma exacerbation. This review will discuss the strong link between asthma and infections and the potential mechanisms to translate these infections into asthma.

Epidemiology

The role of early life infections in the development of asthma results from complex interactions between pathogens, genetics, and environmental factors such as tobacco smoke. Acute respiratory infections are common in asthmatic patients. The early stages of asthma, bronchiolitis or early wheezing affect 10–30% of children depending on definition and 15–25% of children experience recurrent wheezing before school age (3). Thus, wheezing illnesses and asthma pose a significant health and socioeconomic burden globally. Viral infections are common during early childhood development. Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively (4). So, it is not surprising that these 2 illnesses overlap. Almost all wheezing episodes in the first few years of life occur with viral infections, most commonly rhinovirus (RV) and respiratory syncytial virus (RSV), but enterovirus, bocavirus, parainfluenza virus, coronavirus, influenza virus, and adenovirus have also been implicated (5). Bronchiolitis is a common condition characterized by inflammation of the small bronchioles and surrounding tissue in children up to 2 years of age and can affect up to 20-30% of infants in the first year of life and up to 10-20% of infants in the second year of life. (5) Moreover, viral respiratory infections have been implicated in up to 80% of wheezing episodes and asthma exacerbations (6).

Keywords: infections, asthma, children

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0393-974X (2022)

These studies demonstrate the relationship between viral infections and the development of wheezing and/or exacerbation of asthma. Within the range of these respiratory viral pathogens, the most common viruses identified during an asthma exacerbation are RV (44%–88%), RSV (2%–20%), and parainfluenza virus (2%–11%) (7). It is also important to note that around 10% of these cases have coinfection with more than 1 virus, usually with RV being the other virus identified(8). RSV is the most common cause of bronchiolitis in the first year of life, usually occurring in winter (9). RV is the most often detected virus with wheezing in older infants and toddlers (10).

Respiratory Syncytial Virus (RSV)

RSV is also known as one of the leading causes of severe respiratory infection in infants, and several studies have strongly suggested a causal role for RSV in the development of asthma and allergen sensitization (11, 12). For example, a multicenter cohort study conducted by Hasegawa and colleagues showed the relationship between viral load in RSV-infected children and clinical symptoms of asthma. (13) Another study by El Saleeby et al., demonstrated that high levels of RSV titer were associated with more severe respiratory viral disease outcomes (14). These studies confirmed the relevance of RVS infections in developing asthma and asthma symptoms.

Rhinovirus (RV)

RV is the second most common cause of bronchiolitis in infants younger than 12 months (15). RV infections can present year-round, with a peak incidence in late autumn and early spring (3). Rhinovirus is a non-enveloped positive-strand RNA virus in the family Picornaviridae, genus Enterovirus, classified into three species (RV-A, B, and C) (3). Over 170 distinct RV genotypes include 80 RV-A, 32 RV-B, and 65 RV-C genotypes. RV-A and RV-C is often associated with wheezing illness in infants than RV-B (15); RV-C is associated most often with both inpatient and outpatient asthma exacerbations, but also demonstrate that high titer of RV-A could lead to significant exacerbation of the disease (8). RV-C has also been the most common RV species among hospitalized and intensive care unit asthma patients (16). Overall, the clinical impact of RV-A is close to RV-C among asthmatic subjects, whereas the impact of RV-B is much weaker. However, cohort studies have shown that RV-B infections may slightly increase the risk of exacerbation in children whose asthma is of greater severity (3, 17).

Influenza

Influenza circulates both as a seasonal infection and an occasional pandemic. The seasonal variety causes asthma exacerbations but seems to be a minor contributor to the overall burden of asthma disease development. During the 2009 influenza pandemic (pH1N1), asthma was the most common comorbidity among patients, accounting for 22% to 29% of all hospitalized patients with influenza. (18,19) Although asthma was overrepresented in patients hospitalized with pH1N1, at least 1 study found that having asthma led to a more rapid recovery from the viral infection (20). Supporting the idea that asthma might have a protective advantage in the recovery from pH1N1, a retrospective chart review of 2 case series found that those with asthma who were hospitalized with pH1N1 were less likely to have pneumonia, need mechanical ventilation, or die compared with those admitted without asthma (21). So, although influenza may be associated with asthma exacerbations, it also seems that asthma may protect against pandemic influenza mediated morbidity and mortality.

The mechanisms linking respiratory viral infections to the development of asthma and its exacerbation are actively being studied; Some link it to the role of innate lymphoid cells (ILCs), translating RSV infection into atopic disease. ILCs can be classified based on their transcriptional regulation and cytokine production into ILC1, ILC2, and ILC3 cells, which largely emulate the adaptive CD4[1] T helper 1 (Th1), Th2, and Th17 cells, respectively. ILC2s produce high IL-13 and IL-5, 2 cytokines known to play important roles in asthma. IL-5 is a required cytokine for eosinophil development, and several new asthma treatments have been developed to block its effects (22). IL-13 has been implicated in IgE synthesis, mucus hypersecretion, airway hyperresponsiveness, and fibrosis (23).

Atypical bacterial infections

Infections with atypical bacteria also seem to play a role in inducing and exacerbating asthma in both children and adults. Several studies suggest that atypical respiratory pathogens such as *Chlamydophila pneumoniae* (CP) and *Mycoplasma pneumoniae* (MP), and fungi like *Aspergillosis* may contribute to the pathogenesis of asthma (24-26). Related to CP Von and colleagues (27) investigated the relationship between severity of asthma, CP titers, and antibodies specific for CP's heat shock protein (chsp60), and their association with asthma. Severe and moderate asthma was significantly associated with elevated anti-chsp60 IgA, suggesting chronic infection in these patients with more severe asthma (27). An additional study reported that IgE against CP is strongly and positively associated with asthma severity, suggesting a role of anti-CP IgE (and by extension, CP) in the pathogenesis of asthma (28). Conversely, the role of MP in asthma has been less intensively investigated than CP. MP has been associated with recurrent wheeze and may be a coinfection with respiratory viruses.

Children with asthma were more likely to have MP-specific IgM than those without asthma (39% vs 0%), (29) suggesting an increased exposure and colonization of MP in those with asthma. Emerging data suggest that Streptococcus, Moraxella, and Haemophilus genera species are also associated with respiratory illnesses and asthma development (30, 31). Hypopharyngeal colonization with *S pneumoniae, Helicobacter influenza*, or *M catarrhalis* in neonates was reported to increase the risk for recurrent wheeze and asthma early in life (32) In neonates, colonization with *H influenza* or *M catarrhalis* (or both) significantly associated with persistent wheeze (HR, 2.40; 95% CI, 1.45–3.99), severe acute exacerbation of wheeze (HR, 2.99; 95% CI, 1.66– 5.39), and hospitalization for wheeze (HR, 3.85; 95% CI, 1.90–7.79) (32). Children colonized by these bacteria as neonates had a higher prevalence of asthma by 5 years of age and increased beta-agonist reversibility compared with those not colonized as neonates with these organisms. Other bacteria such as *Helicobacter pylori* and *Bordetella pertussis* have been associated with asthma. *B pertussis* has not been associated with the development of asthma but may infect asthma patients more frequently. Similar to viral infections, the mechanisms by which atypical bacterial drive the inception of asthma are being studied.

Early infection with CP or MP leads to a higher risk for asthma through induction of type 2 airway inflammation, mucus cell metaplasia, and airway hyperreactivity, all hallmarks of asthma. For instance, CP infection was shown to induce a Th2 immune response and both airway eosinophilia and neutrophilia, leading to permanent alteration of lung structure and function (33).

Risk factors

Understanding the risk factors for progression from wheezing as an infant to the development of asthma is important for targeted asthma prevention strategies and for offering anticipatory guidance after an initial wheezing episode. Although many viruses have been associated with wheeze as an infant, most data suggest that the greatest association is between RV and RSV recurrent wheeze and the development of asthma (34,35). Severe acute bronchiolitis or early wheezing is associated with an increased risk of subsequent asthma (3). This disease may persist until early adulthood, and the strongest association with worse long-term prognosis was observed in children infected with RV-C and RV-A virus.

Risk factors can differ for allergic and non-allergic asthma. Lukkarinen, et al. studied the infants hospitalized with first-time wheeze and found that RV-induced wheezing, alone or with sensitization and eczema, predicted atopic asthma at school age. On the other hand, parental smoking RSV infection with first wheeze and wheezing before one year of age were risk factors for non-atopic asthma at school-age. (10) These differences in risk factors highlight important mechanistic differences between RV-induced and RSV-induced disease, with RV induced wheezing being a more important risk factor in atopic individuals

Many studies have demonstrated that early RV-induced wheezing is an important asthma risk factor (36,37). Some studies estimate that 60% of children who wheeze with RV in the first 2 years of

life will develop asthma. In the childhood Origins of Asthma (COAST) study, a birth cohort study that prospectively followed children who had at least one atopic parent, infants who wheezed with RV alone were more likely to develop asthma than those who wheezed with RSV alone or RSV and RV (7). The risk of asthma has been shown to increase markedly in infants with RV-triggered wheeze who also have evidence of allergen sensitization. Although it is unclear if sensitization or viral infection came first, it seems most likely that allergic sensitization itself is a risk factor for RV induced wheezing (38-40).

In the COAST study, early life aeroallergen sensitization preceded RV infection, leading the authors to hypothesize that allergic sensitization may be what drives viral wheezing (38). There are many pathways between RV infections and allergic inflammation that can explain the increased risk of asthma that occurs with RV-wheezing and atopy, and these are briefly reviewed in the mechanism section below. The COAST study found that the number of RV wheezing episodes is most closely associated with asthma risk (41). However, most studies reflect the belief that RV-induced wheeze is a significant risk factor for the development of asthma, and likely frequent infant RV-wheezing episodes confer a higher risk. A meta-analysis examining 15 original articles has shown an association between RV-induced wheezing and the development of childhood asthma (42).

Although a link between RSV and asthma has long been suspected, the association is less clear. There have been conflicting results regarding the importance of RSV infection in the development of asthma. Sigurs et al. found an increased rate of asthma in children hospitalized with RSV in infancy; contrary to this study, COAST did not identify an association between RSV in infancy and the development of asthma in school-age (43). Most notably, sensitization does not seem to be a risk factor for infants who develop RSV induced wheezing (44) but is an important risk factor in infants who have RV-triggered wheeze. Hence, RSV will be less significant in atopic populations, such as in the COAST study group.

The severity of RSV infection is likely the most significant risk factor for the development of asthma following RSV-induced wheezing. Despite this growing body of evidence showing a pivotal link between RV and RSV induced wheezing episodes and development of asthma, it remains unclear if infection with RV and/or RSV in infancy contributes to the development of asthma or is simply a marker of asthma susceptibility (39,45). Many dynamics come together to produce asthma, including host and environmental factors. It has been demonstrated that children with severe virus inducted bronchiolitis were born with increased bronchial responsiveness compared to children without bronchiolitis (38), and asthmatic patients have an altered epithelial immune response to RV (46). Many posit that infant RV-wheezing episodes occur in infants predisposed to develop asthma (3).

The host factors that contribute to severe viral infection, predispose to atopy and respond to viral wheezing with enhanced airway damage may be significant risk factors for the development of asthma. There is likely a complex interplay between viral infections, sensitization and environmental exposures in a susceptible host that promote asthma (47).

Mechanisms of asthma exacerbations

Asthma exacerbation is defined as an acute or subacute worsening of asthma symptoms and lung function compared to the patients' usual health status. Exacerbations usually occur in response to various external agents, including respiratory viruses, bacteria, allergens, air pollutants, smoke, and cold or dry air; however, it is estimated that up to 85-95% of asthma exacerbations in children and 75-80% in adults are linked to viral infections. However, most viral infections are not associated with acute exacerbations, and cofactors, including bacterial and allergic inflammation, have been described to increase exacerbation severity.

