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Official Journal of the Italian Society of Otorhinolaryngology Head and Neck Surgery

Organo Ufficiale della Società Italiana di Otorinolaringologia e Chirurgia Cervico-Facciale

105th Congress of the Italian Society of Otorhinolaryngology Head and Neck Surgery

Official report

Emerging and re-emerging infectious disease in otorhinolaryngology

Patologia infettiva emergente e riemergente in otorinolaringoiatria

F. Scasso, G. Ferrari, G.C. De Vincentiis, A. Arosio, S. Bottero, M. Carretti, A. Ciardo, S. Cocuzza, A. Colombo, B. Conti, A. Cordone, M. De Ciccio, E. Delehaye, L. Della Vecchia, I. De Macina, C. Dentone, P. Di Mauro, R. Dorati, R. Fazio, A. Ferrari, G. Ferrea, S. Giannantonio, I. Genta, M. Giuliani, D. Lucidi, L. Maiolino, G. Marini, P. Marsella, D. Meucci, T. Modena, B. Montemurri, A. Odone, S. Palma, M.L. Panatta, M. Piemonte, P. Pisani, S. Pisani, L. Prioglio, A. Scorpecci, L. Scotto di Santillo, A. Serra, C. Signorelli, E. Sitzia, M.L. Tropiano, M. Trozzi, F.M. Tucci, L. Vezzosi, B. Viaggi

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F. SCASSO[1], G. FERRARI[2], G.C. DE VINCENTIIS[14], A. AROSIO[3], S. BOTTERO[16], M. CARRETTI[14], A. CIARDO[2], S. COCUZZA[5], A. COLOMBO[6], B. CONTI[7], A. CORDONE[1], M. DE CICCIO[2], E. DELEHAYE[2], L. DELLA VECCHIA[3], I. DE MACINA[8], C. DENTONE[8], P. DI MAURO[5], R. DORATI[7], R. FAZIO[2], A. FERRARI[13], G. FERREA[8], S. GIANNANTONIO[15], I. GENTA[7], M. GIULIANI[14], D. LUCIDI[15], L. MAIOLINO[5], G. MARINI[14], P. MARSELLA[15], D. MEUCCI[16], T. MODENA[7], B. MONTEMURRI[15], A. ODONE[10], S. PALMA[12], M.L. PANATTA[14], M. PIEMONTE[12], P. PISANI[6], S. PISANI[7], L. PRIOGLIO[1], A. SCORPECCI[15], L. SCOTTO DI SANTILLO[1], A. SERRA[5], C. SIGNORELLI[9 10], E. SITZIA[14], M.L. TROPIANO[16], M. TROZZI[16], F.M. TUCCI[17], L. VEZZOSI[9 11], B. VIAGGI[4]

[1] SOC Otorinolaringoiatria, ASL 3 Genovese, Ospedale P.A. Micone, Genova, Italy; [2] SOC Otorinolaringoiatria, ASL 5 Genovese, Ospedale P.A. Levante Ligure, La Spezia, Italy; [3] Clinica Otorinolaringoiatria, Ospedale Macchi, ASST Settelaghi, Varese, Italy; [4] SOC Neuroanestesia e Rianimazione, A.O.U. Careggi, Firenze, Italy; [5] Clinica di Otorinolaringoiatria, Università degli Studi di Catania, Catania, Italy; [6] SOC Otorinolaringoiatria, Ospedale Cardinal Massaia, Asti, Italy; [7] Dipartimento di Scienze del Farmaco, Università degli Studi di Pavia, Pavia, Italy; [8] SOC Malattie Infettive, ASL 1 Imperiese, Ospedale di Sanremo, Italy; [9] Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Italy; [10] Facoltà di Medicina e Chirurgia, Università Vita-Salute San Raffaele, Milano, Italy; [11] Dipartimento di Medicina Sperimentale, Università degli Studi della Campania Luigi Vanvitelli, Napoli, Italy; [12] SOC Otorinolaringoiatria, Azienda Sanitaria Universitaria di Udine (ASUIUD), Italy; [13] Direzione Sanitaria, AOU Parma, Italy; [14] UOC Otorinolaringoiatria, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy; [15] UOC Audiologia e Otochirurgia, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy; [16] UOC Chirurgia delle Vie Aeree, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy; [17] UOS Chirurgia Cervicale ORL, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy

SUMMARY

Emerging and re-emerging infectious disease in otorhinolaryngology (ENT) are an area of growing epidemiological and clinical interest. The aim of this section is to comprehensively report on the epidemiology of key infectious disease in otorhinolaryngology, reporting on their burden at the national and international level, expanding of the need of promoting and implementing preventive interventions, and the rationale of applying evidence-based, effective and cost- effective diagnostic, curative and preventive approaches. In particular, we focus on i) ENT viral infections (HIV, Epstein-Barr virus, Human Papilloma virus), retrieving the available evidence on their oncogenic potential; ii) typical and atypical mycobacteria infections; iii) non-specific granulomatous lymphadenopathy; iv) emerging paediatric ENT infectious diseases and the prevention of their complications; v) the growing burden of antimicrobial resistance in ENT and the strategies for its control in different clinical settings. We conclude by outlining knowledge gaps and action needed in ENT infectious diseases research and clinical practice and we make references to economic analysis in the field of ENT infectious diseases prevention and care.

KEY WORDS: HPV • EBV • HIV • Mycobacteriosis • Bacterial resistance • Nanomedicine

RIASSUNTO

La diagnosi e terapia delle infezioni in ambito ORL ha presentato nell'ultimo ventennio crescenti criticità legate ai cambiamenti demografici e alle errate abitudini terapeutiche con la comparsa di nuove infezioni e il ripresentarsi di vecchie credute scomparse. Su queste osservazioni abbiamo voluto fare il punto sulle nuove e vecchie malattie infettive prendendo in considerazione gli aspetti epidemiologici delle infezioni ORL del nuovo millennio da cui emerge l'importanza della prevenzione vaccinale. Si è focalizzata l'attenzione sulle infezioni virali, in particolare HIV EBV e HPV, in tutte le loro manifestazioni ORL con particolare riguardo alla patologia oncologica. Sono state analizzate le modalità di infezione cellulare e i punti chiave che portano alla trasformazione neoplastica. Il problema delle linfoadenopatie granulomatose aspecifiche, tubercolari tipiche e atipiche ha evidenziato l'importanza di protocolli medico-chirurgici variabili ed influenzati dai germi e dalla responsività del soggetto e in questo ambito non è emersa differenza tra popolazione adulta e pediatrica. Si è posta attenzione alle patologie infettive del'infanzia che maggiormente impegnano nella diagnosi e terapia per le possibili gravi complicanze: rinosinusiti, otomastoiditi, papillomatosi laringea. Per quanto riguarda le infezioni batteriche si rileva il problema drammatico dei germi multiresistenti: enterobacteriacee, pseudomonas auriginosa, germi gram positivi analizzando i meccanismi di resistenza; il trattamento di queste infezioni si basa sull'utilizzo mirato di nuove molecole ad alto costo ma trova la migliore chance nella loro prevenzione sia con norme igenico sanitarie che con un utilizzo razionale EBM degli antibiotici. Vengono presentate anche le nuove frontiere dell'antibioticoterapia e le tecnologie di nanomedicina ed infine i non meno importanti effetti di cost-effectiveness.

PAROLE CHIAVE: *HPV • EBV • HIV • Micobatteriosi • Resistenze batteriche • Nanomedicina*

LIST OF ACRONYMS AND ABBREVIATIONS

ACIP: Advisory Committee on Immunization Practices
Ag NPs: Silver nanoparticles
AIDS: Acquired Immune Deficiency Syndrome
AIRC: Italian Association for Cancer Research
AJCC: American Joint Committee on Cancer
AMR: Anti-Microbial Resistance
AMS: Anti-Microbial Stewardship
AOM: Acute Otitis Media
AORRP: Adult Onset Recurrent Respiratory Papillomatosis
ARS: Acute Rhinosinusitis
BAL: Bronchoalveolar lavage
BCG: Bacillus Calmette-Guérin
BKB: Benzalkoniumbromide
BLBLIs: Beta-Lactam/Beta-Lactamase Inhibitor combinations
CANS: Computerized Navigation System
CDC: Centers for Disease Control and Prevention
CF: Cystic Fibrosis
CMV: Cytomegalovirus
CNS: Central Nervous System
COPD: Chronic Obstructive Pulmonary Disease
CPE: Carbapenamase-Producing Enterobacteriacee
CRE: Carbapenem-Resistant Enterobacteriaceae
CRP: C-reactive Protein
CRS: Chronic Rhinosinusitis
CRT: Concomitant ChemoRadiotherapy
CS-PVA: Chitosan (CS)- Poly(vinil-alcool)
CT: Computed Tomography
DNA: DeoxyriboNucleicAcid
EA: Early Antigen
EAC: External Auditory Canal
EBER: Epstein-Barr virus-Encoded small RiboNucleic Acid
EBNA: Epstein-Barr Nuclear Antigen
EBV: Epstein-Barr Virus
EGFR: Epidermal Growth Factor Receptor
EMA: European Medicines Agency
ENT: Ear Nose Throat – Otorhinolaryngology
ESR: Erythrocyte Sedimentation Rate
ESBL: Extended spectrum beta-lactamase producing enterobacteriaceae
ESS: Endoscopic Sinus Surgery
FC: Cystic Fibrosis
FDA: Food and Drug Administration
FISH: Fluorescence In Situ Hybridization
FNAC: Fine Needle Aspiration Cytology
G: Graphene
GERD: GastroEsophageal Reflux Disease
GO: Graphene Oxide
HAART: Highly Active Antiretroviral Therapy
HIV: Human Immunodeficiency Virus
HL: Hodgkin Lymphoma
HNSCC: Head and Neck Squamous Cell Carcinoma
HPV: Human Papilloma Virus
HSV: Herpes Simplex Virus
hVISA: Heterogeneous Vancomycin intermediate-resistant Staphylococcus Aureus
HW: Health Workers
ID: Infectious Disease
IGRA: Interferon-Gamma Release Assays
IMRT: Intensity Modulated Radiation Therapy
IRIS: Immune Reconstitution Inflammatory Syndrome
JORRP: Juvenile Onset Recurrent Respiratory Papillomatosis
KD: Kawasaki Disease
KFD: Kukugi Fujimoto Disease
KPC: Carbapenem-resistant Klebsiella
KS: Kaposi Sarcoma
LAC: Lupus Anti-Coagulant
LCL: Lymphoblastoid Cell Line
LMP: Latent Membrane Protein
MDROs: Gram negative multi resistant germs
MIC: Minimum Inhibitory Concentration
miRNA: micro RNA
MOTT: Mycobacterium Other Than Tuberculosis
MPRV: Measles, Parotitis, Rubella and Chickenpox
MRSA: Methicillin-resistant Staphylococcus Aureus
MTBC: Mycobacterium Tuberculosis Complex
MTHFR: MethileneTetraHydroFolate Reductase
NBI: Narrow Band Imaging
NHL: Non Hodgkin Lymphoma
NK/T: Extranodal natural killer/T-cell (lymphoma)
NMR: Nuclear Magnetic Resonance
NPC: Nasopharyngeal Carcinoma
NPs: Nanoparticles
NTM: Non-Tuberculous Mycobacteria
NTs: Nanotubes
NVPP: National Vaccination Prevention Plan
OHL: Oral Hairy Leukoplakia
OKS: Oral Kaposi Sarcoma
OSCC: Oral Squamous Cell Carcinoma
OM: Otitis Media
PAS: Para-Aminosalicylic Acid
PA NPs: Phosphatidylcholine-decorated Au nanoparticles
PCR: Polymerase Chain Reaction
PDA: Polydopamine
PDS: Polydioxanone
PESS: Pediatric Endoscopic Sinus Surgery
PEG-PECA: Polyethileneglycol (PEG) – coated polyethylcyanoacrylate (PECA)
PET: Positron Emission Tomography
PET-CT: Positron Emission Tomography – Computed Tomography
PEUU: Poly(EsterUrethane) Urea
PNPV: National Vaccine Prevention Plan