Viral infections are particularly important when kids return to school after summer and spring breaks (48). Many viruses have been associated with asthma exacerbations, including RSV, RV, and influenza and, to a lesser extent, coronavirus, parainfluenza, adenovirus, and metapneumovirus (49). However, RV is the most common virus associated with

childhood asthma exacerbations, probably because RV is the most prevalent of the respiratory viruses. RV can cause a wide spectrum of diseases, ranging from asymptomatic to severe infection requiring hospitalization. HOWEVER, most RV infections in children with asthma do not lead to an exacerbation. Several factors have been shown to predispose to an exacerbation, most notably of which is a history of atopy. Studies in the Emergency Department have shown that detection of respiratory virus, allergen sensitization (50,51) and eosinophil inflammation are risk factors for acute wheezing. Furthermore, RV-C might be more strongly associated with severe asthma exacerbations and hospitalizations due to a stronger inflammatory response eliciting the virus (52).

Several mechanisms why asthmatics are predisposed to viral infections have been proposed. (53,54) One of the proposed reasons is damaged epithelium, which may increase susceptibility to infections and lead to airway obstruction.

Cellular mechanisms involved in viral response include disruption of the epithelial barrier and tight junctions, impaired apoptosis, increased cell lyses, deficient Th1 response and reduced IFN-γ production, and enhanced expression of viral receptors (55). Viral infection also induces proinflammatory cytokines, including interleukins and mediators or remodelling (VEGF, FGF) (56). Viruses attach to their unique cellular receptors: intercellular adhesion protein 1 (ICAM-1) is used by the majority of RV-A, and RV-A uses all RV-B types, low-density lipoprotein receptor family members (LDLR), cadherin-related family member 3 (CDHR3) is used by RV-C, and CX3CR1 is used by RSV (57-59). Interestingly, the corresponding gene for CDHR3 has been linked to childhood asthma with severe asthma exacerbations (60). Airway epithelial cells form a barrier to the outside world and are the front line of mucosal immunity. Cytokines and inflammatory mediators associated with allergic inflammation induce epithelial barrier disruption. Additional factors that influence the severity of the viral infection and the risk of asthma exacerbation are allergic sensitization and the airway microbiome. For example, RV infection and allergic sensitization synergistically increase the risk of exacerbation (3). Atopic asthma with allergic sensitization can be associated with reduced virus-induced IFN responses, increased viral shedding, and decreased viral clearance (3). Moreover, bronchial epithelial cells of atopic asthmatics have been shown to have increased RV replication, deficient IFN-γ release, and enhanced cell lysis (61).

Several studies have shown that viral infections precede bacterial infections in the airways. This phenomenon may occur for several reasons: viruses may induce expression of airways receptors used by bacteria, viruses may disrupt the epithelial barrier, and they increase the release of inflammatory cytokines and mediators, causing increased inflammation and risk of asthma exacerbations (62). In addition, patients with asthma are frequently colonized with bacteria in the lower and upper airways (63), and acute wheezing in infants has been associated with both viral infections and airway microbiota dominated by bacterial pathogens (64).

Bacterial infections may impair mucociliary clearance and increase mucus production. However, evidence linking bacterial infections to acute asthma exacerbation is limited. Viral infection, specifically RV infection, has been associated with the production of IL-25 and IL-33, which promote type 2 airway inflammation and remodelling primarily through driving the production of type 2 innate lymphoid cells (65). These cells can produce large amounts of type 2 cytokines and drive the atopic response. A recent publication has also shown that IL-33 can dampen innate and adaptive T_H1-like and cytotoxic responses in response to viral infection, which may be another factor in driving virus-induced asthma exacerbations (66).

Genetic polymorphisms of asthma and virus susceptibility

Asthma is a highly heritable disease with heritability estimates from twin studies of more than 50% and even higher for childhood asthma (67). Genetics is, therefore, one important tool for understanding the disease mechanisms of asthma. Our knowledge of the genetic background of asthma has increased rapidly during recent years through methodological advances allowing so-called genome-wide association studies (GWAS) where

millions of genetic variants covering the entire genome can be tested without a prior hypothesis about underlying mechanisms.

The first asthma locus discovered in GWAS approximately 10 years ago was the chromosome 17q21 locus (68). It is still the strongest known asthma locus, and it is particularly associated with childhood-onset asthma and severe exacerbations (69). Interestingly, the 17q21 asthma locus is strongly associated with an increased risk of early wheezing episodes triggered by viruses, including rhinoviruses, and children with early viral wheezing have a much higher risk of later asthma if they carry 17q21 risk variants (70). The biological mechanisms associated with the 17q21 locus are still incompletely understood and might involve several genes in the region, including ORMDL3, GSDMB, GSDMA, PGAP3, ERBB2, and IKZF3 and several cell types, including immune and airway epithelial cells (69). However, most follow-up studies have focused on ORMDL3 as the causal gene with proposed mechanisms related to sphingolipid synthesis (71), regulation of eosinophils (72), and regulation of the ICAM1 receptor, which might explain the susceptibility for rhinovirus infections (73).

Another asthma risk gene, CDHR3, was discovered in a GWAS of childhood asthma with recurrent acute hospitalizations before 6 years of age (74). Experimental studies have later demonstrated that CDHR3 functions as a rhinovirus-C receptor and is highly expressed in differentiated bronchial epithelial cells. In addition, the association between CDHR3 risk variants and rhinovirus-C respiratory illness was subsequently confirmed clinically in birth cohorts. These findings suggest that the mechanism associated with CDHR3 variants is at least partly explained by an increased risk of rhinovirus-C respiratory illnesses and that targeting CDHR3 might be a strategy for preventing rhinovirus-C triggered asthma exacerbations.

In order to understand the genomics of asthma and virus infections, we need more studies that combine information on gene variants, gene expression, and epigenetics in relevant cells and assess during acute symptoms with information on specific infectious triggers. Such studies are challenging but can reveal unknown disease mechanisms and functional subtypes of the disease, which is essential to personalize and improve treatment and prevention of disease, similar to what occurred in other (different) pediatric conditions (75,76).

Prevention and Treatment

Understanding the role of viral infections in asthma development has been the basis for attempts to prevent disease development and reduce the severity and frequency of asthma exacerbations. (77, 78). Currently, no safe and effective human vaccines exist for RSV or RV. The major obstacle is the antigenic diversity of more than 100 serotypes of rhinovirus, which means that creating a successful vaccine is an extremely challenging task (79). Palivizumab, a monoclonal antibody directed against RSV and able to reduce the rate of severe RSV infections, has been found to decrease early episodes of recurrent wheezing in non-atopic children (but not atopic children), when administered to high-risk preterm infants (80-82). The differences between the two populations are consistent with the observation that RSV appears to be more important in non-atopic asthma. However, despite the reduction in early wheeze, there was no reduction in the rate of asthma (83, 84).

Another RSV antibody, motavizumab, administered to a high-risk group in the first year of life, reduced the risk of hospital admissions for wheezing by 87% but did not lead to a reduction in medically treated wheezing between 1 and 3 years of age (85). Taken together, these studies demonstrate that monoclonal antibody therapy directed against RSV in preterm infants can reduce bronchiolitis and recurrent wheezing. In addition, studies have demonstrated that oral steroids use in hospitalized infants with the first wheeze can decrease the risk of recurrent wheeze and asthma in children affected by RV (86-90). Two separate randomized trials exist in which oral corticosteroid, prednisolone, has been applied to wheezing children with RV aetiology. Intriguingly, prednisolone application was shown to decrease the time to the physician-confirmed relapse within the following year (by 20-30%) and time to the initiation of asthma controller medication within the following 5 years (by 30-40%) in these children (91, 92).

Noteworthy, high RV genome load in the placebo-treated wheezing children was associated with the development of a new wheezing episode within 100 days in every case and the initiation of asthma medication within the next 14 months in every case. These results indicate that systemic anti-inflammatory treatment of the first RV-induced severe wheezing episode may markedly decrease the subsequent risk for asthma. In general, RV-induced wheezing is more responsive to oral steroids, whereas RSV-induced wheezing is less likely to respond to oral steroids (91, 93).

Omalizumab, a monoclonal antibody that prevents IgE from binding to its receptor, has been effectively used to treat moderate to severe asthma, reducing the number of asthma exacerbations and seasonal variation in exacerbations (94, 95). Indeed, either year-round or seasonal pretreatment treatment with omalizumab led to a decrease in seasonal exacerbations and a decrease in RV in nasal secretions, especially amongst children with severe asthma; this would suggest that anti-IgE might similarly block asthma exacerbations to how it can inhibit airway allergen-induced asthma. An alternate explanation is based upon the observation that reduction of IgE led to increased type I IFN production from plasmacytoid dendritic cells, ex vivo (96), this would suggest that anti-IgE might increase anti-viral immunity.

Consistent with both of these observations, treatment with omalizumab decreases the severity of asthma exacerbations associated with RV (97).

CONCLUSIONS

Viral infections are important triggers of acute wheezing episodes in infancy and the inception and exacerbation of asthma. The two most common respiratory viruses associated with the development and/or exacerbation of asthma, RSV and RV, seem to have disparate mechanisms through which they lead to disease. RSV appears to drive asthma in infants without a pre-existing history of atopy, whereas RV is more likely to lead to asthma in children who have already developed an allergic phenotype. Understanding the differences in these mechanisms will be important as we explore potential primary prevention strategies and novel therapeutics to prevent viral-induced wheeze, asthma, and development of atopy.

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The role of skin infections in atopic dermatitis in children

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Atopic dermatitis (AD) is a chronic skin disease characterized by inflammation, dry skin, itching and an alteration of the skin barrier. It appears recurrently and, typically, by alternating periods of exacerbation with states of apparent clinical well-being. Although it can develop at any age of life (1), AD occurs more frequently in pediatric age, involving 10% to 30% of children in advanced countries (2, 3). AD is known to be associated with an increased risk of developing other allergic disorders, including food allergies, asthma, allergic rhinitis (4), in a process called *atopic march* (5-10). It is also important to discuss the complications, especially the infectious ones, which represent a significant cause of morbidity in patients with AD.

Skin infections in atopic dermatitis

Patients with atopic dermatitis have an increased risk of developing skin infections, evolving into invasive systemic events. This susceptibility results from host factors, such as alteration of the skin barrier, dysregulation of innate and adaptive immunity, skin dysbiosis and trauma caused by scratching, and virulent pathogenic factors, such as the production of superantigens and toxins. All this involves creating a vicious circle in which the inflammatory state underlying the AD is fueled by the infectious insult (11) (Fig. 1).

In the last decade, researchers have actively investigated the role of the skin microbiome in patients with AD. Kong et al. found that increasing AD severity is inversely related to decreasing microbial diversity (12-14). Microbial diversity during AD flares is dependent on the presence or absence of recent AD treatments, with even intermittent treatment linked to greater bacterial diversity than no recent treatment. Treatment-associated changes in bacterial skin diversity suggest that AD treatments diversify skin bacteria preceding improvements in disease activity.

The most common skin infections in AD are caused by *Staphylococcus aureus* (15), followed by *Streptococcus pyogenes* (16, 17). Both pathogenic species can develop invasive infections. In *S. Aureus* infections, the most severe virulence factors are due to the production of Staphylococcal enterotoxins (superantigens) SEA, SEB, SEC, SED, SEE and toxic shock syndrome toxin 1 (TSST-1) (16). Staphylococcal enterotoxins interact with the specific variable regions of T cell receptor β chains (TCR Vβ), leading to cytokine production and mitogenic

Keywords: skin, children, atopic dermatitis

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0393-974X (2022)

response in T cells (18). Recently, it was found that enterotoxin Y (SEY) predominantly activated human T cells with a particular TCR Vα profile (19). In addition, superantigens are responsible for producing specific Immunoglobulins E (IgE), which stimulate basophils and mast cells to release cytokines and chemokines, resulting in AD exacerbations (20). Furthermore, *S. aureus* expresses several molecules that contribute to the intensity of symptoms, including δ-toxin, which stimulates mast cells and α-toxin, which causes lymphocyte apoptosis and damages keratinocytes (especially when the levels of IL4 and IL 13 are increased such as in atopic diseases) (16, 21, 22). For the first time, Moran et al. demonstrated that secreted staphylococcal virulence factors could be quantified with a Western dot blot assay at the protein level directly from skin swabs obtained from the skin of atopic dermatitis patients, eliminating the need to culture *Staphylococcus Aureus* (23).

Particularly severe are the forms caused by methicillin-resistant *Staphylococcus Aureus* (MRSA), which produces more superantigens and colonizes the skin of AD patients in 12% of cases (24). The most frequent forms of skin infections include impetigo, cellulitis and skin abscesses. Impetiginized lesions present as yellowish-brown, honey-coloured crusts surrounded by an erythematous base or as fluid-filled blisters (bullous impetigo). Non-purulent forms include erysipelas and cellulitis. These infections usually start in a focal area of the skin and can spread rapidly to cover large body surface areas. In these cases, patients may develop fever and bacteremia.

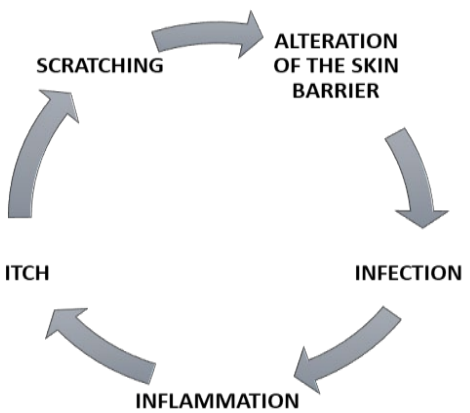

Fig. 1. *The cycle of the atopic skin.*

Although less common, patients with AD are also at risk for viral infections (25), including *eczema herpeticum* (EH), *eczema vaccinatum*, and *eczema coxsackie*. An infection causes EH with *Herpes simplex* virus infection (HSV), and it affects 3% of AD patients, especially those with earlier onset, severe AD, and increased risk of atopic diseases (food allergies and asthma) (26). EH may result in disseminated vesicles, viremia, fever, malaise, keratoconjunctivitis, encephalitis and septic shock. A history of skin infections with *S. aureus* is also a significant risk factor for the development of EH among AD patients (26). Another life-threatening viral infection in AD is *eczema vaccinatum* (EV), caused by smallpox vaccination, and a vesiculopustular rash characterizes it. A more recently described viral skin infection of AD is *eczema coxsackie* (EC), due to coxsackieviruses in the enterovirus group. AD patients with EC may present with oral sores and papules on palms and soles like the hand, foot, and mouth disease.

Prevention and management of infectious complications in patients with AD

Prevention of the infectious complications of AD should focus on controlling AD flares and the establishment of a routine of maintenance skincare (27). To control AD flares, patients should first avoid irritants and allergens that may trigger AD. Common irritants include soaps and detergents with fragrances that are harsh and drying. Wearing loose nonabrasive clothing is also useful in preventing trapping of perspiration and minimizing skin irritation. In addition to avoiding allergens, AD patients should maintain their skin hydration. All patients are recommended to shower/bathe in warm water for 15 min with a wet towel used to cover areas of the body not covered by water to help seal in moisture. Maintenance of adequate skin moisture using a daily regimen can reduce the need for topical corticosteroids. The need for antibiotics in patients with severe AD exacerbations remains controversial (28). The main concern with the overuse of antibiotics in AD exacerbation is the potential development of bacterial resistance and dysbiosis (29).

Acute-phase response markers such as CRP and ESR may help determine the need for antibiotics in patients with severe AD exacerbation who are suspected of having infections. Future studies should also address whether anti-inflammatory treatments, especially targeting type 2 inflammation, may benefit AD patients with active infection. The management of impetigo is shown in Fig. 2.

In localized cases, it is recommended to remove the crusts and apply topical antibiotics (ozenoxacin cream 1%, mupirocin 2% ointment, fusidic acid 2% cream and retapamulin 1% ointment). Systemic antibiotics are necessary for widespread or localized forms with one or more of these characteristics: age <2 months, >10 vesicles, size >36 cm2, compromised immune status, for at least 7 days. In non-MRSA impetigo cases, oral dicloxacillin, cephalexin, erythromycin, amoxicillin/clavulanate and cefadroxil can be prescribed. In patients with a recent history of MRSA colonization or confirmed infection, consider using antibiotics that cover MRSA, including vancomycin IV +/- rifampin, trimethoprim-sulfamethoxazole, clindamycin or doxycycline, until wound culture and susceptibility are available. If there is a lack of clinical response in 3 days, a wound culture should be considered to assess antibiotic susceptibility, underlying conditions should be ruled out, and antibiotic therapy should be revalued (30) (Table I).

It is not recommended to use antibiotics in noninfected AD due to the potential development of bacterial resistance. Managing viral infections first requires supportive care and identification of the viral agent. If EH and EC are severe, IV acyclovir should be initiated immediately. Oral acyclovir can be used in less severe EH (31, 32).

Additional targeted approaches in the future will require a more delicate dissection of mechanisms underlying the disease, similar to other pediatric conditions with not fully elucidated pathophysiology (33, 34).

Table I. *Impetigo: antibiotic therapy in localized and widespread MRSA and not-MRSA infections. PO= per os, IV= intravenous.*

ANTIBIOTIC THERAPY IN IMPETIGO		
LOCALIZED INFECTION	NOT MRSA INFECTION	MRSA INFECTION
TOPICAL ANTIBIOTICS: -Ozenoxacin cream 1% BID/ 5 days, -Mupirocin 2% ointment TID/ 7-10 days, -Fusidic acid 2% cream TID/ 7-10 days, -Retapamulin 1% ointment BID/ 5 days.	SYSTEMIC ANTIBIOTICS: -Dicloxacillin PO 4 times daily for a week -Cephalexin PO 4 times daily for a week Amoxicillin/Clavulanate PO BID 7 days - Cefadroxil PO BID for 7 days	SYSTEMIC ANTIBIOTICS: -Vancomycin IV +/- Rifampin BID for 10 days -Clindamycin 3 times daily PO for 7 days -Trimethoprim-sulfamethoxazole PO BID for 7 days -Doxycycline BID PO for at least 7 days

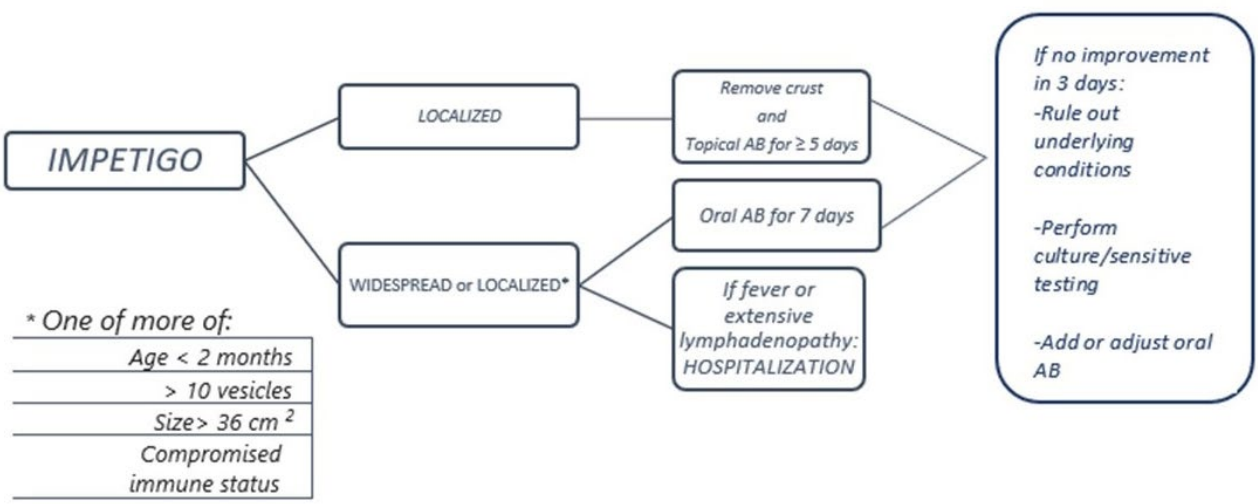

Fig. 2. *Management and treatment of impetigo. **AB**=antibiotics.*

CONCLUSION

Atopic skin is predisposed to infection by pathogenic microbes, particularly Staphylococcus aureus and the herpes simplex virus. This predisposition to infection is caused by an abnormal skin barrier due to a combination of genetic, environmental and immunological factors. Therefore, the reduction of skin infections is a cornerstone in AD therapy32.

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Pediatric COVID-19 – a review

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In December 2019, in Wuhan (Hubei, China), the first coronavirus disease 2019 (COVID-19) cases due to severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) have been reported. On March 11th, 2020, the World Health Organization (WHO) declared the pandemic. On July 1st, 2021, more than 180 million people had developed the disease, with more than 3.9 million deaths. In Italy, the most updated data show that, among all cases (n= 4.218.979) and deaths (n=125.058) due to COVID-19, 5.5% (n= 231.338) with 11 deaths concern the 0-9 years age group, while 9.6% (n= 406.460) with 15 deaths concern the 10-19 years age group. This review aims to present a comprehensive overview of COVID-19 in children.

In December 2019, in Wuhan (Hubei, China), the first coronavirus disease 2019 (COVID-19) cases due to severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) have been reported. On March 11th, 2020, the World Health Organization (WHO) declared the pandemic. On July 1st, 2021, more than 180 million people had developed the disease, with more than 3.9 million deaths (1). In Italy, the most updated data (as of June 9th, 2021) show that, among all cases (n= 4.218.979) and deaths (n=125.058) due to COVID-19, 5.5% (n= 231.338) with 11 deaths concern the 0-9 years age group, while 9.6% (n= 406.460) with 15 deaths concern the 10-19 years age group (2). Worldwide, the pediatric population accounts for approximately 7% of the total number of confirmed cases, with distribution by age group that progressively increases from neonatal to adolescent age, with half of the cases occurring in children aged 1-14 years, while cases in the first year of life are rare. Since the first months into the pandemic, it became clear that peculiar features characterize pediatric COVID-19. This review aims to present a comprehensive overview of COVID-19 in children.

Clinical symptoms of pediatric COVID-19

In the last year, accumulating literature showed that children and adolescents appeared the population least at-risk for developing the critical disease (3, 4). Once infected, the risk for a child to develop severe disease requiring hospitalization is 25 times lower than in adults (0.1% *vs* 2.6%), and the risk of death is 500 times lower than in adults (0.001% *vs* 0.5%), as based on a French study (5).

Clinical manifestations of COVID-19 in children range from asymptomatic to mild, moderate, severe, and critical (6). Symptomatic children usually present with fever, myalgia, rhinitis, sore throat, cough, and gastrointestinal symptoms such as vomiting, diarrhoea, and abdominal pain. Severe COVID-19 in children is rare and is generally associated with dyspnea/ tachypnea, central cyanosis, oxygen saturation <92%, tachypnea; gastrointestinal symptoms; impaired

Keywords: children; SARS-CoV-2; COVID-19

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0393-974X (2022)

consciousness; seizures; sepsis; shock; or multi-organ dysfunction (6).

The prevalence of clinical symptoms in the pediatric population ranges widely among studies because differences influence case definition and diagnostic criteria: dry cough, fever, pharyngitis, and rhinorrhea (3, 4). Vomiting, diarrhoea, headache, myalgias and syncope have also been reported. In a US study, 93% of adult patients reported at least one of the following symptoms fever, cough, and shortness of breath, while one of these symptoms was described in 73% among children. Specifically, 71% of adult cases reported fever compared with 56% of pediatric cases, 80% of adult cases reported cough compared with 54% of pediatric cases, and 43% reported dyspnea compared to 13% of children with confirmed cases of infection (7).