PCR: Polymerase Chain Reaction
PPE: Personal Protective Equipment
pRB: retinoblastoma protein
RT-PCR: Real Time Polymerase Chain Reaction
RARS: Recurrent Acute Rhinosinusitis
RNA: RiboNucleicAcid
RRP: Recurrent Respiratory Papillomatosis
RT: Radiotherapy
SAP: Surgical Antibiotic Prophylaxis
SCC: Squamous Cell Carcinoma
SF: Regenerated Silk Fibroin
SIO: Italian Society of Otorhinolaryngology
SSIs: Surgical Site Infections
TB: Tuberculosis
TCAB1: Telomerase Cajal body protein 1
TDM: Therapeutic Drug Monitoring
TLM: Transoral Laser Microsurgery
TNM: Tumor, Nodes, Metastases Classification System
TORS: Trans Oral Robotic Surgery
TST: Tuberculin Skin Test
URI: Upper Respiratory Infection
US: Ultrasound
URT: Upper Respiratory Tract
VCA: Viral Capsid Antigen
VISA: Vancomycin intermediate-resistant Staphylococcus Aureus
WHO: World Health Organisation

1. Introduction

The Italian Society of Otorhinolaryngology (SIO) issued a comprehensive report on Infectious disease in ENT around 30 years ago.
Since then, new demographic, epidemiological and clinical trends, as well as advances in research, diagnosis and cure have emerged impacting the burden and characteristics of Ear Nose Throat (ENT) infectious diseases. These include, among others, migration, antimicrobial resistance, and accumulated evidence on the role of infections in the pathogenesis of neoplasia.
In this context, we saw the scope and rationale of carrying out a comprehensive review on emerging and re-emerging infectious disease in otorhinolaryngology, focusing on key epidemiological, clinical and preventive features and we would like to thank the SIO for sharing this interest in the subject and entrusting us with the honour and the burden of this research.

2. ENT infections in the new millennium: outline of epidemiology and prevention

Infectious diseases in the field of ENT affect various age groups and represent a major problem for public health, since they include a wide spectrum of diseases, which range from self-limiting or mild clinical pictures to severe or fatal ones, featuring complications and morbidity, which compromise fundamental physiological functions such as hearing, smell, taste, phonation and breathing and may cause permanent disabilities [1].
The high frequency of ENT infections demands investments in public health, because these diseases have an effect both on the organisation of health services, in terms of access to treatment and availability of ENT specialists, and on consumption of economic and pharmaceutical resources due in part to antibiotics [2 3].
ENT is one of the areas where use and misuse of antibiotic treatment has resulted in enormous issues relating to antibiotic resistance and increasing complexity of medical management of more resistant and more virulent pathogens [4].
Loading of infectious diseases in the head and neck area is not uniformly distributed on a world level and it is higher in low-income countries, because of scarcity of ENT specialists, higher prevalence and severity of the infections, disorders of the immune system (HIV, malnutrition) and widespread incidence of risk behaviour (cigarette smoking, consumption of betel and alcohol) [5].
In high-income countries in the last century there was an epidemiological and demographic transition involving the shift from high mortality due to infectious diseases to increased mortality through chronic degenerative disease, particularly in the ageing population, even though ENT infectious diseases still have a considerable impact.
Cases of acute infectious disease occur daily in Otorhinolaryngology: it is estimated that AOM and infections of the upper airways are among the two most common reasons for a visit to the doctor every year and that in Europe the incidence of AOM among infectious etiopathogenesis handled in ENT examination rooms amounts to 268 cases per 1000 patients/year [4].

2.1 ENT vaccine-preventable diseases and hearing impairments

It is currently estimated that 328 million adults and 32 million children (equal to approximately 5.3% of the world's population) live with an invalidating hearing loss and that approximately 15% of the world's population is affected by various degrees of hearing impairment [6].

Prevalence of people affected by hearing loss on a world level is not uniform: in high-income countries prevalence is low (0.5% in children and 4.4% in adults), while in the African and Asian Regions greater impact may be found (2.4% in children in Southern Asia and 8.8% of adults in Central Asia) [7].
The WHO includes among the principal causes of deafness chronic ear infections, rubella, measles, parotitis and meningitis and estimates that up to 60% of hearing impairment in infancy could be avoided by paying specific attention to issues linked to childbirth (17%), use of ototoxic drugs (4%) or to other causes (8%) and by means of prevention of the above-mentioned infections (31%) [8].

Measles, Parotitis, Rubella and Chickenpox (MPRV)
The rubella virus is an RNA virus of the Togaviridae family, which can cause prenatal or congenital deafness through vertical transmission of the virus from mother to foetus. Foetal infection in the first three months, especially in the first 8-10 weeks, carries an extremely high risk of causing malformations and hypoacusia, but this risk still exists when the infection is contracted in the second or third trimester [9].
Deafness as a result of rubeolic infection is generally sensorial and bilateral, can already be seen at birth, but may be progressive and may appear in later stages.
Despite the existence of a world-wide plan for elimination of congenital rubella, according to the WHO approximately 100,000 babies per year are born with congenital rubella syndrome, mainly in Africa and South East Asia [10].
In Italy there are still issues linked to congenital rubella: from supervisory data of the years 2005-2013 of congenital rubella infections 160 rubella infections appeared during pregnancy and 75 cases of congenital infection, with an average national annual incidence of 1.5/100,000 live births; two peaks were also recorded, in 2008 and in 2012 (with average annual incidence of 5.0 and 3.6/100,000 live births) [11].
Measles, parotitis and varicella, unlike rubella, are mainly a cause of post-natal or acquired deafness, by causing damage to the cranial nerve VIII and/or cochlear system [9].
The measles virus is an extremely contagious paramyxovirus, which causes permanent bilateral deafness simultaneously with cutaneous rash in 1/1000 cases, because of infection in the inner ear that leads to destruction of the cochlea and the nerve fibres. Parotitis is also caused by a paramyxovirus, which in 5/10,000 cases causes an infection which, through damage to the cochlear ducts and degeneration of the organ of the Corti and the cochlea, leads to deep, permanent hypoacusia, usually unilateral, associated with aseptic meningitis, sometimes coupled with tinnitus, ataxia and vomiting. Varicella zoster is a herpes virus which, in its acute stage, causes severe bilateral deafness by destroying the nerve and sensory cells of the neuroepithelium by means of a neuro labyrinthitis [9].

Meningitis
Bacterial meningitis in paediatric age is one of the most common causes of acquired deafness: it is estimated that after a meningitis from Streptococcus pneumoniae and from Neisseria meningitidis there is a risk of hypoacusia of 22% and of 8% respectively [12].
Haemophilus influenzae type b can also cause severe bacterial meningitis in children, which in 3-6% of cases proves fatal and in approximately 20% of cases causes neurological complications and permanent deafness [13].
Cases of bacterial meningitis aggravated by neurological complications and deafness are also found in adults: in high-income countries annual incidence of bacterial meningitis is 0.8-2.6/100,000 adults, while in low-income countries it may be up to 10 times higher [14].
In Europe and the United States, the majority of bacterial meningitis in adults is caused by Streptococcus pneumoniae (70-80%) and by Neisseria meningitidis (10-20%) [15].

Otitis media and chronic infection of the ear
Otitis Media is one of the most common infectious etiopathogenesis in the world: approximately 80% of children under the age of three years has an acute episode of OM and 40% are affected by repeated episodes [16]. OM can be subdivided clinically into subcategories: AOM, recurrent AOM, secretory OM and chronic suppurative OM can be caused by either virus or bacteria. The micro-organisms mainly involved are Streptococcus pneumoniae, nontypeable Haemofilus influenzae, Staphylococcus aureus, Moraxella catarrhalis and Streptococcus pyogenes [3].
OM is a problem for public health because it is one of the most common diseases in infancy, causing approximately 51,000 deaths per year in children under 5 years of age [17]; it represents one of the major causes of morbidity and deafness (it is the third most important cause of hearing loss on a world-wide level, with a prevalence of 30.82 out of 10,000 subjects) [16]; it frequently requires surgical treatment and it is the most frequent cause of prescriptions of antibiotics [3].
Chronic OM is responsible for a number of extracranial complications (facial paralysis, subperiosteal abscess, labyrinthine fistula etc.) and causes important sequelae (paralysis of the facial nerve, profound hearing loss) [5].
In spite of the availability of antibiotics, bacterial meningitis secondary to acute and chronic OM continues to be

an important cause of morbidity and mortality [16 18]; it is one of the reasons why Healthcare professionals, including Otorhinolaryngologists, should promote antipneumococcic vaccination in children and in adults over 65.

2.2 The increasing role of manifestations in the head and neck area of infections in immunodepressed subjects

Immunodepression exposes patients to increased risks of infections and mortality from cancers [19], sometimes correlated with the action of oncogenic viruses (EBV, HPV) [20-22].
Immune system depression currently represents the fourth cause of disability on a world-wide level, but there is a continuous increase in subjects facing immunodepression, as certified by the WHO estimates, according to which immunodepression could become the second cause of disability in the world by 2030 [19]. The causes of immunodepression are mostly of a secondary nature, caused by increased incidence and prevalence of sufferers from AIDS, of patients who have to face long periods of treatment with immunomodulatory drugs, of patients with immune system imbalance linked to neoplasias and its treatment by chemotherapy and radiotherapy and of patients with post-transplant induced immunodepression [23].
In these subjects infections frequently appear, caused by opportunist diseases, which in immunocompetent subjects would be normally controlled by the numerous barriers and by the immune system, but which in subjects with immunodeficiency cause infections and sometimes aggravation of the clinical picture which can also prove to be lethal [23].
The ENT area not only constitutes, with its own epithelia and anatomically, the first barrier against infections, but it is also rich in tissue involved in immune reactions, owing to the numerous lymphatic stations (the complex lymph node chain in the neck and the lymphoid tissue of the tonsils and adenoids are only the best known examples).
Therefore it becomes important to consider the infections from diseases that were until a few years ago considered infrequent in the ENT area but which today appear in new and more serious ways.

Cytomegalovirus (CMV)

Infection from CMV is quite common. This virus is a member of the Herpesviridae family, which is transmitted through contact with bodily secretions and by sexual means and affects 60-90% of the population, causing clinical pictures that can range from absence of symptomatology in a slightly feverish and fatigued mononucleosis-like form down to serious clinical pictures and symptomatologies that can involve the CNS, the salivary glands, the lymph node stations and other internal organs [24].
CMV usually causes symptoms immediately after the appearance of the infection and remains quiescent (inactive) in various tissues for the whole lifetime [25].
However, various stimuli can reactivate the quiescent CMV and cause the disease: especially in immunodepressed subjects the disease is generally due to viral reactivation.
The majority of subjects are asymptomatic, while others show illness and develop high temperatures; on the contrary, subjects with a compromised or immature immune system, as in the case of a foetus, may develop serious symptoms, including blindness and deafness [24].
Congenital CMV (maternal-foetal transmission) can lead to a highly complex symptomatology, including important loss of hearing, included as first non-genetic cause of loss of hearing during pregnancy [26]. Typical signs in the immunodepressed patients who undergo reactivation of the latent virus are antibiotic-resistant sinusitis, with exacerbations of local invasiveness and the classic picture of serious CMV lymphadenopathy.
CMV lymphadenopathy normally has a benign course and does not require treatment in immunocompetent subjects, while in the immunodepressed it may involve severe cases with variable prognoses. Specific complications of these forms of CMV lymphadenopathy involving the numerous lymph node stations of the various cervical chains include: meningitis and encephalitis, pneumonia, hepatitis and enteropathies [27].

Toxoplasma gondii

Toxoplasma gondii, an obligate intracellular parasite, is the agent for toxoplasmosis, which is a widespread disease in most of the world; the definitive hosts of the protozoi are felines (including cats), while other mammals (including humans) are intermediate hosts [28].
Humans can be infected by transplacental transmission, by ingestion of cysts or by organ transplants, while direct human-human transmission has not been demonstrated. Rigorous detection in the USA [29] has shown that 11% of the population between the ages of 6 and 9 years is positive for Toxoplasmosis, which may be present in the subject in the form of tachyzoites, bradyzoites (tissue cysts) or oocysts. The manner in which the toxoplasmosis manifests itself depends on the condition of the immune system of the patient. In both children and adults who are immunocompetent the infection is asymptomatic. Considering the importance in the discipline of otorhinolaryngology of the diagnostic course of lateral-cervical and occipital masses/nodules and taking account of the basic diagnostic principle according to which masses on the neck may be correlated with situations of inflammation, either congenital or

neoplastic, a major review of the literature [30] in 2015 concluded that a diagnosis of toxoplasmosis is rarely taken into consideration by doctors, even when a patient shows an isolated cervical or occipital lymphadenopathy. Lymphadenitis from toxoplasmosis is a self-limited disease in an immunocompetent subject, with resolution in 1 or 2 months at most, without antibiotic treatment [31]. It is extremely important, however, to make a specific diagnosis of this infection in immune compromised patients, given that it may have severe developments or even lead to fatal outcomes [32].

In these cases, therefore, it is essential to apply specific treatment protocols with high dosage and long term treatment. The otorhinolaryngologist must therefore take this diagnostic assumption into consideration and absolutely must not underestimate it, given that this specialist is generally the first one to assess the so-called "lateral cervical lump".

Lymph node located tuberculosis (TB)

Possible location of TB in the head and neck area represents a diagnostic challenge for otorhinolaryngologists on a worldwide level, and not only in areas of high/very high prevalence of TB, with one of the reasons being high risk of misdiagnosis with a carcinoma [33].

To get a better understanding of manifestations of TB at the cervical level, scientific literature in recent years has focused on accurate analysis and assessment of its various clinical manifestations, methods of presentation, improved and more appropriate diagnostic techniques, comorbidity and treatment protocols. In this respect a recent retrospective study [34] concludes that the majority of patients with head/neck located TB in most of the cases show signs and symptoms of lymphadenitis (86.53%), followed by manifestations determined by laryngeal, adenotonsillar, deep spaces of the neck and the salivary glands localisation. Needle aspiration biopsy is recognised as the gold-standard procedure for diagnosis of tubercular lymphadenitis, together with serology (which may, however, occasionally show aspecific profiles) [27 35]. If the outcome is positive, even if the course is occasionally improved, it is absolutely necessary to assess possible coinfection from HIV (26% of patients) and any possible association with tubercular lesions (cavernous or not) at the level of lung parenchyma (16.3% of patients).

It is therefore important to keep this diagnostic hypothesis firmly in mind, when faced by pictures of lateral cervical or subepithelial nodules. As has been widely foreseen by numerous Epidemiologists since the early 2000s, so-called "Head&Neck TB" is no longer an infrequent case in diagnostic analysis of lateral cervical tumefaction and lymphadenitis. That was valid once, especially in the areas that classically had greater prevalence of TB and TB-HIV coinfection, but even today, in the light of epidemiological and demographic changes, this diagnostic hypothesis must always be taken into consideration facing cervical lymphadenitis.

Leishmaniasis

Leishmaniasis is a parasitic infectious disease that is endemic in more than 90 countries in the world, with an incidence in the global population that the CDC estimates to range from 700,000 to 1,200,000 subjects infected per year [36]. The pathogens involved may be at least 20 species of protozoan parasites. These parasites are transmitted to humans through the bite of females of infected phlebotomus (vector). The sand fly (pappataci) or phlebotomus is actually a small insect, similar to a mosquito, but three times smaller (2-4 mm), which lives mainly in hilly areas, even though its presence has been registered in recent years in the large plains of Italy [37]. An infected female, when it bites to feed on blood, can transmit Leishmania Infantum, the parasitic protozoan that is responsible, in Italy, for human visceral and cutaneous leishmaniasis and canine leishmaniasis. Transmission never occurs through direct contact person to person or dog to dog, nor even through contact of a person with an infected dog [36]. The majority of infected people will never develop any symptomatology, while, on the contrary, in patients with various degrees and causes of reduced immunocompetence the following pictures may appear: classic manifestations of localized cutaneous leishmaniasis, or diffuse cutaneous leishmaniasis, or more invasive manifestations ranging from mucocutaneous leishmaniasis up to severe visceral leishmaniasis also known as Kala Azar. There is an intermediate form of manifestation, which appears with large lymph node tumefactions, frequent at the level of the lateral cervical chains, which, in the history of the disease, may represent a sign of reactivity in the immunocompetent person or the stage preceding diffusion and mucous-visceral invasion. More than 90% of new cases reported by the WHO in 2014 occurred in 8 countries: Brazil, Ethiopia, India, Somalia, South Sudan, Sudan, Algeria and Colombia. Epidemic forms of visceral leishmaniasis in Eastern Africa (Ethiopia, Kenya, South Sudan and Sudan) caused increased mortality and morbidity in 2014 and 2015 in the communities affected. Recently, however, bigger epidemics of cutaneous leishmaniasis have affected various areas of Afghanistan and Syria [38]. It should not be forgotten, though, that the WHO, in the year 2006, recorded the highest frequency of severe visceral leishmaniasis with 9000 cases/year in Southeast Asia. In epidemic areas le-

sions are frequently found involving the head and neck area at a pediatric age. An important study on the population of Lebanon (an endemic area) showed that 44% of patients affected by leishmaniasis bore disease lesions at the head and neck level only, while 13% bore them in association with lesions in other sites [39]. These lesions are considered almost totally as cutaneous or muco-cutaneous lesions, involving the exposed zones of the face and therefore potentially within reach of the vector phlebotomes. It should also be borne in mind that, if the initial lesion located in the head/neck is of large dimensions, verrucous or ulcerated appearance, the progression to widespread cutaneous leishmaniasis, or even to profound leishmaniasis, is considered to have higher probability. Italy is not an endemic area, but sporadic cases may appear and they are undergoing a slight increase. As a consequence of this epidemiological picture and of the highly unstable geopolitical situation in the high endemic areas (Lebanon, Libya, Syria, Turkey, Iran), which has lead to massive migratory movements and spreading of the vector (phlebotomus) in recent years, including in our areas, health operators have to take into consideration diagnostic hypotheses attributable to Leishmania, in the face of potential pathognomonic pictures that must therefore be acknowledged. Last but not least we must consider the severe impact that this infection can have on immunodepressed subjects, where possible diffusion at the mucous visceral level may even cause profound ulcers with potentially lethal damage to internal organs. These conclusions have been confirmed by a number of studies [40], generally carried out on populations resident in endemic areas or originating from such areas.

Severe forms of Pseudomonas infections

Infections from Pseudomonas aeruginosa, a Gram negative bacteria which is widespread in the environment, are typical in widely known disease pictures familiar to the ENT specialist. These infections are particularly frequent in hospital environments [41], where the bacterium can play the role of transmissible or opportunist infectious disease, also affecting the tissues of the hearing organ.

Pseudomonas infections have undergone a considerable percentage increase in severe forms, both in terms of initial severity of the clinical picture and of development of antibiotic resistance. In the area of the ear, Pseudomonas causes various types of otitis: OM, chronic purulent OM, otitis externa and malignant otitis externa.

Also, because of the increase in immune deficiency and the number of fragile and polypathological patients, there are increasingly frequent complex cases of malignant otitis resistant to the classic antibiotic treatment protocols. In effect, more than 95% of cases of malignant otitis externa caused by Pseudomonas are found in patients affected by AIDS or by advanced forms of diabetes with a complex pathological picture. It is in these very cases that we have the highest frequency of serious and severe progression in osteomyelitis of the cranial base and the temporomandibular joint.

HIV correlated manifestations

The incidence and prevalence of HIV infection as well as of the AIDS correlated syndrome is increasing in some Regions [42]. It therefore becomes important to consider also those pathological pictures regarding the head and neck area, both as specific and non-specific manifestations [43]. Consideration must definitely be shown for possible locations in the cervical area of the KS or the Non Hodgkin Lymphoma with extranodal location (oral cavity and maxillary sinus). Opportunist infections, however, are typical in this area and are prone to frequent complications and the cause of particularly difficult cases to treat, for example manifestations of relapsing laryngitis and pharyngitis and with poor response to treatment for Pseudomonas aeruginosa, or the other opportunist infections from viruses or protozoa analysed above or even severe infectious pictures from EBV or CMV. In adults with full-blown AIDS, however, the more frequent infectious head/neck diseases are oropharyngeal candidiasis, chronic rhinosinusitis and cervical lymphadenopathies. The literature includes citations, although less frequent, of other HIV correlated diseases: recurrent OM, vestibular deficiency, hyposmia and hypogeusia, lymphoepithelial parotid cysts, tuberculous retropharyngeal abscesses, non-malignant nasopharyngeal lymph node hypertrophy. Lastly, it is important to remember that some studies have demonstrated a very strong correlation between HIV, HPV and tumours, not only in the uterine cervix and anorectal tract, but also in the head/neck area, typified by greater severity of the lesions and a stronger aggressiveness [44].

2.3 Oncogenic potential of ENT infections

Starting from the assumption that 5% of deaths in the world from malignant tumours have a lesion seated in the head/neck area as their primary cause [45], it is highly relevant to consider the role of infectious factors among the etiopathogenetic factors. The literature definitely confirms that consumption of alcohol and tobacco should be considered principal causes of the onset of at least 75% of malignant neoplasms of the oral cavity, oropharynx, hypopharynx and larynx, but there is agreement within the scientific community that in the remaining 25% of cases

the largest share, even though this is still in the stage of quantifiable definition, is directly correlated with oncogenic consequences of two essential viral infections: HPV and EBV [46].

Human Papillomavirus (HPV)

Definitely most of the studies focused into the oncogenic potential of HPV, a double-stranded virus of DNA. Infections with genotypes that are more often associated with oncological disease are linked to HPV 16 and 18, are widespread all over the world and their causal correlation with squamous cell carcinomas in various areas has been widely demonstrated [47]. HPV 16 and, although with less correlation strength, HPV 18 are among the most important genotypes with oncogenic effects causing neoplastic diseases located prevalently in the oropharynx, which affect specifically the tonsillar epithelium and the base of the tongue [48]. These two genotypes have specific tropism for the basal cells of the stratified epithelia [49] and, as a further confirmation of this, the AIRC [50] officially included HPV-16 in 2007 as an oncogenic factor involved in these tumours [51].