A multicenter cohort study involving 82 participating healthcare institutions across 25 European countries reported five hundred eighty-two subjects with PCR-confirmed SARS-CoV-2 infection (8). Four hundred fifty-four (78%) were contributed by tertiary or quaternary healthcare institutions, whereas fifty-four (9%) had been diagnosed in secondary and seventy-four (13%) in primary healthcare settings. The median age was 5.0 years (IQR 0·5–12·0), and the sex ratio was 1.5 males per female. One hundred forty-five (25%) had pre-existing medical conditions. Three hundred sixty-three (62%) individuals were admitted to the hospital. Forty-eight (8%) individuals required ICU admission. More recently, a retrospective cross-sectional study analyzed children diagnosed with COVID-19 from healthcare organizations in the United States (9). Among 12.306 children with lab-confirmed SARS-CoV-2 infection, 16.5% presented with respiratory symptoms (cough, dyspnea), 13.9% had gastrointestinal symptoms (nausea, vomiting, diarrhoea, abdominal pain), 8.1% had dermatological symptoms (rash), 4.8% had neurological (headache), and 18.8% had other non-specific symptoms (fever, malaise, myalgia, arthralgia and disturbances of smell or taste). The hospitalization frequency was 5.3% in the study cohort, with 17.6% needing critical care services and 4.1% requiring mechanical ventilation. Following propensity score matching, the risk of all outcomes was similar between males and females. However, the risk of hospitalization was more significant in non-Hispanic Black [RR 1.97 (95% CI 1.49–2.61)] and Hispanic children [RR 1.31 (95% CI 1.03–1.78)] compared with non-Hispanic whites.

Laboratory findings in the pediatric population with SARS-CoV-2 infection

The abnormal laboratory findings commonly detected in the adult population are less frequently reported in the pediatric population. Both leucopenias with lymphocytopenia and lymphocytosis have been reported (11). Moreover, eosinopenia has been frequently detected in patients with SARS-CoV-2 infection (12). Increased C-reactive protein (C-RP) and interleukin (IL)-6 values were detected in 13.6%-20% of the cases, especially in severe forms (11). High levels of procalcitonin were significantly associated with the severity of COVID-19, suggesting bacterial superinfection (11). High levels of C-RP (>10 mg/dL), leucopenia, increased lactic dehydrogenase creatine kinase, D-dimers, and ferritin values are also significantly associated with the severity of COVID-19 (11).

Instrumental findings in the pediatric population with SARS-CoV-2 infection

Data on radiographic features associated with severe/critical COVID-19 pneumonia are sparse. Cases of interstitial pneumonia have been detected in asymptomatic children. Bronchial thickening and ground-glass opacities are the most common radiologic features reported in children (13). In a case series involving 10 children, unilateral patchy opacities were observed in 4/10 (40%) of the cases (14)). Other authors described unilateral and bilateral opacities in children with COVID-19, and bilateral opacities appeared to be more typical in the pediatric population (15). Bilateral peripheral and/or subpleural ground-glass and/or consolidative opacities in the lower lobes of the lungs are the most common abnormalities found on CT. The “halo” sign was reported in about 50% of cases and was generally observed in the early phase of the disease, defined as a focal consolidation with a rim of surrounding ground-glass opacity. Successively, it progresses to ground-glass (progressive phase) and can develop into consolidative opacities (developed phase). Fine mesh reticulations and “crazy paving” signs have also

been reported, even if less frequently in the pediatric population compared to adults. Pleural effusion and lymphadenopathy have been rarely described (15,16).

Risk factors for developing severe forms of SARS-CoV-2 infection in the pediatric population

Extremes of pediatric age (in particular, age 0-3 months or >20 years) and comorbid conditions have been reported to be risk factors for developing severe COVID-19 (17-19); The most frequently reported comorbidities include congenital or acquired cardiovascular and pulmonary diseases, medical complexity (e.g., due to neurologic impairment, developmental delays, or genetic syndromes including trisomy 21), diabetes mellitus, neoplasia, obesity, congenital or acquired immunodeficiencies and hemoglobinopathies (18).

Of note, the unprecedented global effort to define COVID-19 pathological mechanisms has quickly led to a better understanding of the virus-host interactions and the host requirements for immunity against SARS-CoV-2 infection (20,21). In this context, a recent study found that ~ 10% of adult patients with severe COVID-19 have high levels of neutralizing autoantibodies (autoAbs) against type I IFNs in their serum (22). The impact of these autoAbs was demonstrated by the inability to detect IFN in patients' sera and their capacity to prevent anti-viral immune responses *in vitro*. Interestingly, Zhang et al. studied 659 individuals who developed severe COVID-19 and found that ~3.5% of patients harboured germline loss-of-function variants in genes previously found to be important for host defence against influenza or other viral infections (e.g., bi-allelic loss of function mutations of IRF7 or IFNAR1, heterozygous mutations in TLR3, TICAM1, TBK1, or IRF3), (23) due to the key role of these genes in the type I IFN signaling pathway. These results are currently tested in different and more numerous cohorts.

Overall, these studies defined a crucial and non-redundant role for type I IFNs in immune control of SARS-CoV2 infection, thus preventing severe COVID-19. Furthermore, these findings have paved the way for identifying possible therapeutic interventions. In particular, the early administration of IFN-β in individuals at risk may theoretically alleviate the lack of type I IFN signaling in the first phase of COVID-19 disease by rapid induction of an anti-viral state, and may mitigate the natural evolution of SARS-CoV-2 infection to potentially fatal disease.

Therapy

SARS-CoV-2 infection is generally milder in children than in adults, and a substantial proportion of children with the disease have asymptomatic infection. Therefore, most children with SARS-CoV-2 infection will not require any specific therapy and can be managed with supportive care alone (24-26).

Generally, supportive treatment includes adequate fluid intake and additional oxygen supplementation, if needed. However, patients can require more intensive care and respiratory support through non-invasive ventilation or mechanical ventilation. There are no pediatric data from placebo-controlled randomized clinical trials and limited data from observational studies to inform the development of pediatric-specific recommendations for the treatment of COVID-19. The National Institutes of Health (NIH) COVID-19 Treatment Guidelines contains a specific section with special consideration for children (27).

The major recommendations regard the use of anti-viral therapy (Remdesivir) and steroids. Remdesivir is recommended for: (i) hospitalized children aged ≥12 years with COVID-19 who have risk factors for severe disease and have an emergent or increasing need for supplemental oxygen; (ii) hospitalized children aged ≥16 years with COVID-19 who have an emergent or increasing need for supplemental oxygen regardless of whether they have risks factors for severe disease; (iii) in consultation with a pediatric infectious disease specialist, remdesivir can be considered for hospitalized children of all ages with COVID-19 who have an emergent or increasing need for supplemental oxygen. Moreover, the COVID-19 Treatment Guidelines Panel recommends using dexamethasone for hospitalized children with COVID-19 who require high-flow oxygen, non-invasive ventilation, invasive mechanical ventilation, or extracorporeal membrane oxygenation. For pediatric patients, the dexamethasone dosing regimen is dexamethasone 0.15 mg/kg/dose (maximum dose 6 mg) once daily for up to 10 days.

The availability of effective vaccines has dramatically changed the approach to COVID-19 pandemics. In this regard, the Italian Society of Pediatrics (SIP) has recently released a position paper that includes the main recommendations for the vaccination in the pediatric population (currently, ≥ 12 years of age). SIP recommends:

1) in line with current ministerial recommendations, Covid-19 vaccination for all children and adolescents aged 12 years and older without contraindications for specific age-approved vaccines;

2) the use of any Covid-19 vaccine (provided that EMA and AIFA approve it), according to the timing and mode of administration provided for the specific age groups;

3) the administration of COVID 19 vaccine even without adhering to specific time intervals compared to the vaccines provided by the current National Plan for Vaccine Prevention, except for the minimum time necessary to assess any adverse events (15 days);

4) in case of children with a history of previous SARS-CoV-2infection, an interval of at least 90 days between the diagnosis or the administration of any monoclonal antibodies, and the first administration of Covid-19 vaccine;

5) not to prescribe medications aimed at preventing any postvaccine adverse events;

6) to guide adolescents and their families towards a free and informed vaccination pathway;

7) to inform parents about how to manage the most frequent post-vaccine signs and symptoms, but especially about the timing to contact their doctor of reference to benefit from further specific information;

8) to strongly reiterate to adolescents and their families the value of continuous and constant compliance with rules for containment and spread of SARS-CoV-2, even after vaccination and until specific indications are formalized by the national regulatory bodies.

CONCLUSION

After one year into the pandemics, our understanding on pediatric COVID-19 has exponentially improved. However, several unmet needs remain. It will be fundamental to fully characterize the immunopathological bases of SARS-CoV-2 infection in children to identify possible biomarkers for severe disease and the development of the Multisystem Inflammatory Syndrome in Children (MIS-C), the post-infectious inflammatory syndrome related to SARS-CoV-2. Moreover, long-term complications after COVID-19 have been reported in adults, while few data are available for the pediatric population.

Finally, several trials are assessing the efficacy and safety of the COVID-19 vaccine in children under 12 years of age. Studies designed for the pediatric population are needed further to understand the peculiar features of COVID-19 in children.

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Hot topics in pediatric chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a heterogeneous chronic inflammatory condition of the paranasal sinuses and nasal passage. CRS may significantly compromise patients' quality of life and may represent an alert sign for other underlying systemic diseases. CRS is now considered an umbrella diagnosis for several diseases with distinct mechanistic pathways (endotypes) and variable clinical presentations (phenotypes). Therefore, it is necessary to integrate medical and surgical skills and evaluate and treat the patient through a multidisciplinary approach. Only two biologicals (omalizumab and dupilumab) are approved in adults with severe CRS with nasal polyps. Clinical trials conducted in children and adolescents are needed to reinforce the strength of available efficacy and safety data of biologic therapies in the adult population and children with CRS with nasal polyps.

Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory disease involving the mucosa of the nose and one or more sinuses of at least 12 weeks duration. Because the mucosa of the nose and sinuses form a functional and anatomic continuum, the sinuses are always involved in nasal disease.

In 2020, the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) defined CRS (with or without nasal polyps) in children as the "presence of two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip): ± facial pain/pressure and/or ± cough; for ≥12 weeks". These signs and symptoms are necessarily supported by evidence of inflammation on nasal endoscopy and typical features on imaging (TC/RMN) (1). Nasal polyps (NPs) are inflammatory outgrowths of sinonasal tissue. These masses are characterized by inflammatory mucosa oedema and inflammatory infiltrates of the polyp and the surrounding mucosa. They often have the shape of a grape cluster due to the force of gravity and have a gelatinous appearance, yellowish, pink-grey sometimes, and NPs can be visible already on anterior rhinoscopy (2).

Most commonly, NPs present as bilateral or monolateral inflammatory lesions originating in the ethmoid sinuses and projecting into the nasal airway beneath the middle turbinate. Although NPs are observed in various clinical conditions, including cystic fibrosis and malignancy, they are more frequently associated with a subset of chronic rhinosinusitis (CRS) aptly named chronic rhinosinusitis with nasal polyps (CRSwNP). In this condition, NPs are benign and typically develop bilaterally in the sinonasal cavity (3, 4).

CRS represents a significant health problem

Keywords: chronic rhinosinusitis, nasal polyps, children, adenoids hypertrophy, endotype, phenotype

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0393-974X (2022)

affecting 5-28% of the general population and 2-4% of children. Among all patients with CRS, only approximately 25% to 30% and from 30% to 70% patients with CRSwNP have asthma. NPs occur in 5% of allergic asthma, while they occur in 13% of those with non-allergic asthma (5-8). However, CRS is associated with significant morbidity and decreased quality of life, making this disease clinically important to identify, evaluate, and treat.

History

NPs were first noted about 4000 years ago in Egyptian literature. Due to mummification in which cranial contents were removed through the nose, Egyptians were well versed in nasal cavity procedure. Further on in history, the Greek Hippocrates became known as the father of rhinology and medicine. He observed and documented many otorhinological conditions, including nasal polyps. Hippocrates referred to these growths in the nose as "polypus" as they resembled sea-polyps (9).

Anatomical features

CRS is most prevalent in adults because sinuses are not fully formed until adolescence. In the pediatric age, only the maxillary and ethmoid sinuses are present from birth, the frontal and sphenoid sinuses develop later, and all develop the size of the adult around adolescence. Homeostasis of sinonasal drainage is borne by the patency of the Sinus Ostia, the presence of enough mucous production, and an efficient ciliary function. In the absence of proper mucus production and ciliary mucus clearance, systemic diseases such as cystic fibrosis and primitive ciliary dyskinesia must always be excluded, respectively.

A critical anatomical point sustaining homeostasis of the paranasal sinuses is the osteomeatal complex. The osteomeatal complex is an important area of sinus drainage situated in the middle meatus where secretions from the maxillary, the anterior ethmoid, and the frontal sinuses converge. Major structures of the osteomeatal complex are the natural ostium of the maxillary sinus, the infundibulum, the uncinate process, the hiatus semilunaris, and the ethmoid bulla. Obstruction of the osteomeatal complies is often the starting point for sinus disease in the pediatric population (10-11).

On the other hand, in the pediatric age, adenoid hypertrophy associated with anatomic abnormalities usually contributed to CRS development. The adenoids are part of Waldeyer's ring and have an important immune defence role. They undergo hypertrophy in a paraphysiological way, which usually regresses after 6-7 years of life. Adenoid hypertrophy prevalence is 34% in the general pediatric population (higher in children with ear, nose, and throat issues such as OSAS). Often in children adenoids, hypertrophy is characterized by a bacterial reservoir and causes a

Table I. *Favoring factors and associated systemic disease.*

Favoring factors	**Systemic disease**
Frequent upper airways infections	Cystic fibrosis
Anatomic sinus abnormalities (small sinus ostia)	Primary ciliary dyskinesia
Immaturity of the humoral immune system	Immune deficiency
Biofilm formation in the sinus tissue	Vasculitis
Enlarged adenoidal pads harboring biofilm	
Allergy	
Rhinitis	
Trauma	
Foreign body	
Gastroesophageal reflux	
Tobacco smoke	

posterior nasal obstruction with mucous retention, a major mechanism implicated in CRS development. The most common pathogenic organisms identified in the adenoids include Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenzae, and group A streptococci. These same bacteria are similar to the common organisms found in acute and chronic sinusitis in children (12-13).

Favoring factors and associated systemic disease

In the pediatric age, the most recent literature has identified several favoring factors and associated systemic disease for the development of chronic rhinosinusitis (Table I) (7, 14, 15). Patients with Kartagener's syndrome, primary ciliary dyskinesia, Young's syndrome, Eosinophilic granulomatosis with polyangiitis (EGPA) (previously Churg Strauss Syndrome), and cystic fibrosis frequently have a long history of CRS. Atopy with or without asthma is a suspect favoring factors of development CRS. In patients with allergic rhinitis, the nasal mucosa's swelling can alter the nasal ostium's physiological ventilation (16). Allergic rhinitis may represent comorbidity of CRS also in the pediatric population, complicating the diagnosis and therapeutic management (17).

In a recent retrospective study in 4044 pediatric patients with CRS, the prevalence of allergic rhinitis was very high (27%), more than the other disease. On the other hand, there is conflicting information regarding the relevance of allergic sensitizations in CRS development. While some studies show an association, others have shown no significant association. A clear and definitive causal relationship between allergic rhinitis and CRS has not been established, especially in children (18-19).

Chronic rhinosinusitis and asthma are not to be regarded as simply localized disease processes but part of a systemic inflammatory disease affecting the respiratory tract. According to recent literature, there is a strong correlation between the severity of asthma and the clinical and imaging features of rhinosinusitis. In 2021 Tosca et al. had undergone 128 asthmatic patients to nasal endoscopy and found rhinosinusitis features in more than 50% of the patient (20-21). CRS may aggravate asthma control and worse functional respiratory tests, namely spirometry (22).

In a subgroup of the asthmatic patient with aspirin, sensitivity up to 90% has CRSwNP. A triad of symptoms (bronchial asthma, CRSwNP, and aspirin intolerance) is referred to as Samter's triad or ASA-triad. Several authors have recently demonstrated the contribution of various microorganism infections (viral/fungal/bacterial) and environmental factors such as smoke exposure, pollutants, and toxins in CRS pathogenesis. Cigarette smoking was associated with a higher prevalence of CRS in European countries (23).

Pathogenesis and endotype

The exactly pathogenetic mechanism underline of CRS with or without nasal polyps remains unclear, but researchers have proposed several hypotheses. First, it is hypothesized that a defective mucosal barrier could lead to increased exposures to inhaled allergens, microbes, and particulates regularly inhaled that, in the setting of a dysregulated host immune response, could promote chronic inflammation. In healthy conditions, the epithelial cells that line the sinonasal mucosa form a physical barrier to protect the host from inhaled respiratory pathogens and particulates and play critical roles in mucociliary clearance and host defence. However, in CRS, the mucosal barrier is defective; epithelial cells do not function normally, increasing tissue permeability. This condition determines the activation of self-limited immune-defence, characterized by a cellular and cytokine repertoire targeting one of the three classes of pathogens (viruses, parasites, and extracellular bacteria and fungi) (Fig. 1).

There are many types of white blood cells (eosinophils, neutrophils, basophils, mast cells, innate lymphoid cells type-2, T cells, and B cells) that are elevated and play a role in CRS inflammation (24, 25). In this contest, three different endotypes driven by different cytokine and chemokine pathways are observed. A neutrophilic flogosis characterize type 1 and type 3 endotypes; type 2 are eosinophilic. Type 2 is the most studied, and this inflammation appears to be associated with remodelling and formation of polyps and appears to be more frequently associated with forms resistant to conventional

therapy. Endotyping facilitates the development of individualized treatment plans, which reduces cases of undertreatment or overtreatment and thus fewer surgeries and side effects from therapy. Endotyping is also extremely useful in choosing a suitable biologic treatment (26-27). So far, very few studies have explored the distinguishing features of CRS in childhood due to the challenge to recruit enough patients and the complexity of diagnosing at this age. Recent omics advances in large-scale consortia have provided valuable new insights into CRS sub-phenotypes and underlying cellular processes but mainly focused on adults.

Histopathological investigation into sinus mucosa of young children with CRS demonstrated neutrophils and eosinophils predominance in younger and older children, respectively. In summary, the likely, defective mucosal barrier, microorganism colonization, and consequent immune response play important roles (28-38).

Clinical features and phenotype

Patients with CRSwNP must report the presence of anterior or posterior rhinorrhea, nasal congestion, hyposmia, and/or facial pressure or pain lasting for more than 12 weeks.

Cough is another typical symptom of pediatric age; it can be wet or dry and worsens at night. The suggested mechanism for triggering cough in rhinosinusitis is the post-nasal drip (the drainage of secretions from the nose and paranasal sinuses into the pharynx) (39). On the other hand, hyposmia is more typical in adults than in pediatric patients due to difficulty recognizing the symptom. Nasal obstruction is frequent in the pediatric age, which causes oral breathing and sleep disorders breathing. In some patients, nasal secretions, purulent or mucoid, can be absent because they are posteriorly into the oropharynx.

Nasal polyps in patients with CRSwNP have a marked negative effect on the quality of life (QoL), including physical function, social function, and mental health, in addition, the severity of symptoms have been linked to the size and number of nasal polyps and the extent of obstruction.

The Visual analogue scale (VAS) is a score used in the Rhinology field to quantify patients' symptoms severity is the Visual analogue scale (VAS). Markers to grade the severity are mild (0-3), moderate (3-7),

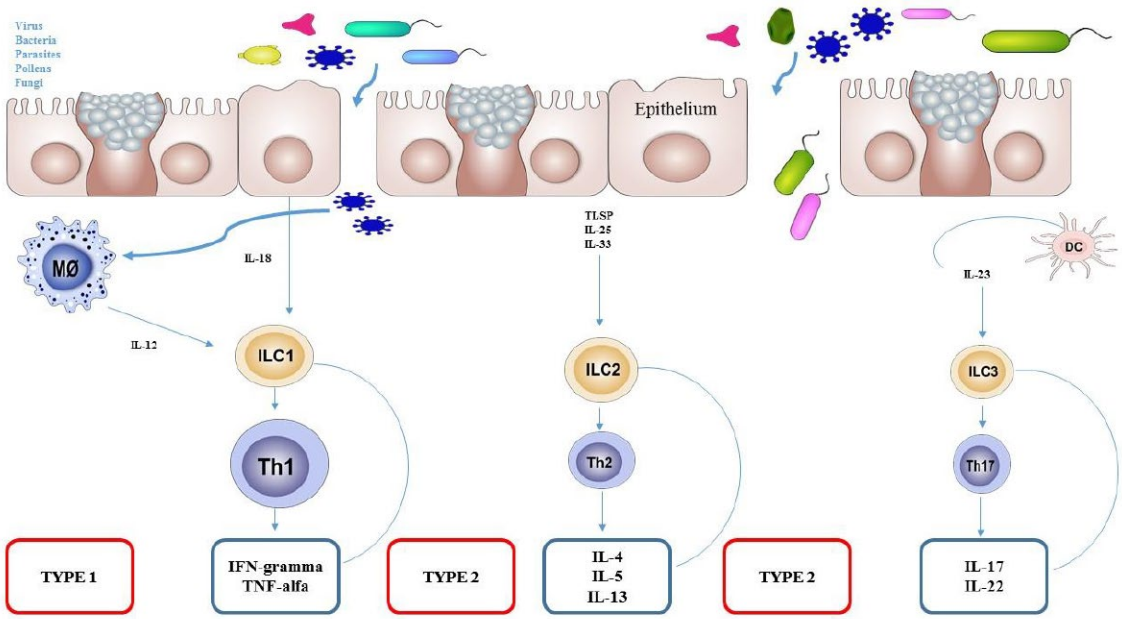

Fig. 1. *The different types of response when the barrier is breached, depending on the triggers (virus, bacteria, parasite, fungi, pollens). The immunological response is characterized by a rapid response mediated by the lymphocyte's subset (**ILC-1, ILC-2, ILC-3**) that active the delayed T helper subset (**Th1, Th2, and Th17** respectively). **MØ**: Macrophage; **ILC**: Innate lymphoid cells; **TLSP**: Thymic stromal lymphopoietin; **TH**: T helper; **INF-γ**: gramma Interferon-γ; **TNF-α**: alfa Tumor Necrosis Factor-α.*

and severe (7-10); this can be used in primary care to predict response to treatment and assess the need for a referral. Symptom severity can be measured by questionnaires such as the 22-Item Sinonasal Outcome Test (SNOT-22). The test evaluates symptoms and social or emotional issues associated with the condition. These are evaluated from 0 to 5, with 0 signifying no problem and 5 being the worst extent of a problem (40-41).

A new and relevant classification system of CRS phenotype is being proposed in EPOS 2020 guidelines. This system differentiated primary CRS from secondary CRS and divided each subgroup into localized or diffuse disease based on the anatomical distribution1. Primary CRS phenotypes depend on the underlying pathologic mechanism, type-2 or non-type 2. For example, allergic fungal rhinosinusitis (AFRS), a type-2 phenotype, can be developed in children with allergic fungi rhinitis. The clinical manifestation is characterized by proptosis and polyposis, and usually, these patients presented with typical radiographic findings such as unilateral sinus opacification with the non-erosive expansion of the sinuses (42).

In the same way, a new phenotype of chronic rhinosinusitis with nasal polyposis in the adult patient has recently been described: central compartment atopic disease (CAD). This clinical phenotype is distinguished by the high prevalence of allergy and the low prevalence of asthma in affected patients. The pathognomonic clinical features are the oedema of the middle turbinates and nasal polyps in patients with allergic sensitization to inhalants (43-44).

Eosinophilic chronic rhinosinusitis (eCRS), the last type-2 phenotype, is rare in children and is difficult to treat as it is associated with a high recurrence rate. Non-type 2 phenotypes are isolated sinus disease and noneosinophilic CRS, typically in obese and middle-aged women. Conversely, secondary CRS phenotype results from localized disease (odontogenic disease, mycetomas, fungal balls) and systemic disorders (45).