It is interesting to note that the literature over the last 10 years has brought to light a trend of increased incidence of HPV correlated carcinomas of the oropharynx, while the share of incidence caused by other etiopathogenetic factors and risk factors are clearly decreasing [48 52]. This was also confirmed by a rigorous systematic review of 2017, which concluded that global spread of HPV infections is dramatically changing the epidemiological scenario of carcinomas in the head and neck area [53]. This epidemiological situation constitutes an enormous prevention challenge, but this aspect will be better analysed in the paragraph on "Prevention", with analysis of the transmission methods and existence of instruments of prevention [54 55].

Epstein-Barr Virus (EBV)

EBV is a Herpes virus, being a member of the numerous family of the DNA Herpesviridae. This virus is encountered by 90% of the world's adult population [46] and has been shown today to be associated with various malignant diseases. In the head and neck area it can remain in latent form inside the epithelial cells for several years, but its potential reactivation can give a strong impetus and contribution to the development, growth and acquisition of invasiveness of certain specific diseases, particularly nasopharyngeal carcinoma. An important recent study, pursuing a line of research that has been well developed over this decade [56], has provided the first evidence of the molecular pathogenetic mechanism with which EBV causes the typical beginning of induced oncogenic cell division in nasopharyngeal carcinomas (through overexpression of TCAB1) [57]. In addition to this, there is a role of EBV in the genesis of malignant tumours in the salivary glands [58]. An epidemiological element that stands out is its greater incidence in Asia, compared to Europe and America. In this respect, an important review in 2017 attempted to assess incidence of malignant neoplasia in the salivary glands with correlations between types of neoplasia and geographical area. This study [59] reported that the incidence of tumours in salivary glands were EBV correlated in 45.1% of cases occurring in patients in the world, but with the following geographical distribution: 44.2% of all malignant tumours in the salivary glands of American patients, 70% of Asian patients and 11.8% in Europeans. The multicultural nature of society, as well as globalisation related to transcontinental transfer of people, are aspects that give reason to reflect on the importance of the etiological hypothesis of EBV infection also on our continent.

2.4 ENT nosocomial infections and antibiotic resistance

Nosocomial infections, also known as "infections acquired in hospital and/or during procedures of assistance", are infections acquired during hospital stays, but which were not present even at an incubation stage at the time of admission. Infections that show up more than 48 hours after hospital admission are usually considered nosocomial infections. Antibiotic resistance, on the other hand, means the phenomenon deriving from various biological and biochemical mechanisms causing, in the last instance, reduction in effectiveness of anti-microbial drugs, with the emergence and spread of diseases resistant to the most effective, safest drugs. The emergence of resistant strains is, of course, a natural phenomenon, but it is exacerbated by inappropriate medical and veterinary use of antibiotics. The increase of microbial resistance has become a well known and widespread issue of worldwide public health [60 61]. These two phenomena, antibiotic resistance and their inappropriate use, are dramatically linked inasmuch as they do not just overlap clinically in hospital patients (and therefore in a certain segment of debilitated or immune suppressed subjects), but they are causally linked to each other. Best use of antibiotics (an alternative way of defining appropriateness in prescribing them) and best practices of hygiene and prophylaxis on premises and in procedures of medical assistance should be the two keys to successfully curbing these rampant phenomena. The Otorhinolaryngologist has to prescribe antibiotics daily for treatment purposes, manage perioperative antibiotic prophylaxis and carry out surgical operations in conditions that are frequently contaminated, if not occa-

sionally dirty-contaminated. For head and neck surgery the scientific literature has focused for a long time on best practice evidences, given both the typical features of the operating field and the potential gravity of complications of infections of the surgical site in that area. A very recent German study [62] showed how various protocols for antibiotic prophylaxis in head and neck tumours surgery led to significantly different outcomes, both in morbidity and in mortality of patients, precisely as a consequence of a reduction of nosocomial infections, either of a general category (pneumonia, infections of the urinary tract, sepsis) or of the surgical site (purulent discharge, abscess, cellulitis). A further study [63] in June 2017 demonstrated that the link between appropriateness in prescriptions of antibiotics and nosocomial infections does not concern only major oncological surgery. This study deals with the appropriateness of dosages, times and methods of antibiotic prophylaxis for minor paediatric operations such as tonsillectomy, adenoidectomy, otosurgery of the middle ear and tympanum; moreover it gives confirmation of data collected in Italian hospitals stating that "use of SAP reduces intraoperative wound contamination in pediatric surgery, thus minimizing the risk of SSIs". The authors come to the conclusion that, in this subdiscipline as well, prevention of nosocomial infections is almost completely in the hands of and down to the choices of the otorhinolaryngological surgeon.

2.5 Aspects of prevention

Nowadays, prevention requires an approach that is increasingly multi-disciplinary: the hygienist and the epidemiologist, on the basis of proved scientific evidences, implement their preventive actions which have to be carried out by different technical and professional figures (i.e. surgical washing of hands must be carried out by operating theatre operators, perioperative antibiotic prophylaxis becomes a technical/professional choice of the operating surgeon who is taking care of the patient) [64].

Preventative measures are aimed at promotion and safeguarding of the health of the subject and of the community, which need educational and empowerment measures addressed to colleagues, operators, patients, relatives and the whole of society, but they are at the same time an instrument to warrant economic sustainability of health systems. Appropriate investments in prevention, and implementation, for example, of programmes for vaccination on a national scale not only improve the quality of life of a very large number of people, but also generate direct and indirect savings [65 66].

In the field of ENT, too, three different levels of prevention can be identified: primary prevention, aimed at healthy subjects, to promote their state of health and prevent them from developing diseases; secondary preventative measures, aimed at identifying sick people at a preclinical or early stage of disease, in order to be able to make a positive change in the development of a disease; and lastly tertiary preventative measures aimed at improving the quality of sick subjects.

Primary prevention

One of the most important and effective primary measures of prevention is definitely vaccinoprophylaxis, which should be promoted and incentivised by all health operators.

The new PNPV 2017-2019 includes the classic vaccines of ENT interest, against measles, parotitis, rubella, H. influenzae type b, pneumococcus, meningococcus C-ACYW135, diphtheria influenza, with the addition of new vaccines against varicella and meningococcus B, and it extends the recommendation for HPV vaccination also to adolescent males. The PNPV 2017-19, the Calendar for life 2016 and the scientific Societies also lay down the offer of active, free vaccination against influenza, pneumococcus and Herpes Zoster for the over 65s and patients at risk (immunodepressed, heart conditions, diabetics, COPD sufferers) [68 69].

Since 2013 in Italy a gradual fall has been recorded in vaccine coverage [70-72]. This fall, partly due to vaccine hesitancy and amplified by the Fluad case [73-75], leads to the risk of reappearance of diseases that have been eliminated and is causing epidemic outbreaks on a large scale for diseases that were under control or for which there is a plan for elimination, as shown by the current resurgence of measles, which has already caused 3672 cases (263 in health workers) from January to July 2017, sometimes complicated by otitis (4%), and 3 deaths

To combat the risks from the fall in vaccine coverage and limit the epidemic phenomena under way, the vaccines included in the PNPV 2017-19 have also been added to the Essential Levels of Assistance and a Decree was recently issued under which obligatory vaccination against diphtheria, tetanus, poliomyelitis and hepatitis B has been extended to a further 6 vaccinations, against measles, parotitis, rubella and varicella, Haemophilus influenzae type B and whooping cough.

Primary prevention actions have also been implemented for tuberculosis: vaccination with BCG, which requires careful risk-benefit analysis, more sustainable building, with greater attention paid to health and, for HIV positive subjects, prevention with isoniazid and antiretroviral therapy [76 77].

Primary prevention measures also include health educa-

tion to inform the population on prevention methods, on modality of transmission and potential risk factors of infectious diseases, as well as on aspects of promotion of adequate hygienic behaviour to be adopted in private life in work areas and in hospitals (good hygienic standards, washing hands, appropriate use of PPE, application of good practices in care and correct perioperative prophylaxis protocols, proper use of antibiotic treatment, health supervision).

It is also fundamental to understand the knowledge possessed by HW, with regard to prevention opportunities proposed and scientifically validated for all levels of this discipline, not only because HWs are those who can and must put some of the measures into action, but also because they are the ones who have to promote prevention among the patients [78 79].

The first fact-finding report was recently published in Italy and Europe on 262 Italian ENT specialist doctors, to understand their level of knowledge and attitudes of HWs on the issue of HPV infection. It was revealed from this study that ENT specialists have a good level of knowledge of HPV infection and a positive attitude towards prevention, especially towards vaccination. These results must form a useful basis for planning, implementing and assessing specific continuous education programmes on the subject of prevention of HPV infection [78].

Secondary and tertiary prevention

Secondary prevention measures include screening of all newborn babies and especially audiological screening on birth (with a probe emitting low intensity sound), to make early identification of infants affected by moderate or profound congenital hypoacusia (>= 40 dB HTL between 0.5 and 4 kHz), in order to complete diagnosis by the third or fourth month and operate by the sixth, during the period of neural plasticity [80].

For control of tuberculosis there are ongoing specific programmes of supervision and control of the disease, which must be reserved for high risk groups (where incidence of disease is greater than 50/100,000).

The main objective of these programmes is identification of infected subjects (Mantoux and Quantiferon Tests), administration of preventive chemotherapy in cases of positive tests, to combat progression of infection into tubercular disease, and diagnosis of pulmonary forms of tuberculosis (Chest X-Ray) [81].

Other secondary prevention measures are targeted on identification of precancerous forms or tumours in the initial stage in the head and neck area passing on to identifying HPV infections and mutations on a genetic level (EGFR, TP53, p14, p16), but it will be necessary, in any case, to identify new biomarkers and gain clearer understanding of the genetic mechanisms and of cancerization, in order to be able to prevent and/or to better treat these pathologies [82].

Tertiary prevention measures include any measures aimed at improving quality of life for patients with hearing deficiency, improving their communicative potential, teaching sign language and making specific devices available (e.g. Cochlear implants).

Tertiary prevention includes any measures aimed at controlling the more complex outcomes of a disease and consists of clinical-therapeutic control of chronic or irreversible diseases, to prevent or restrict the onset of later complications and invalidating outcomes. Tertiary prevention consists of:

- management of functional deficiencies and disabilities (by means of rehabilitation or care measures, delegated to family, social and work reinstatement, and to improving the quality of life);
- follow-up and rehabilitation pathways post-infection or post-operative;
- choices regarding surgical techniques or removal extensions in the operative area, intended to prevent recurrence or progression of the specific pathology, including taking into consideration its etiologic agent (e.g. lymphadenectomy associated with operation for resection of a tumour of the head neck);
- choices of adjuvant or neoadjuvant therapies (chemotherapy or radiotherapy) intended to prevent recurrence or progressions of the specific pathology and in consideration of its etiologic agent.

3. HPV infection and related head and neck cancer

HPV are a very heterogeneous group of circular double-stranded DNA viruses, enclosed in an icosahedral capsid made up of 72 capsomeres with no viral envelope. The viral DNA contains a sequence of 7 early genes (E1-7) and 2 late genes (L1-2). The early proteins serve the purpose of encouraging cell growth and replication, while the transcription products of the L1 and L2 genes represent two structural genes for the virus, which go to make up the two proteins needed for formation of the viral capsid.

More than 100 different types of HPV are known. These may be catalogued on the basis of their genetic analogies, which generally correspond to a different tissue tropism. Viruses with mucous tropism are those capable of expressing greater oncogenic potential and are therefore defined as high risk HPV. The best known and most common are the subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52,

56, 58, 59, 68. HPVs with cutaneous tropism generally have less oncogenic potential and are responsible for benign cutaneous lesions, such as papillomas, verrucas and condylomata. They are therefore defined as low risk HPV and the most common of these are the subtypes 6, 11, 42, 43, 44.