Diagnosis

Diagnosis is multidisciplinary. Nasal endoscopy is used to confirm the diagnosis of nasal polyps, and anterior rhinoscopy is useful in the visualization of large polyps. Nasal endoscopy allows evaluating the presence of polys, oedema, and drainage; furthermore, it allows performing culture or biopsy when indicated. The biopsy is usually useful only when polyps are observed on one side. However, this histopathological data could be useful in terms of prognosis. Tissue eosinophilia is linked to high rates of recurrence.

In allergic rhinosinusitis, allergy tests need to be performed via patient interviews, skin prick tests, or blood tests. Because CRSwNP in children is often associated with cystic fibrosis, there is a need to perform sweat tests to observe chloride levels. CT and MRI visualize the pathological changes of the nose and sinuses. Pre-surgically, CT excellent complementary tool in displaying CRSwNP is used as it gives the best visualization of the bony structure but exposes the child to radiations. The Lund Mackay scoring system is dependent on CT scan results. The focus is the opacification of the sinus system and osteomeatal complex. There is a scoring scale from 0 to 2 where 0 = absence of opacification, 1 = partial opacification and 2 = complete opacification. A score is given for both left and right, and the maximum score per side is 12. MRI provides superior imaging of soft tissue compared with CT scans, and MRI is useful in the event of intracranial complications (16, 46, 47).

Treatment

CRS has multiple treatment considerations, including traditional medical therapy, biologics, and surgery. The first-line treatment for CRS is medical therapy, with nasal corticosteroids and nasal irrigation to reduce inflammation and oedema and antibiotics for eradicating microorganisms. Oral corticosteroids are usually prescribed and unresponsive for patients. Investigation of the cause and pathogenesis of the nasal polyps is imperative as this has a bearing on the route of treatment chosen. Specialists treat benign nasal polyps conservatively and only turn to surgery when medical therapy fails. Treatment is very personalized and varies with each individual (48).

Biological therapy

Chronic rhinosinusitis is the "umbrella" term, such as asthma, anaemia, arthritis, describing a heterogeneous disease with different phenotypes and endotypes. Therefore, in the adult patient with poorly

controlled CRS treatment with biologic should be considered. Two biologics targeting type-2 pathway underlying CRSwNP are currently approved only in an adult population: dupilumab and omalizumab (49-52). Dupilumab, a fully human IgG4 monoclonal antibody directed against the IL4 receptor alpha subunit, was the first biological drug approved by food and drug administration (FDA) for severe CRSwNP in adult patients unresponsive to surgery end/or systemic corticosteroids (53). Omalizumab, a humanized monoclonal anti-IgE, was recently approved by the European medical agency (EMA) and FDA to treat severe CRSwNP in adult patients.

There are conflicting data in several case series in the pediatric population that suggested an effective role of omalizumab in a patient with CRSwNP, particularly in a patient with other allergic comorbidities (54-55). Large-scale future studies are needed to better understand the possible role of biological drugs in treating pediatric patients with uncontrolled CRSwNP.

Conflict of interest statement

The authors declare no conflict of interest.

Financial support

No financial support was received for this work.

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Papillomavirus skin infections and children. An overview on cutaneous and anogenital warts treatment

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Human papillomavirus (HPV) skin infections manifest mainly as cutaneous (CWs) and anogenital warts (AGWs). They are quite frequent in children, and genital infections raise problems concerning the nature of transmission, including the possibility of child sexual abuse. HPV skin infections may resolve spontaneously, and the treatment choice should consider the efficacy and tolerability of different therapies. This paper provides an overview of treatments available in children for CWs and AGWs: cryotherapy, salicylic acid, photodynamic therapy, surgery and mechanical therapies, nitric-zinc complex, podophyllin, imiquimod, intralesional immunotherapy, sinecatechins, topical antivirals, such as cidofovir, and other therapies. HPV vaccines have changed the incidence of HPV-related diseases, especially in HPV-naïve people, but their effectiveness in already infected patients is being investigated.

Human papillomavirus (HPV) infections affect up to 10-20% of school-aged children (Fuller 2017). Skin infections manifest as cutaneous warts (CWs), including common, plantar and flat warts, and anogenital warts (AGWs) (1). AGWs are the most frequent sexually transmitted disease and affect up to 3% of sexually active teenagers (2).

Transmission routes

HPV transmission can occur through skintoskin or skintomucosa contact and be sexual or non-sexual (2).

One of the most frequent non-sexual ways is contact with fomites, fingers, mouth (2). The horizontal one is the main transmission route of common warts. Auto-inoculation or hetero-inoculation by parents or caregivers may account for AGWs in female virgins or pediatric patients without a history of child sexual abuse (CSA) (2,3). Another explanation for non-sexually transmitted AGWs in children is vertical transmission: the mother may transfer HPV to the newborn through the amniotic fluid, or the placenta, or contact via the maternal genital mucosa during natural birth (2). The rate of CSA-related AGWs is unknown, ranging from 0 to 80% and increases directly with age, being greater in children older than 4 years (3). The presence of cutaneous HPV types in pediatric AGWs may suggest a high rate of horizontal transmission(4). Only 58.5% of AGWs have been related to mucosal types in children, particularly HPV6; while 43.4% have been described as due to cutaneous HPV types, such as 2, 27 and 57 (4).

Treatments

There is no consensus about first-line therapy in

Keywords: papillomavirus, skin, anogenital warts

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0393-974X (2022)

the pediatric population. Treatment choice should consider the efficacy and tolerability of different therapies (1). Duress or pain related to therapeutic procedures shouldn't overcome advantages associated with the healing of the disease. Since the natural resolution is possible in 63.6% of cases at 2 years (5), the watch and wait approach may be considered but should be avoided if warts persist for more than 2 years or are symptomatic (3). Treatments can be divided into destructive and immunostimulatory methods, considering that most destructive procedures may require local or general anaesthesia (1, 3).

Cryotherapy

Spray gun or cotton wool application of liquid nitrogen, at −196°C, are equally effective (5). Cotton wool application is useful in sensitive areas such as the face and improves patient compliance (5). The interval between each session should be 2 to 3 weeks. Clearance rates (CRs) range from 51.6% to 68.2% (5). Adverse effects (AEs) of cryotherapy are pain and blistering (5). Application of eutectic lidocaine/prilocaine 5% cream prior to cryotherapy doesn't seem to reduce pain in AGWs (6). Cryotherapy for AGWs may be less tolerated than CWs and should be avoided in children.

Salicylic acid (SA)

SA is not recommended in children younger than 2 years or for treatment of AGWs, because its absorption is greater in these children and through mucous membranes, leading to systemic toxicity, namely "salicylism". For the same reason, application to wide surface areas should be avoided too (5). Nevertheless, SA is a useful topical drug for treating pediatric CWs, alone or in association with other therapies (5). CRs of monotherapy range from 18% to 100%, while combination therapy with glycolic acid, lactic acid, or podophyllin-cantharidin leads to complete resolution in 60-100% of cases (5). When appropriately used, SA has minimal AEs, mainly skin irritation (5).

Photodynamic therapy (PDT)

In addition to antitumoral and anti-inflammatory effects (7, 8, 9). PDT, using topical 5-aminolevulinic acid (ALA) as a photosensitizer, may kill different microbes through the production of reactive oxygen species and immunomodulation (9).

PDT is effective in children with difficult or recalcitrant CWs, particularly flat facial warts (10, 11). In this group of patients, conventional PDT and daylight PDT have shown similar results, with CRs respective of 73.3% and 80% after three treatment sessions (12).

Using 20% 5-ALA solution, PDT has been recently tested for pediatric perianal AGWs with high CRs (13). It has been proved to be effective also in recalcitrant AGWs. Generally, three to six sessions are required (13). Mild to moderate pain during light exposure, irritation and hyperpigmentation are the main AEs (9, 12, 13).

Surgery and physical therapies

Surgical techniques should be considered for treatment-resistant pediatric warts or extensive diseases like giant condyloma acuminatum (Buschke-Lowenstein tumor) (6). Electrocautery and CO2 laser are used in pediatric CWs with CRs respective of 50% and 57% (5). Treatment with pulsed dye laser in a pediatric group with recalcitrant warts has shown CRs of 75%, and both perineal/perianal and facial warts have had a complete resolution in 100% of cases(14). Scarring and pain are common AEs of surgical and mechanical therapies.

Nitric-Zinc Complex

The nitric-Zinc complex is a topical solution containing nitric acid, zinc, copper, and organic acids that induce a painless caustic effect on warts, leading to their "mummification" (15). It is already used in adults for cutaneous and genital warts, while in children, elevated CRs have been reached in periungual (87,5%) and palmoplantar warts (71%) (15). However, its usefulness for pediatric AGWs has not been evaluated.

Podophyllin

Podophyllotoxin, also called podofilox, is a natural antimitotic agent derived from the root of Podophyllum sp (6). Although it is not recommended in children because of the risk of systemic toxicity, topical podophyllotoxin 0,5% has been successfully

used to treat AGWs in the pediatric population, with burning as the most common AE (6).

Topical antivirals

Cidofovir, an antiviral agent against DNA polymerase, has been widely investigated to treat warts (6). Topical cidofovir 1-3% has been reported to be effective in recalcitrant CWs and AGWs in the pediatric population, with not common AEs, mainly local irritation (6).

Imiquimod

Imiquimod 5% cream, induces an inflammatory response by activating toll-like receptor 7(16). It is not recommended in children younger than 12 years, but it has shown CRs of 53% in preadolescent children with AGWs (16). Duration of therapy may range from 2 to 6 months, and AEs are generally mild, ranging from irritation to severe inflammation (16).

Intralesional immunotherapy

Candida antigen, mumps antigen, the combined measles, mumps, and rubella (MMR) vaccine, tuberculin purified protein derivative (PPD), and bacillus Calmette-Guerin (BCG) vaccine can be used through intralesional infiltration to induce a response against HPV by cell-mediated immunity, particularly in case of pediatric recalcitrant or multiple warts (17). CRs range from 23.3% to 95.2% (17), with peaks of 73% and 80% in children with AGWs treated respectively with MMR vaccine or Candida antigen(18). AEs are minimal, including flu-like symptoms and injection site pain (17, 18).

Sinecatechins

Sinecatechins, derived from *Camellia sinensis* (green tea), is a promising therapy for AGWs and CWs in children, even though their mechanism of action is still unknown (19). Applying sinecatechins 10% ointment has reduced the number and/or size of warts in 67% of pediatric patients, while 16.7% have experienced complete resolution (19). However, a mild local irritation has been reported in 29.2% of cases (19).

Sinecatechins 15% ointment has been recently tested for AGWs in preadolescent children with an 89% total response and low AE-profile (16).

Other therapies

The following therapies may be effective for cutaneous and genital warts, but data in children are limited: trichloroacetic acid, pyruvic acid, retinoids, cantharidin, diphenylcyclopropenone, squaric acid dibutyl ester, oral cimetidine and zinc sulfate (5, 6).

Prevention

HPV vaccination, through quadrivalent (6,11,16,18), bivalent (16, 18) and the more recent 9-valent HPV vaccines, has resulted in a marked reduction of HPV-related diseases, such as AGWs or cervical high-grade squamous intraepithelial lesions (HSILs)(2,20). HPV vaccination is advised for 9-year-old children or older (21). About the role of vaccines in already infected people, opinions are contrasting. A recent meta-analysis shows that HPV vaccines do not prevent recurrences of AGWs (21). Some studies suggest the effectiveness of HPV vaccines to prevent recurrences of anal or cervical HSILs among men and women, but further investigations are ongoing (20).

CONCLUSION

CWs and AGWs are very common worldwide. They represent a therapeutic challenge, especially in pediatric patients, because of low compliance with the currently available treatments and the high percentage of recurrences (up to 77% in AGWs) (6). The paucity of randomized clinical trials in pediatric patients renders it impossible to evaluate the best therapeutic option for HPV lesions. The genetic mechanism underlying papillomavirus skin infections in children are still not completely understood. However, in many pediatric conditions, experimental research revealed modulating metabolic processes or genetic mechanisms in various diseases (22-30). Host-response interactions or sub-cellular alterations due to genetic defects are increasingly recognized by using a wide array of technologies in several pediatric conditions with important consequences on diagnosis, prognosis and treatment of patients (31-37). Concerning pediatric HPV infections, the final choice for follow up and treatment is the result of the number, size, shape and localization of cutaneous or genital warts, the confidence of the physicians with

the different types of treatments, and the preferences of the parents. Moreover, some physical treatments require dedicated and expensive instruments, which may be not available in an outpatient setting. The fruitful collaboration between physicians, children and relatives is essential to reach the desired outcome.

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Pediatric virus-associated erythema multiforme the state of the art and proposal for virological screening

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Erythema multiforme (EM) is a mucocutaneous hypersensitivity reaction frequently triggered by infections or drugs. EM is divided into major, with severe mucosal involvement, and minor, with minimal or absent mucosal involvement. Virus-associated erythema multiforme (VAEM) accounts for the majority of EM cases and represents a distinct form concerning Mycoplasma pneumonia-induced rash and mucositis (MIRM) (1). Typically, EM patients are young adults. However, children are affected in 20% of cases (2).

Epidemiology and etiology

The incidence of EM in the pediatric population is not known; it is more common in girls (3), although recurrent forms show a male predominance (4). The mean age of affected children is 5.6 years, ranging from 0.1 to 17 years (5). Precipitating factors are detectable in the majority of cases. Vaccines are a frequent trigger in infants, while infections are predominant from childhood (5). Herpes simplex virus (HSV) is considered to account for most cases of VAEM in adulthood but not for children. Several studies reported a low association of EM with HSV, about 0-18% of cases (2, 3, 5), while non-specific febrile illness or non-mycoplasma upper respiratory tract infection prior to the appearance of EM has been described in the 24-36% of cases (2, 3). Varicella-zoster virus (VZV) (6), Cytomegalovirus (CMV) (7), Epstein Barr virus (EBV) (8), Adenovirus (7), parvovirus B19 (9), ORF virus (10), Sars-CoV-2 (11) have been reported as triggers for EM pediatric cases.

Typically, viral infections precede EM by a few days up to 4 weeks (6-11). Drugs may elicit EM in children with congenital HIV infection (12). However, children experience recurrences in 14-16% of cases (3, 5) within an average of two years (3). Heinze et al. described a median age of onset of 9.1 years (range 0-18 years) and an average of two recurrences each year (4). The rate of HSV infections in recurrent EM is higher than at first presentation and reach 61% of cases (5). Nevertheless, tests for HSV are not performed in all cases (4). Negative cases of HSV may be due to other viruses or subclinical HSV infection: in patients with idiopathic recurrent EM, HSV-DNA has been detected in lesional skin biopsies (13).

Keywords: erythema multiforme, children, virus-associated erythema multiforme

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0393-974X (2022)

Pathogenesis

Physiopathology of VAEM is yet not completely known, and only the mechanisms of HSV-associated EM (HAEM) have been investigated. After a clinical or subclinical HSV infection, macrophages and/or CD34+ Langerhans cell progenitors engulf HSV, causing viral DNA fragmentation (14). DNA fragments, notably the viral *pol* gene, are transported to distant skin sites by peripheral blood mononuclear cells, with a skin-homing receptor called cutaneous lymphocyte antigen (14, 15). Specific CD4+ Th1 cells respond to viral antigens with the production of interferon-γ, leading to an inflammatory cascade (14, 15). We may presume that, like HSV, also other viral antigens could induce an immune response triggering autoreactive T-cells. The pathogenetic mechanism of HAEM is different compared to drug-associated EM. The latter is mechanistically distinct from HAEM because drug-induced EM inflammation is mediated by TNF-α and not IFN-γ (14, 15). A similar auto-immune mechanism has been proposed for other pediatric VAEMs. A child with negative Parvovirus IgM but positive Parvovirus IgG developed an EM-like eruption after being stung by a wasp (9). Authors speculated that an antigen shared by parvovirus B19 and bee sting, namely phospholipase A2, might be involved in the reactivation of the virus (9).

Clinical presentation

Targetoid rash is the distinctive cutaneous appearance of EM. A target lesion is an individual lesion less than 3 cm in diameter with a regular round shape and well-defined border (16). It is compared to a cockade because it is composed of two concentric rings around a dusky or blistering centre (16). The outer ring is erythematous, while the intermediate is paler, raised and edematous (16). Targets may be atypical, with only two zones and/or a poorly defined border (16). The targetoid rash typically spreads on the extensor surfaces of limbs and acral regions and the volar and dorsal aspects of hands and feet, neck and face. Skin lesions develop rapidly in a few hours or days. They are not pruritic, but a burning sensation may be present. Table I reassumes clinical features of EM and its common differential diagnoses, particularly MIRM (1) and Steven-Johnson syndrome (SJS) (17). In children,

Table I. *Clinical feature of EM minor, EM major, MIRM and SJS*

	EM minor	**EM major**	**MIRM**	**SJS**
Cutaneous lesions	Typical targets Atypical targets	Typical targets Atypical targets	Typical or atypical targets Blisters Epidermal detachment (<10% of body surface area)	No typical targets Blisters on macules Epidermal detachment (<10% of body surface area)
Mucosal involvement	No	Yes	Prominent	Severe
Distribution	Acral	Acral	Extremities Trunk Face	Widespread
Symptoms	Burning sensation Pruritus	Pain Fever Impaired food intake	Pain, Fever, Cough, Positive auscultatory findings	Pain, Fever, Dehydration, Multiple organ dysfunction

EM*: *Erythema multiforme;* ***MIRM**: Mycoplasma pneumonia-induced rash and mucositis;* ***SJS****: Steven Johnson syndrome*

EM minor is more frequent than EM major (2, 5) and infants have oral involvement only in one out of ten cases (5). Nevertheless, EM major is predominant in recurrent forms (5).

Mucosal involvement is characteristic of EM major. Oral membranes are more frequently involved than those ocular or genital. Symptoms of EM major are more aggressive than minor form, with pain, fever, and inadequate food intake. In a considerable proportion of individuals, the erythema multiforme illness spectrum is still a significant cause of severe vision loss. The ocular manifestations range from mucopurulent conjunctivitis, chemosis, and lacrimation to perforating corneal ulcers. Because of the recent increase in the number of cases reported and the high percentage of individuals with ocular lesions, ophthalmologists must be aware of the ocular implications of erythema multiforme. During the acute phase of the disease, systemic steroids appear to have little influence on the development of ocular symptoms (18).

Diagnosis

The diagnosis of pediatric EM is based on clinical features. A skin biopsy may be helpful in cases of difficult recognition, but it is performed in only 10% of reported pediatric EM cases (5). Interface dermatitis with basal vacuolar degeneration is the typical histopathological finding (15). Direct immunofluorescence in EM is not specific. However, it may be performed to rule out auto-immune bullous diseases (15).

The lower proportion of HSV association in children than in adults and the evidence of other viral associations warrants a broader microbiological screening, particularly in this age group. Considering that EM may be a sentinel of severe systemic viral infections, we propose that each EM patient be subjected to a virological screening, which is reassumed in Table II. The research of viral aetiology should be adapted to the individual anamnestic, clinical and laboratory characteristics of each patient

Table II. *Proposal for virological screening in virus-associated erythema multiforme.*

HSV	Anti-HSV IgM, IgG antibodies PCR skin swabs Tzanck smear from skin lesions In case of recurrent EM: Anti-HSV antibodies PCR from skin biopsy specimens
VZV	Anti-VZV IgM, IgG antibodies PCR from skin swabs DFA on skin biopsy Viral culture from vesicles
CMV	Anti-CMV IgM, IgG antibodies
EBV	Anti-VCA IgM Anti-VCA IgG Anti-EA IgG
Adenovirus	PCR swabs from affected sites (throat, eyes)
Parvovirus B19	Anti-Parvovirus B19 IgM, IgG antibodies
ORF virus	PCR swabs ORF biopsy
SARS-CoV-2	SARS-CoV- 2 RT-PCR nasopharyngeal swabs

***HSV**: herpes simplex virus; **VZV**: varicella-zoster virus; **CMV**: cytomegalovirus; **EBV**: Epstein-Barr virus; **SARSCoV-2**: severe acute respiratory syndrome coronavirus-2; **PCR**: polymerase chain reaction; **DFA**: direct fluorescent antibody assay; **anti-VCA**: anti-viral capsid antigen; **anti-EA**: anti-early antigen; **RT-PCR**: reverse transcriptasepolymerase chain reaction.*

and performed after other causes, such as drugs or M. pneumoniae, have been ruled out.

Treatment

The lesions of EM typically tend to resolve spontaneously over 1-2 weeks, even if the forms with mucosal involvement last for longer (15). Hospitalization is more common in pediatric recurrent EM than in adult forms, with one-third of children needing admission (4). Supportive care is the basis of the treatment of pediatric EM (5). Topical corticosteroids help relieve cutaneous burning sensation or pain from mucosal erosions. Antiseptic washes and anaesthetic solutions are also used in the oral cavity (15). Ocular involvement requires specialist consultation. Severe cases are mainly treated with systemic steroids and anti-viral, but steroids in pediatric EM are controversial (5). Steroids are administered to limit the duration and severity of symptoms, especially in EM major, but at the cost of a high percentage of adverse events, including immunodepression and enhancement of further episodes of recurrent EM (4). Anti-virals seem not to alter the clinical course of acute EM (15), but acyclovir helps limit EM recurrences in adulthood (19). Anti-viral prophylaxis may be considered in first-line also in children, regardless of HSV test results because, even at this age, HSV is the most common trigger for recurrent EM, and anti-viral therapy is safe and well-tolerated (4). Anti-viral prophylaxis with acyclovir or, better yet, with its prodrugs with greater bioavailability, such as valacyclovir, should be continued at least 6-12 months (20).

Nevertheless, relapses may occur in 20-25% of cases, even with adequate anti-viral treatment (20). Pediatric recurrent EM not responsive to anti-viral agents may require treatment with immunosuppressant and anti-inflammatory agents, such as dapsone, intravenous immunoglobulin, and azathioprine (4). Notably, children have a lower response to immunosuppressive therapies than adults (4).

Conclusion

Pediatric VAEM has specific clinical and etiopathogenetic features that make it different from other forms of EM, especially MIRM. Besides HSV, new viruses associated with pediatric EM cases emerge, not least the novel coronavirus. Therefore, a virological screening for VAEM is advised for other pediatric virus-related exanthems. Pediatric VAEM research still awaits the development of precise biomarkers or identifying definitive genetic polymorphisms to carefully assess the individual course of the disease or possible targeted treatments. Interestingly, several previously not completely understood paediatric conditions or risk factors have been linked to biomarker researchers often based on omic technologies (21-26). Several different genes and cellular mechanisms have been identified in recent years, and this strengthened the importance of omic sciences in defining the clinical spectrum and the therapies tailored to many childhood disorders (27-31). Genes that modulate response to environmental factors and/or influence metabolic substrate or cellular molecular events have been revealed causative for previously neglected pediatric diseases (32-38).

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COVID-19 ocular manifestation in children

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has had far-reaching health repercussions. The infection can be asymptomatic, mild, or life-threatening, depending on the severity of the illness. Almost every organ in the body might be affected. Different signs of infection in the eye have been reported. The most common symptom is conjunctivitis, which can appear at any phase of the infection. Ophthalmic signs may appear immediately after COVID-19 infection or may appear several weeks later. COVID-19 in children may be substantially different from COVID-19 in adults in terms of exposure history, clinical features, and ocular manifestations. COVID-19-related ocular abnormalities in children are generally mild, children recover rapidly, and these disorders are not connected with any long-term problems. Children represent 1% of COVID-19 cases in most published datasets, with a much lower risk of developing severe disease or mortality. Because most children present with mild forms of acute respiratory infections, researchers initially focused on children with underlying/comorbid conditions and rare fatalities. The most prevalent clinical signs in children with active COVID-19 are fever and cough, accompanied by lethargy, myalgia, rhinorrhoea, sneezing, sore throat, headache, disorientation, diarrhoea, vomiting, and stomach pain. The majority of children infected with COVID19 had a family member who had the disease and developed symptoms before the child. As a result, we gathered information on COVID-19 ocular symptoms, focusing on those that had recently been published (1).