The virus has a tropism for squamous epithelia, and replicates by following the state of differentiation and maturation of the keratinocytes. Viral DNA localises the level of the basal cells, where it may remain in a latent state at the nuclear level or it may trigger a self-replication process by entering the replication mechanisms of the epithelial cell [1].

HPV has been isolated in the oral cavity of approximately 6-10% of children and adolescents and, according to various statistics, in between 5 and 8% of the healthy adult population.

Some studies carried out on identification of viral DNA in the oral cavity of a broad cohort of healthy subjects in the USA, revealed a prevalence of approximately 7.3% of all HPVs and of 4% of those at high risk, with significantly higher prevalence in the male sex than in the female (10.1% *vs* 3.6% for all HPVs and 6.8% *vs* 1.2% for those at high risk) [2 3]. In a recent work, Orosco reports the results of a screening for oral infection from HPV carried out on 9256 subjects aged between 18 and 69 years, which detected HPV infection in 8.1% of the subjects, including 55.7% high risk HPV and 55.3% low risk, with 11% of subjects with both types of HPV, and which revealed that HPV infection is positively associated with the number of partners for oral sex but not with the female sex or socioeconomic class [4].

The most frequent clinical manifestations of HPV infection are:

- **squamous papilloma** is the most common benign epithelial neoplasia of the oral cavity, the lesions may be found everywhere in the mouth, but especially on the ventral surface of the tongue and in the lingual frenulum area, on the palate and on the mucous surface of the lips [5]. They may appear at any age as whitish exophytic lesions with a rough verrucous surface. They are often caused by HPV-6 and HPV-11 infection. Treatment is surgical, any recurrence should raise the suspicion of development towards malignant neoplasia;
- **verruca vulgaris**, most frequently caused by HPV-2 and HPV-4, represents one of the principal manifestations of HPV infection on the skin and in the oral cavity. On the level of the oral cavity it is usually found on the keratinized surfaces of the lips, gums and palate. The verrucous oral lesions are contagious, typical in children, but may be encountered at any age. The lesion from verruca vulgaris initially expands rapidly in dimension and then stabilises, often for many years. From a clinical point of view, differential diagnosis between verruca vulgaris and squamous papilloma does not assume any relevant importance, since the treatment for the two lesions is the same: surgical excision;
- **focal epithelial hyperplasia**, otherwise known as Heck's disease, is associated with HPV-13 and HPV-32 infection. It mainly affects children, but is increasingly often found also in HIV positive patients. It localises in the mucosa of the lips and tongue, with multiple and sessile nodular lesions. The lesions often disappear after a long time without the need for any treatment and the risk of recurrence is minimal;
- **condyloma acuminatum** can affect the oral mucosa, larynx, trachea, genitals and the perianal region. Oral condylomata are generally associated with HPV-2, HPV-6 and HPV-11 and are most frequently localised on the labial mucosa, the soft palate and lingual frenulum. They are often correlated with orogenital contact. Condylomas are difficult to treat, in order to significantly reduce possibility of recurrence it is advisable to remove surgically all lesions simultaneously.

Localisation in the larynx, **laryngeal papillomatosis**, caused by HPV infection of subtypes 6, 11, 16, 18, 31, 33, is especially important and because of its distinctive epidemiological, clinical and therapeutic characteristics will be dealt with later, in the chapter dedicated to emerging ENT infectious disease in paediatrics (Chap. 8).

Apart from the above-mentioned manifestations, HPV infection can also cause precancerous or cancerous lesions. HNSCC is the sixth most frequent tumour in the world, with incidence of approximately 400,000 new cases per year [6]. Tobacco smoking and alcohol certainly represent the best known and recognised risk factors; reduced consumption of these, which has been seen over recent decades, should therefore have meant reduced incidence of HNSCC. This does not correspond, though, with what is actually observed, especially in western countries (USA and Europe), where an increase has been seen, especially in tonsillar and lingual base tumours. Moreover, the mean age of patients affected by this type of tumour has fallen (40-55 years) and frequently the exposure to traditional risk factors is anamnestically negative. The idea that we are facing a different etiology for these tumours is now widely spreading and there is a body of evidence in support of an important role for HPV in their genesis.

However, in spite of high prevalence of HPV in the oral cavity in the general population, the incidence of tumours of the oral and oropharyngeal cavity are, in any case, quite low. Even though the incidence of HPV-

correlated oropharyngeal carcinoma, which represents the most frequent HPV-correlated tumour among the HNSCC, increased by 225% from 1988 to 2005, it is currently about 2.6 cases per 100,000 residents [7]. It is therefore obvious that not all HPV carrying subjects face development towards neoplasia. The existence of viral DNA obviously may represent a risk factor and an initial condition potentially capable of developing into a neoplastic pathology, but the factors that determine this development still remain to be clarified. In any case, the low incidence of tumours compared to the wide spread of the virus in the population seems to suggest that care should be taken not to exceed in alarmism concerning the risk of contagion in subjects who are partners to patients with HPV-correlated HNSCC [8].

The data relating to prevalence of the virus in head and neck tumours is highly variable. A meta-analysis by Kreimer has provided a summary of the various data available in literature and shows that viral DNA is present in approximately 35% of oropharyngeal tumours and in 24% of tumours of the oral cavity and larynx. Viral subtypes 16 and 18 alone are responsible for more than 90% of HPV-correlated cases [9].

However, identification of the viral DNA in neoplastic tissue does not demonstrate per se an etiopathogenetic link between HPV and tumour. To establish the correlation with certainty, methods are required that are not limited to show the presence of viral DNA, but which rather demonstrate its oncogenic activity [10 11]. It will be shown below which methods currently represent the diagnostic gold standard in this sense.

What determines prevalence of HPV at the level of the oral cavity? The role of this virus in the etiology of cervical cancer has rapidly given the impression that sexual habits might represent a risk factor for spread of the virus. In particular, oral sex seems to be notably correlated with the condition of viral infection [12-16], but what determines the expression of oncogenic activity has not yet been completely clarified. It is probable that many factors interact with the presence of the virus to allow development towards neoplastic transformation.

Inadequate hygiene of the oral cavity seems to be a potential risk factor in conditioning development of HPV-correlated HNSCC [16]. Cigarette smoking also seems to increase risk of viral oncogenesis, even if, as recalled above, many patients afflicted by HPV-correlated HNSCC are non-smokers. The idea has been growing, recently, that oncogenic potential of the virus depends not only on the high risk HPV genes, but also on the state of expression in the host cell of a series of transcription factors capable of modulating the viral activity [17]. This might explain the large biological variability in determining insurgence of tumours.

The viral DNA contains a sequence of 7 early genes (*E1-7*) and 2 late genes (*L1-2*). Early proteins serve the purpose of encouraging cell growth and replication; in particular, the E6 and E7 proteins represent the two main oncoproteins of the virus, since they are capable of inducing neoplastic transformation of keratinocytes. These interfere with two oncosuppressor mechanisms present inside the cell. E6 interacts with p53, which is normally capable, following damage to DNA, of blocking the cell cycle and inducing apoptosis. Viral protein E7 is capable of interacting with another important protein known for its oncosuppressor effect, i.e. pRB. The latter, through the formation of a link with the transcription factor E2F, is capable of arresting progression of the cell cycle from G1 to S, therefore inhibiting cell replication. Viral protein E7 blocks formation of the pRB-E2F complex and therefore allows proliferation of tumorous cells. Unlike early proteins, the transcription products of the genes *L1* and *L2* represent two structural proteins of the virus, which are necessary for the formation of the viral capsid.

To express its oncogenic potential, HPV has to infect cells in the replication stage. This means it has to use the DNA polymerase and, as mentioned above, probably several other transcription factors of the host cell. The infection of the mucosa and skin appears at the level of the basal cells, since these are the only ones capable of replicating. Basal cells of mucosa at the level of the tonsillar crypts are more exposed to the action of the virus than to other regions, where these cells are usually coated with several cell layers. This might explain, at least in part, greater incidence of HPV-correlated carcinomas at the level of the tonsils. Once the basal cells are infected, the viral DNA can replicate itself, even if remaining at a plasmid level, and transmit itself to new generation cells. The spread of the virus can therefore extend to the more superficial layers of the mucosa or epithelium. At this level no replication of viral DNA occurs, but there can be seen transcription of late genes, which lead to formation of structural protein of the virus, as well as the presence of complete viral particles on the superficial part of the epithelium. As long as the viral genome stays in plasmid form, anyway, no neoplastic transformation of the cell can be seen. For this to occur, integration of DNA into the cell genome is required. Breakage of the circular DNA of the virus usually occurs in correspondence with the *E2* gene, which has a repressive effect on transcription of *E6* and *E7*. This event therefore enables transcription of the two main viral oncogenes and determines cell transformation towards neoplastic [18-20].

3.1 Diagnosis

The diagnosis of HPV-correlated tumours is currently anatomopathological and aimed at identifying viral oncogenic activity present in tissue samples. In the majority of cases bioptic samples taken from the original tumour and fixed in formalin are available; this represents a limitation for carrying out highly reliable tests, which can only be obtained from fresh material. It should be recalled that the initial manifestation of a HPV-correlated tumour of the head and neck area is not rarely represented by an isolated lateral cervical adenopathy, without the evidence of any primitive tumor. In this case, needle aspiration cytology may represent the first sample for carrying out diagnostic tests and results obtained in this manner seem to show perfect agreement with those deriving from methods based on the use of bioptic samples [21 22].

Identification of viral DNA inside neoplastic tissue is not by itself an indication of HPV-correlated tumour, but it may simply represent a transitory viral infection of the mucosa, without real oncogenic potential. It is therefore necessary to separate the methodologies which detect the presence of the virus in the tissues, which are more appropriate to study the spread and prevalence of the virus in the population, from those which aim to show up products of viral activity that are responsible for the process of neoplastic transformation and which should therefore be used in genuine diagnosis of HPV-correlated tumours. It should be reminded that the former methodologies include genic amplification based on PCR and hybridization in situ, such as FISH. PCR is highly sensitive in detecting the presence of even a few copies of viral DNA, but for the same reason it is not very specific. For these purposes, the use of "Real Time PCR" may be more reliable, since it is capable of performing quantitative analysis of virus load, which is more correlated to a real state of infection. The FISH method is also directed towards identification of viral DNA, but it is performed with RNA probes, complementary to viral DNA, interacting with sections of tissue fixed in formalin and analysed through a fluorescent microscope. At variance with PCR based techniques, this method enables the analysis of the distribution of viral DNA in the cells and in the bioptic tissue and shows greater specificity. This enables distinction of a diffuse nuclear pattern, indicative of the plasmid state of the viral DNA and therefore not yet integrated into the genome of the host cell, from a punctuated pattern, which on the other hand suggests completed genome integration [24]. It also enables a view of a diffuse state of viral infection on cancer cell clones, rather than a state of infection localised to a few isolated cells, which cannot be relevant from a clinical point of view.