COVID-19 Ocular Manifestation

COVID-19 ophthalmic involvement has been observed in several individual case reports and small-scale research.

A. Ocular Surface and Conjunctiva Manifestations

The most prevalent ocular manifestation in COVID-19 patients is conjunctivitis. During the active phase of the illness, COVID-19 might produce severe inflammation and cytokine storm.

Keywords: Covid-19, SARS-CoV-2, eye

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0393-974X (2022)

It is uncertain whether conjunctivitis is caused by the coronavirus or is a symptom of vasculitis. Conjunctivitis was described as one of the Kawasaki-like symptoms in two out of six children with MIS-C in a study conducted at the Children's Hospital of Philadelphia. On presentation, one of them exhibited conjunctivitis and changed mental status and irritation. On the fifth day of his stay in the hospital, another child acquired conjunctivitis (2). Rauf et al. presented a case of a 5-year-old boy with a known COVID infection, which manifested with multi-organ failure and non-purulent bulbar conjunctivitis in one hotspot in Kerala, India. The youngster also had an elevated erythrocyte sedimentation rate (ESR) and myocarditis, all of which pointed to an unusual Kawasaki disease presentation (3). Irritability and limbic sparing conjunctivitis were seen in a COVID-positive 6-month-old full-term infant who was fully immunized and previously healthy (4). Blondiaux et al. found two children with conjunctivitis in a group of four COVID-positive children aged 6- to 12-years-old (5). Riphagen et al. published a case series of eight COVID-positive children ranging in age from 4- to 14-years-old; 5 had conjunctivitis at the time of presentation (6). In a cross-sectional investigation of 17 COVID-positive children aged 1- to 16-years-old with PIMS in New York, Cheung et al. discovered that 11 had conjunctivitis that was evident at the start of the disease course (7). In a smaller study, Heidemann et al. reported 3 individual case reports of children aged 5- to 7-years-old hospitalized with PIMS and COVID. Three to four days after admission, two patients developed conjunctivitis (8). Godfred-Cato et al. recruited 570 children between the ages of 2 weeks and 20 years old in a large-scale analysis of COVID-related MIS-C patients in the United States. Two hundred twenty-six patients had conjunctival hyperemia as a symptom and were treated with a combination of aspirin, IVIG, and/or steroids in the majority of cases (9).

Finally, Ma N et al., in a cross-sectional study conducted in Wuhan (China), discovered that conjunctival discharge, congestion, and eye rubbing were present in 49 of 216 pediatric COVID-19 patients (ages of 2 and 11 years old) hospitalized between January 26 and March 18. Those with systemic coronavirus symptoms or cough were more likely to develop ocular symptoms, which improved or disappeared after using simply eye drops or no treatment at all. Conjunctival discharge, eye rubbing and conjunctival congestion were the most prevalent ocular signs and symptoms (10).

B. COVID-19 Neuro-Ophthalmic Manifestations

In the United States, a 6-year-old male with agammaglobulinemia and hyper IgM syndrome, asthma, and obstructive sleep apnea was diagnosed with right-sided facial nerve palsy. RT-PCR detected SARS-CoV-2, but either HSV or VZV; this is the first example of Bell's palsy in a child who has been infected with SARS-CoV-2 (11).

C. COVID-19 Orbital Manifestations

Dacryoadenitis is a viral infection and is the most common cause of a painful lacrimal gland mass. In a healthy 10-year-old girl, acute dacryoadenitis was identified as a late consequence of the SARS-CoV-2. The patient had previously had contact with COVID-19-infected individuals, and his IgM and IgG antibody tests were positive. Another testing for autoimmune disorders and infectious diseases came back negative. The virus can enter the lacrimal gland via the lacrimal ductules or by direct hematogenous dissemination in the early stages of the disease. Later, the virus-induced immune response may affect the lacrimal gland, causing inflammation. Systemic steroids work well for acute dacryoadenitis (12).

Another case report described a confirmed positive COVID-19 in two adolescent boys (12-year-old) who suffered abrupt onset unilateral, progressive, painful orbital oedema. In addition, COVID-19-induced upper respiratory congestion is thought to impede mucociliary clearance, resulting in subsequent sinus blockage and bacterial infection. However, the course of the disease in children is relatively moderate, with most asymptomatic or minimal symptoms (13-14).

D. COVID-19 Retinal findings

The non-invasive imaging method optical coherence tomography (OCT) is utilized to detect retinal alterations during systemic disease. Retinal findings have been discovered on OCT and retinal

fundus imaging in COVID-positive people who often have conjunctivitis and ocular surface disease. A case of central retinal vein occlusion in a 17-year-old female with confirmed COVID-19 infection was presented by Walinjkar et al. (15). It is impossible to link the progression of COVID-19 disease to its severity. High-dose steroids may help normalize inflammatory indicators and coagulation indices in the early stages. An anti-vascular endothelial growth factor (anti-VEGF) is used in the established phase to treat the condition. There was no previous history of thrombotic events or anticoagulant use for this patient.

Early anticoagulant prophylaxis should be recommended in patients with systemic comorbidities and severe COVID-19 infection. COVID19 may predispose patients to be arterial and venous thrombosis.

Symptoms of vitreoretinal inflammation, such as vasculitis or vitritis, are common in viral retinitis. In an 8-year-old kid who had previously tested positive for COVID-19, a splinter-retinal haemorrhage encircling the optic nerve, as well as vitritis-like hyperreflective patches, were observed. Nonpurulent conjunctivitis, several vitritis-like hyperreflective patches, and moreover conjunctival hyperemia with a periorbital rash COVID-19 positive result were found in the posterior vitreous of both eyes in a 10-year-old girl. (16)

CONCLUSION

The likelihood of unusual viral presentations in pediatric patients is highlighted in our case study.

The symptoms of the SARS-CoV2 virus in children are largely unknown. Respiratory sickness, leukopenia, thrombocytopenia, myocarditis, interstitial pneumonia, Kawasaki disease, and vasculitis have been observed in juvenile patients. Although more research is needed to thoroughly understand the significance of SARs-CoV2 infection in patients during the current coronavirus disease 2019 pandemic, we propose that practitioners consider it as a possible atypical presenting symptom of infection.

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Clinical manifestation of Congenital Ocular Toxoplasmosis

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To the Editor,

Toxoplasmosis, a prenatal infection caused by the parasite Toxoplasma gondii, is a leading cause of morbidity and mortality in children (1). It is found worldwide; however, its incidence varies by place, depending on climate and population behaviours. Toxoplasmosis is transmitted vertically in pregnant women during primary infection, and maternal sickness is usually unrecognized. During pregnancy, congenital toxoplasmosis is passed from the mother to the fetus through the placenta. If the mother was infected during pregnancy or immunosuppression reactivates a prior infection, toxoplasmosis can be transmitted transplacentally. In immunocompetent women who have been infected with Toxoplasma and gained immunity prior to pregnancy, the transmission of Toxoplasma to a fetus is extremely rare. Even if the mother shows no signs or symptoms of illness, this can happen. When pregnant women become sick in the first trimester, the likelihood of fetal infection rises to 15–20 %, 25 % in the second trimester, and 65–70 % in the third trimester. The fetuses who are infected sooner are the most vulnerable. Newborns with congenital toxoplasmosis frequently show no indications of the systemic disease at birth, but a more thorough examination may reveal severe ocular and neurological abnormalities (2). Even patients with a subclinical infection are at risk for long-term complications, such as retinochoroiditis and neurological problems. The most common signs of congenital toxoplasmosis are ocular abnormalities. The presenting sign of ocular toxoplasmosis is hazy vision, linked with active lesions and symptomatic vitreous inflammation. Scotomas are proportional to the size and location of retinochoroidal scars during the parasite's dormant stage. The classic ocular presentation of toxoplasmosis is a nidus of fluffy white, focal necrotizing retinitis or retinochoroiditis close to a variably pigmented chorioretinal scar. Severe vitritis often obscures the active lesion. The severity of anterior uveitis can range from a mild inflammatory response to a full-fledged inflammatory response, obscuring posterior segment involvement. The inflammation in the anterior uveitis can be granulomatous or non granulomatous (3). Congenital cases can also include strabismus, microphthalmia, cataracts, optic atrophy, and nystagmus. Multifocal retinochoroiditis, low-grade or absent vitreal infiltration, an active lesion larger than 2 disk diameters without an associated retinochoroidal scar, absence of a retinochoroidal

Keywords: toxoplasmosis, children, eye

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0393-974X (2022)

scar, bilaterality, optic disk involvement, choroiditis without retinitis, hemorrhagic vasculitis, serous retinal detachment, and retinal neovascularization are some of the more unusual findings. These lesions may be present at birth or develop later in life. It is important to note that ocular involvement is significant because it frequently affects the macular region. Prenatal and neonatal treatment continued during the first year of life has been linked to a considerable decrease in the disease's prevalence and severity. However, there is now a heated debate concerning the benefits of treating congenital toxoplasmosis; therefore, it is crucial to understand the disease's natural history (4). In conclusion, the exact time of toxoplasma infection that leads to eye illness is unknown. However, current evidence suggests that postnatal toxoplasmosis infect more people than prenatal toxoplasmosis; this has far-reaching ramifications for public health. Knowing the relative contribution and severity of ocular toxoplasmosis infection in children might help with counselling and public debate about the need for child screening programs; screening and health information focus on reducing the risks of toxoplasmosis due to prenatally acquired infection, primarily to lower the risk of ocular morbidity in the long run.

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In healthy children, *Herpes zoster ophthalmicus* has a good prognosis

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Herpes zoster ophthalmicus (HZO) is an infection of the fifth cranial nerve's ophthalmic division caused by the *Herpes zoster* virus. It is more common in older people and is rarely observed in children. Autoimmune disease, HIV infection, and cancer are the most commonly documented systemic diseases related to zoster virus infection in children. Other risk factors include intrauterine varicella infection or exposure during the first year of life, which can also happen to otherwise healthy people. Oral or intravenous antivirals and topical steroids for ocular symptoms are used in the same way for *Herpes zoster* in adults. Thankfully, postherpetic neuralgia is not as frequent in children as in adults. However, co-management with a paediatrician is critical to rule out underlying systemic reasons. Though *Herpes zoster* in children is uncommon, it does happen and can cause ocular issues. In healthy children, HZO appears to have a benign course. However, it is linked to severe problems, including ocular problems and subsequent vision loss. Hutchinson's sign and anterior uveitis were discovered to be predictors of vision loss and necessitated strict monitoring.

Furthermore, in children, long-term corneal hypoesthesia, implying irreversible nerve and ganglion damage, can be one of the consequences of HZO. Reduced corneal sensitivity is crucial for the long-term ocular care of these children since it is a risk factor for problems in ocular surgeries and contact lens usage. Examples of ocular involvement are acute hemiplegia, sclerokeratitis with anterior uveitis, central retinal artery blockage, and optic atrophy. Glaucoma secondary to uveitis in *Herpes zoster* ophthalmicus has also been reported. Meningoencephalitis, ocular neuritis, a sixth nerve palsy, and stroke have all been documented as probable HZO consequences in the literature. A stroke is more likely to occur if zoster is present in the eye. Vaccination against VZV effectively lowers complications, hospital admissions, and the disease's socioeconomic effects. *Herpes zoster* appears to be less common in children who have had varicella vaccination than in paediatric patients who have had varicella infection (1.3 per 100 000 person-years vs 20 per 100 000 person-years) (1-4).

Antiviral drugs are safe, even when used at high dosages. Children's studies, on the other hand, are scarce. In addition, the acyclovir derivatives valacyclovir and famciclovir are not approved for use in children.

Keywords: Herpes zoster, children, infection, eye

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0393-974X (2022)

CONCLUSION

HZO is uncommon in children, with most cases linked to immunosuppression or varicella infection contracted during pregnancy or the first year of life. HZO, on the other hand, can develop in otherwise healthy children; this emphasizes the relevance of PCR in confirming the diagnosis of HZO in children early in the clinical phase, allowing treatment with acyclovir to begin immediately to avoid any sight-threatening complications.

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