The election method representing the current gold standard for diagnosis of HPV-correlated carcinomas is based on research into neoplastic tissue of the mRNA transcription of viral oncogenes, in particular of E6 and E7 [10 11]. However, this method is not particularly widespread, since it necessarily requires a fresh, frozen tissue sample, as it is not applicable on tissue fixed in formalin. Its application is therefore often limited to research activity carrying out studies taking into consideration sensitivity and specificity of alternative diagnostic methods. In this sector, a lot of attention is focused on detection of biomarkers that are correlated to the carcinogenic process and are induced by viral activity. An important role is currently being played by immunohistochemical methods capable of detecting an overexpression of p16 protein. This protein may be considered a surrogate marker of viral oncogenicity. In effect, this protein is always expressed in tumours of proven viral correlation and its histochemical detection therefore enables achievement of 100% diagnostic sensitivity. However, more than 10% of p16+ tumours lackany viral DNA detection both by the FISH and PCR methods [23-27]. The specificity of immunohistochemistry for p16 alone cannot therefore be considered satisfactory.

To overcome this diagnostic shortcoming, a two-step diagnostic strategy has been proposed, envisaging preliminary immunohistochemical assessment for detection of p16 in paraffin embedded tissue, exploiting the high sensitivity of this method in identifying HPV+ tumours, and subsequent performance, only for p16+ tumours, of PCR analysis for identification of DNA of high risk HPV. Combined analysis of these two methods would make it possible to reach close to 100% sensitivity and specificity [23 29]. This strategy therefore seems at the moment to restore widespread agreement even in the absence of more sophisticated methods.

The current importance of methods for the identification of p16+ tumours also resides in the fact that this marker seems to be closely linked to the prognosis of cancer pathology, rather than to the presence of the HPV virus itself. It is actually known that the survival curve of patients who are carriers of HPV positive tumours is generally better than that of non-HPV correlated HNSCC carriers. In reality, according to some authors, the subgroup of patients who are carriers of p16+ and HPV- tumours show survival curves similar to those of patients with p16+ and HPV+ tumours and both are better than those of carriers of HNSCC p16- [24]. This therefore suggests that actually it is not the presence of the virus itself that affects the prognosis, but rather the state of expression of the p16 protein and the extent to which it is a consequence of HPV infection. In any case it should be reminded that it is not

possible to exclude definitely that the state of p16+ and HPV- tumours may be a consequence of failure to detect, by the common primers used in PCR methods, viral subtypes that have not yet been identified.

New alternative strategies have also been proposed for the diagnosis of HPV correlated tumors: an interesting work should be mentioned that studies the possibility of performing a serologic diagnosis of the state of a HPV correlated tumour [13 28-30]. In this field, there seems to be a particularly promising possibility of using serologic identification of direct antibodies against early virus proteins. In particular, the detection of anti E6 antibodies shows high sensitivity and specificity in cases of oropharyngeal tumours [29]. Research on anti E6 antibodies in non-oropharyngeal HNSCC, however, for reasons that are still rather unclear, shows lower sensitivity (50%) while maintaining high specificity (100%) [31]. A recently introduced method should also be pointed out, which enables detection, with *in situ* hybridization techniques, of viral mRNA for E6 and E7, performed on fixed and paraffin embedded material. Overcoming the need of fresh material for this type of investigation should represent a breakthrough, enabling diffusion of a shared single diagnostic test for identification of HPV correlated tumours [32].

3.2 Clinical picture

HPV related oropharyngeal carcinomas are more frequent in males (M:F = 4:1), of Caucasian ethnicity (4% in blacks, 34% in whites), of ages between 40 and 55 years, therefore younger than HPV negative patients, often non-drinkers and non-smokers and from a high social status [14 15].

A number of studies have shown that HPV positive tumours appear clinically at an early T stage (T1-2) and advanced N stage (N2-3). The lymph node metastases often have a cystic nature and affect multiple and bilateral levels [33]. This clinical characteristic must always be considered in the diagnosis of lateral cervical carcinomatous metastasis from a tumour in an unknown location, since an HPV positive needle aspiration test directs the search for the primitive tumour at the level of the tonsils or the base of the tongue.

HPV positive oropharyngeal tumours also appear with distinct histological characteristics, compared to HPV negative tumours: low or moderate tumour differentiation, lack of keratinization, basaloid aspects and elevated mitotic index [34] (Figs. 3.1, 3.2).

It is also possible to detect radiological differences between HPV positive oropharyngeal carcinomas and negative ones, the former usually presenting as small or even hidden primitive lesions with large, often cystic lymph node metastases and with well defined margins of while in the latter case lymph nodes frequently shows broken capsules and infiltration of adjacent muscles (Figs. 3.3, 3.4).

A number of studies have shown better disease-free and overall survival for patients with HPV positive oropharyngeal carcinomas compared to patients with HPV negative oropharyngeal tumours, showing a lower risk of death in the first case (60% *vs* 80% respectively) [36]. A meta-analysis in 2007 compared patients with HPV negative and positive oropharyngeal tumours, showing in the latter a 49% lower risk of death and 36% lower risk of recurrence [37]. Also, after three years of follow up the risk of progression and the risk of death are 72% and 79% lower in HPV positive compared to HPV negative patients. In a retrospective study carried out on more than 45,000 cases, Khode found a survival improvement of two years in HPV positive oropharyngeal carcinomas compared to the negative ones [38].

The exact mechanism of improved prognosis for HPV positive oropharyngeal tumours is not clear at the moment. Better survival rates are partly attributable to less biological aggressiveness in these neoplasia and greater sensitivity of HPV correlated tumours to combined chemo and radiation treatments and to induction chemotherapy. Better therapeutic responses are believed to be due to the

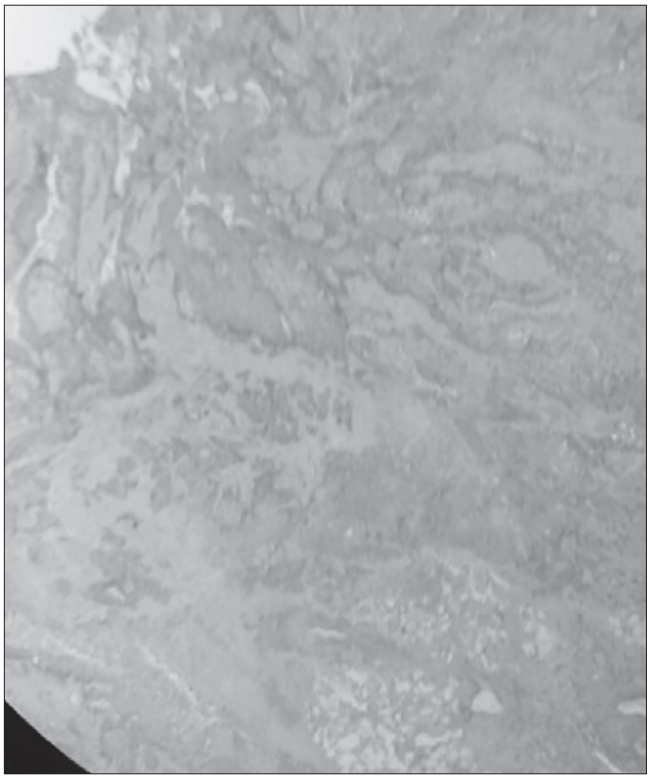

Fig. 3.1. HPV positive tonsillar carcinoma.

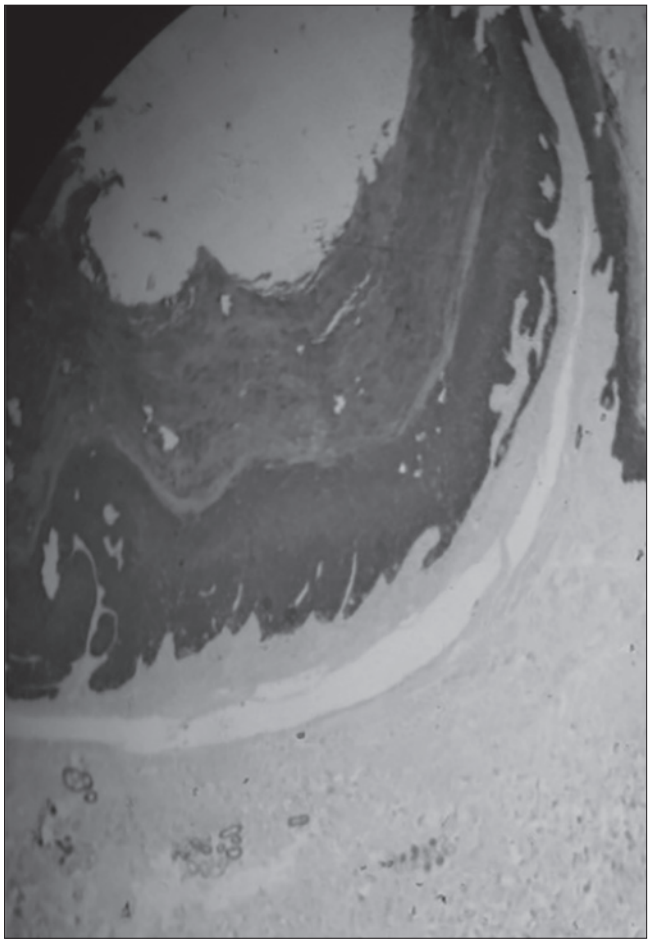

Fig. 3.2. Overexpression of p16 protein in tonsillar carcinoma.

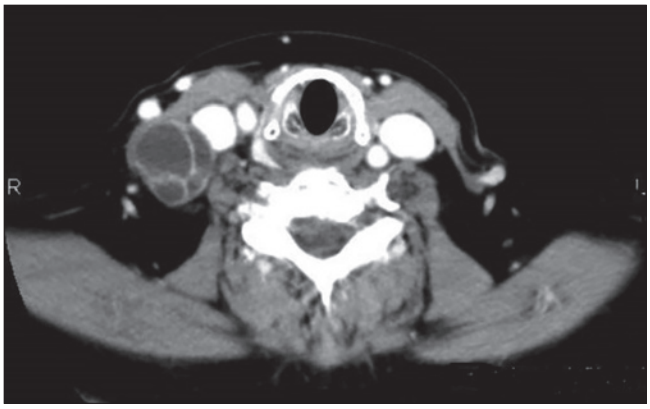

Fig. 3.3. CT picture of lymph node metastases from HPV positive tonsillar carcinoma.

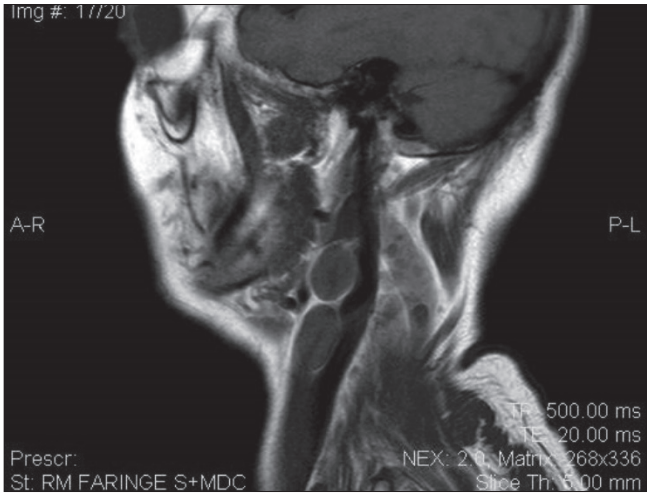

Fig. 3.4. MR picture of lymph node metastases from HPV positive tonsillar carcinoma.

lack of the so called field cancerization effect and a consequent lower rate of genomic damage [39 40].

A recent study by Baruah detected an increase in angiopoietin-1 and vascular endothelial growth factor, both of which are favourable to angiogenesis, in patients with p16 negative head and neck carcinomas, compared to those with p16 positive HPV related; this might explain the decrease in angiogenesis in the HPV positive carcinoma and its consequent better prognosis [41].

Considering their better overall survival and their younger age, de-intensified treatments for HPV positive patients are desirable [43]. This treatment de-intensification might consist of a reduced radiation dose and/or concomitant therapy, or the omission of chemotherapy, replaced by biological therapy, or again the introduction of neoadjuvant chemotherapy to avoid concomitant chemotherapy during radiotherapy.

The growing incidence of HPV correlated head and neck neoplasias and their distinctive clinical and prognostic feature compared to HPV negative ones, has led the AJCC to make a differentiation in the latest edition of the TNM for these tumors. The 8th edition of the TNM classification in 2017 shows a separate classification for p16 negative and p16 positive oropharyngeal neoplasias, regarding both T and N and showing a clearly distinct difference in the prognostic sense for patients diagnosed with a p16 positive carcinoma [43]. Lydiatt quotes as an example a hypothetical patient presenting a 2 cm p16 positive carcinomatous lesion at the level of a palatine tonsil together with 2 positive neck ipsilateral lymph nodes: according to the previous TNM classification, this tumour was classified as stage IV, whereas according to the new classification it belongs to stage I [43-45]. The same author reports that it is absolutely necessary to update the classification of head and neck tumours, because starting from the 1990s, initially in the United States and later elsewhere, incidence

of carcinomas of the tonsil and tongue associated with infection from papillomavirus has undergone an increase of around 5% each year. It is therefore important to differentiate the two types of carcinoma (HPV positive and negative). The only widespread inexpensive and reliable test is represented by the histochemical detection of p16 protein in fixed tissue. This is why the eighth Edition of the TNM classification is based on the overexpression or not of p16 in the tumor. Overexpression of p16 should involve both the nucleus as well as the cytoplasm of the tumour cell. If p16 staining is confined to the cytoplasm only, the test has to be considered as non-specific and therefore negative.
Tables 3.I and 3.II show the classification of p16 positive and p16 negative oropharyngeal T, where, for p16 positive tumours, lack of embedding of the carcinoma *in situ* and the separation of T4 into T4a and T4b can be seen.
The classification of N has also been differentiated into two groups on a clinical and radiological basis. The clinical Ns, both single and multiple, monolateral and less than 6 cm in dimension, having the same effect on survival, are classified as N1. Whereas, in the case of clinically palpable lymph nodes, contralateral to the lesion or bilateral, worse impact will be seen on the survival of the patient and therefore they are classified as N2. Lymph nodes greater than 6 centimetres in dimension, though, represent the stage of higher risk: N3 (Tables 3.III, 3.IV).

3.3 Overview of treatment

In order to be more schematic, treatment will be divided according to the stage of the disease:

Early stages of the disease (T1-T2 N0)

The initial stages of the disease are normally treated with a single method: surgery or RT. There are currently no studies of top scientific quality comparing these two treatment methods within the same population. The decision leading to the choice of the most suitable treatment is generally based on the site and dimension of the tumour, also assessing the residual global functional deficiency.

Surgical treatment

The most modern treatment envisages a transoral approach, with either laser (TLM) or with the aid of a robot (TORS). The oncological and functional outcomes seem to be fully comparable with those of open surgery, that is why the open approach of disease in the initial stage is now a days seldom performed. The risk of hidden lymph node metastases in this stage varies from 10 to 31%, so ipsilateral surgical treatment of the cervical lymph node stations is normally indicated. Surgical treatment of the neck contralateral to the seat of the primary tumour might be indicated in neoplasias that reach the median line or are close to it, in order to obtain pathological staging also of the contralateral cervical side.

Table 3.I. Clinical and pathological T for p16 positive oropharyngeal Carcinoma, Eighth Edition TNM (from Amid et al., 2017 [43], mod.).

T category	T criteria
T0	No primary identified
T1	Tumour 2 cm or smaller in greatest dimension
T2	Tumour larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumour larger than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Moderately advanced local disease; tumour invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate, or mandible or beyond

Table 3.II. Clinical and pathological T for p16 negative oropharyngeal Carcinoma, Eighth Edition TNM (from Amid et al., 2017 [43], mod.).

T category	T criteria
Tx	Primary tumour cannot be assessed
Tis	Carcinoma in situ
T1	Tumour 2 cm or smaller in greatest dimension
T2	Tumour larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumour larger than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Moderately advanced or very advanced local disease
T4a	Moderately advanced local disease; tumour invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate, or mandible
T4b	Very advanced local disease; tumour invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base or encases carotid artery

Table 3.III. Clinical and pathological N for p16 positive oropharyngeal Carcinoma, Eighth Edition TNM (from Amid et al., 2017 [43], mod.).

T category	N criteria
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One or more ipsilateral lymph nodes, none larger than 6 cm
N2	Contralateral or bilateral lymph nodes, none larger than 6 cm
N3	Lymph node(s) larger than 6 cm

Table 3.IV. Clinical and pathological N for p16 negative oropharyngeal Carcinoma, Eighth Edition TNM (from Amid et al., 2017 [43], mod.).

T category	N criteria
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and extranodal extension negative
N2	Metastasis in a single ipsilateral lymph node larger than 3 cm but not larger than 6 cm in greatest dimension and extranodal extension negative; or metastases in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and extranodal extension negative; or metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and extranodal extension negative
N2a	Metastasis in a single ipsilateral lymph node larger than 3 cm but not larger than 6 cm in greatest dimension and extranodal extension negative
N2b	Metastasis in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and extranodal extension negative
N2c	Metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and extranodal extension negative
N3	Metastasis in a lymph node larger than 6 cm in greatest dimension and extranodal extension negative; or metastasis in any lymph node(s) and clinical overt extranodal extension positive
N3a	Metastasis in a lymph node larger than 6 cm in greatest dimension and extranodal extension negative
N3b	Metastasis in any node(s) and clinical overt extranodal extension positive

Radiotherapy

In radical treatment, a total dose equivalent to 70Gy in 35 fractions is normally used. In the case of more laterally localised (tonsils) tumours a prophylactic RT should be carried out on the ipsilateral cervical lymph nodes, while for those mainly localised in the median area, both the cervical areas should be treated. The most frequently used technique is IMRT.

Advanced stages of the disease (T3-T4 N0 and T1-T4 N1-N3)

In recent years a tendency has been seen to offer patients with advanced disease concomitant radio-chemotherapy treatment, as a strategy for organ preservation. A potential worry that might grow from the choice of a protocol for organ preservation arises from the consideration that salvage surgery has proved to have an elevated percentage of success in carcinoma of the larynx but not equally so in other cervical cephalic areas, such as the oropharynx. The choice of treatment for each individual patient derives from the dimension and site of the disease from the potential residual functional deficiency as well as from the preference of the patient and local expertise. HPV status has an important influence on the prognosis and in the future could also affect the choice of treatment.

Surgical treatment

The transoral approach is suited more for the T1-T2 categories but may sometimes be considered for the T3 category, if there is a reasonable chance of achieving negative resection margins. In cases where transoral treatment has contraindications, concomitant radio-chemotherapy treatment should be taken into consideration. Alternatively, an open surgery approach can be considered especially if a reconstruction stage is needed Free flaps are usually preferred, but in such cases the functional results are often less than optimal, especially when adjuvant therapy is indicated. Since there is elevated possibility, greater than 50%, of hidden cervical lymph node metastasis, an elective treatment of the cN0 neck is recommended.

Primary chemoradiotherapy

CRT in loco-regionally advanced stages of disease is an effective therapeutic choice (organ preservation).

RT alone can be taken into consideration for patients unfit for concomitant CRT, especially if aged over 70 years, inasmuch as it is known that addition of concomitant treat-

ment does not add significant benefit in terms of survival. Induction chemotherapy may be considered in patients with advanced oropharyngeal carcinoma (T4, N3, N2c), to reduce the risk of distant metastasis, and for other selected patients with bulky T (T4) and/or lymph node disease (N3), in order to induce cytoreduction, educe the radiotherapy target and consequent toxicity and aid chemotherapy effect before the RT changes the vascularisation. Currently there is no high quality definitive scientific evidence supporting the use of induction chemotherapy for HPV related head and neck tumours. In the future it might have a better-defined role within de-intensified therapeutic protocols and in selected patients.
Concomitant radio-chemotherapy is associated with higher toxicity compared to RT alone, especially regarding deglutition, with considerable impact on quality of life. Technological improvements in RT, including IMRT, have permitted reduction of late toxicity and sparing of the parotid glands and trials are currently ongoing to explore the role of IMRT in improving dysphagia, by means of reduction of doses of radiation administered to the pharyngeal constrictor muscles and the other structures involved in deglutition.
In the presence of an advanced class N (N2-N3) and on termination of concomitant CRT, lymph node evacuation of the neck is proposed, even if there is lack of evidence of greater benefit from surgical treatment of the neck practised after rather than before the CRT treatment. Recent level I scientific evidence showed that a policy of active PET-CT guided supervision allows survival rates similar to those achieved with neck surgery planned as default at the end of CRT. Surgical treatment of the neck is only planned when there is clear or doubtful residual lymph node on PET-CT carried out at 10-12 weeks after the end of CRT. This strategy gives lower morbidity related to less effective treatments and lower cost effectiveness ratio.

Adjuvant radiotherapy and chemoradiotherapy
Indications for adjuvant therapy depend on the common risk factors for recurrence/metastasis that are usually taken into consideration for squamous carcinomas of the head and neck area.

Therapeutic de-intensification
Patients afflicted with HPV related squamous carcinoma have different epidemiological, molecular and clinical features from non-HPV related counterparts, with better radiosensitivity and prognosis. These subjects are also younger and with higher morbidity correlated to treatment, which may negatively influence quality of life. These aspects have led to the development of clinical trials with de-intensified protocols aimed at reducing acute toxicity and especially chronic toxicity in a younger population with greater chance of survival.
At the moment, de-intensified treatments for HPV related oropharyngeal carcinoma are still subject of study and any change, compared to the proven therapeutic standard, in the management of these patients must take place exclusively within the context of clinical trials.

Vaccination
On the back of excellent results achieved in prevention of uterine cervical carcinoma with vaccination programmes on young women, which have been introduced in many countries, there are many current indications for extending anti-HPV vaccination also for males.
In Sweden, where 73% of women are vaccinated, oral HPV prevalence has passed from 9.3% in 2009 to 1.4% in 2014, demonstrating the usefulness of vaccine also in prevention of head and neck neoplasia. If we consider that, in the USA each year there are an estimated 7500 new male cases of HPV related neoplasias, mainly head and neck or anal carcinomas, vaccination extended to males seems to be strongly indicated.
At the current moment of time, there are two vaccines approved by the FDA: one quadrivalent (HPV 6,11,16 and 18) and the other bivalent (16 and 18). These vaccines have shown to be highly promising in primary prevention against HPV related oropharyngeal tumours [46] and for this reason the ACIP and the CDC recommend extending HPV vaccination to both sexes, between the ages of 11 and 26 years [47].

4. EBV and related head and neck cancer

The halo of mystery cloaking the genesis and high incidence of undefined manifestations of tumours in young children in central Africa was the main reason that drove Denis Burkitt, in 1956, to study the clinical details of this phenomenon in the population of Uganda.
The characteristics of diffusion and endemia of this pathology quickly led two English virologists, Anthony Epstein and Yvonne Barr, to the hypothesis of a deterministic involvement of specific microbial agents, with a high level of transmissibility between humans.
The microbiological studies that they carried out on a cellular line of samples made by Burkitt led to the discovery of a "new" viral agent, a herpes virus, which was subsequently named after them as the Epstein-Barr Virus (EBV).
EBV is a DNA herpes virus, 150-200 μm in diameter; its nucleic acid is constituted by an icosahedric capsid, ap-

prox. 100 nmin diameter, packed with more than 172 kb of linear double stranded DNA, with 162 capsomeres. The viral envelope, with its lipoprotein structure, has an irregular shape and is acquired during budding of the virus by the plasma membrane of the host cell.
The viral genome consists of a single short and a single long region, with multiple repeated terminal sequences, which probably allow circularization of the DNA.
The high diffusion capacities of this virus, as already shown by Burkitt, are attributable both to specific characteristics intrinsic to the virus, which allow for isolation even in ethnic gatherings in remote localisations, as well as to the means that enables its channelling: interhuman transmission of saliva, by direct and/or indirect means, is an event that is easily and frequently achieved.
The characteristics mentioned above are, on their own, sufficient to explain the high incidence of infections determined by EBV: it is estimated that approximately 95% of the world population contracts an asymptomatic infection from EBV, lasting the whole lifetime.
New born babies become susceptible to EBV as soon as maternal antibody protection ceases. Many young children contract the infection, even if they do not show any particular symptoms, but an occasional slight feeling being unwell.
The clinical phenomena of EBV infection start to mutate in adolescence, a time of life when EBV infection, in approximately 35 to 70% of cases, is observed in the form of "infectious mononucleosis", whose clinical sign may be of specific concern for otolaryngologists.
Infectious mononucleosis is a proliferating lymph node reticular disease, of a non-neoplastic nature, which according to some authors is attributable to the category of sexually transmitted diseases and whose acute form appears as high temperature, generally above 38°C, lasting for 1-2 weeks, tonsillar hypertrophy and submandibular angle lymphadenopathy. These symptoms may occasionally be preceded, in the viral incubation stage, by a sensation of generally feeling unwell, with widespread asthenia and myalgia [1].
Different importance is assumed, however, in terms of the whole complex of clinical and prognostic features, by all the pathologies of an oncological nature where etiological responsibility of EBV is recognisable.
The deterministic intervention of EBV in the pathogenesis of some neoplastic diseases is, a phenomenon essentially linked to its complex and structured oncopathogenic process, which has not yet been clearly defined.
Viral oncogenesis of EBV has been variously blamed for different neoplastic pathologies, such as:

- oral hairy leukoplakia;
- squamous cell carcinoma of the oral cavity;
- nasopharyngeal carcinoma;
- laryngeal carcinoma;
- Burkitt's lymphoma;
- Hodgkin's disease;
- gastric carcinoma [2].

The regional prevalence of these oncological pathologies is found in the cervical cephalic region, which is, moreover, characterised by high incidence of neoplastic pathologies. Recent estimates worldwide have calculated case frequency of approximately half a million new cases/year, with the number of deaths exceeding 370,000.
EBV, after its penetration into the human organism, binds to its glycoprotein components, gp350 and gp220 and to a specific B-lymphocytes membrane receptor, Cr2, the same one that is linked by the C3d fragment of the complement. This means that the virus can mimic a physiological link, a mechanism by which it can "neutralise" the host defences.
The receptor binding constitutes the prelude to the actual penetration of the virus into the B-lymphocyte, where it achieves viral DNA localisation, a phenomenon which is expressed in two different forms, episomal and integrated. In the episomal form, the DNA, in circular form, remains separated from the human genome complex, while in the integrated form the DNA is incorporated into the host genome [3].
Lymphocyte infection from EBV may be followed by two different types of consequence:

- the start of a viral replication cycle and death of the host cell through lysis, a phenomenon consequent to the release of viral particles capable of infecting other cells;
- determinism of a state of latency, during which the virus does not replicate inside the cell. This state may even last for an extremely long time.

The viral genome localised inside the B-lymphocyte induces synthesis of specific proteins, called EBNA antigens, constituted by a set of at least 6 proteins (EBNA1-6), which interfere with the cell DNA, change the expression of various genes and activate the B-lymphocytes to cause unlimited proliferation (cell immortalisation).
Proliferation of B-lymphocytes is mediated by the EBNA antigens, as well as by three membrane proteins (LMP1-2A- 2B) and by two types of non-polyadenylated RNA (EBER1 and EBER2), to obtain a particular cell line, the LCL.
A special oncopathogenic role is played by EBNA-2 and LMP-1, the first of which is capable of promoting transcription of certain genes, including the oncogene c-fgril whose product intervenes in stimulation of cell proliferation, while LMP-1 acts to positively modulate cell prolif-

eration, both by associating on the cytoplasmic membrane with a protein that sends signals for activating proliferation, and by stimulating activation of the cell oncogene bcl-2, whose product is capable of effectively countering the programmed cell death.
This process causes the EBV infected lymphocytes to shift from a latent state to one of active proliferation in which physiological homeostatic controls are removed, a phenomenon which aids the neoplastic transformation processes.
On the basis of expression of viral proteins and expression of cell surface markers, three different programmes of viral latency have been identified:

- latency I: genic programme typical of Burkitt's lymphoma;
- latency II: genic programme detectable in carcinoma of the nasopharynx, in the NK/T nasal lymphoma, in the lymphomas of primary effusion;
- latency III: genic programme identifiable in some lines of Burkitt's lymphoma and in lymphomas in immunocompromised subjects.

Among EBV antigens especially important from a pathogenical point of view are the VCA and the EAs.
The viral capsid antigen, the first antigen system to be described in infections from EBV, is a complex antigen including the structural components of the viral capsid which are synthesised into an infected cell at the end of the lytic cycle. Anti-VCA antibodies appear after the infection and remain lifelong. EAs are a group of non-structural components whose production does not require viral DNA synthesis. They are part of the synthesised antigens when one of the lymphoid cells is spontaneously activated to viral replication or when the viral replication cycle is induced by exogenous stimuli. The EA consist of a series of polypeptides. Two principal components of EA have been identified as "diffuse" (D) and "restricted" (R) respectively and are defined on the basis of their distribution in the cell and their different solubility in methanol: the R component, unlike the D, is soluble in methanol. The activator of ZEBRA replication is also an EA and other components probably exist which are part of the EA complex, such as viral DNA polymerase, viral DNase and thymidine kinase.
The importance of the EAs is that they can indicate, in vivo, the start of viral replication.
Not all human serums with antibodies against EBV possess antibodies against the EA complex: patients with acute infection or chronic active infection from EBV tend to produce antibodies against EAs, but these antibodies are normally absent from the serums of healthy patients with remote exposure to the virus.

The pathological mechanisms determining the evolution of a viral infection towards oncological pathologies of the cervical cephalic area very often assumes particularly complex variegations, with processes in which the existence may be presumed of a conditioning, favourable substrate, which might be a disorder of the immune system or synergic co-intervention of other viral elements [4].

4.1 Oral hairy leukoplakias

Oral hairy leukoplakia is a typical pre-cancerous lesion featuring the presence of whitish, hyperkeratotic non-detachable patches, with non-regular morphology, generally localised on the edges of the tongue and less frequently on the gums, inside the cheeks and in the lower part of the mouth. These manifestations can typically be seen in cases where the EBV infection appears in immunocompromised patients, such as those afflicted with infection from HIV.
Oral hairy leukoplakia may appear in any stage of the infection process, even if it is more easily seen in subjects who present with a CD4 lower than 200 cells/mm^3 or who have undergone organ or bone marrow transplants [5].
This pathology does not incur transformations of a malignant kind and does not require treatment, even if its presence requires special attention inasmuch as it is indicative of a condition of immune deficiency, a factor which could favour the associated co-intervention of other pathogens.
Diagnosis is based on demonstration of the virus in the superficial epithelial layers in vacuolated cells relatable to koilocytes, cells which suggest viral infection.

4.2 Oropharyngeal squamous cell carcinoma

The frequency of encountering correlation between EBV infection and oropharyngeal squamous cell carcinoma is variously reported in literature, according to the statistics of the various authors, with rates ranging from 0 to 100%. It has been observed, however, that this variability is partly attributable to geographical and ethnic differences between the various populations under examination [6].
Under a diagnostic profile, it has been shown that expression of the virus RNA, EBER, cannot be considered an exclusive marker for this type of neoplasia, since it is widely present also in the severe forms of epithelial dysplasia.
However, different diagnostic weight may be given to the association between the presence of EBER, of LMP1 and of EBNA2.

Numerous studies have observed the co-existence between elevated rates of infection from EBV in subjects with oropharyngeal squamous cell carcinoma, unlike, however, what is found in tissue of healthy subjects belonging to the same study cohorts [7].

It is important to underline that oropharyngeal squamous cell carcinoma localises mainly at the level of the tonsils and base of the tongue, areas of ample lymphatic tissue representation where EBV resides.

Specific analysis concerning co-infection of EBV and HPV in the areas of the tonsils and base of the tongue in patients with oropharyngeal squamous cell carcinoma have found co-infection rates equal, respectively, to 25% and 70% [4].

It appears that oncogenic determinism by EBV in this neoplastic disease may, according to some theories, depend on induction of a process of epigenetic re-programming of the infected tumour cells, a phenomenon which might explain scarce identification of EBV in oropharyngeal squamous cell carcinoma.

4.3 Nasopharyngeal carcinoma (NPC)

NPC is a malignant tumour of the squamous epithelial cells of the nasopharynx, widespread in southern China, even though elevated frequency rates may also be found in Eskimo populations, the Maltese and some regions of North Africa.

Its association with EBV was first presumed on the basis of serum epidemiological investigations, from which it emerged that all patients with non-differentiated nasopharyngeal carcinoma possessed anti-VCA and anti-EA antibodies, especially against component D, and that they possessed, both in serum and in saliva, anti-VCA IgA.

At a later date, the presence of the viral genome was demonstrated in all bioptic samples of the undifferentiated tumour and EBV transforming products was found in the malignant epithelial cells of NPC in vitro cultures [8 9].

EBV reaches the epithelial cells by means of glycoprotein gH, which permits adhesion through a CD21-independent mechanism. Various research works, moreover, have put forward the hypothesis that EBV can infect, during primary infection, also the lymphocytes T and NK, which normally do not express CD21, but which acquire it, when activated by infected lymphocytes B, through synaptic transfer [10]. In subjects with normal function of the immune system, the latent infection at the level of the memory B cells permits only transcription of the EBERs, called latency 0 state.

In the latency 1 state, which is found in Burkitt's lymphoma, the EBV nuclear antigens - EBNA-1 and BamHI A, BARTs are also expressed.

In the latency type II state which is characteristic of Hodgkin's lymphoma and of NPC, EBNA-1, LMP-1, LMP-2, BARTs, and EBERs are expressed.

In latency type III, which is typical of lymphoproliferative disorders, all the latency genes are expressed, including EBNA-2 and EBNA-3. This state of latency is maintained only in conditions of immunodepression, such as in post-transplant or AIDS.

In nasopharyngeal carcinoma a few cells enter the lytic cycle of viral replication while most of them remain in stage II of latency.

The physiopathological relationship and molecular mechanism of EBV-mediated carcinogenesis has not yet been clearly defined. Usually EBV is found in latent form at the level of tumour cells, only rarely giving rise to a lytic form.

The viral production is limited to just three proteins: EBNA 1, latent membrane protein 1 and 2 – LMP1 and LMP2 –, as well as a series of small RNAs – EBER – and miRNA – [11].

Cells infected by EBV should normally be recognised by cytotoxic T lymphocytes specific for EBV, but in NPC this does not happen. Since EBV encodes for viral IL-10, which goes to increase production of IL-1 on the part of the epithelial cells and T lymphocyte helpers, contributing to the tumour growth and avoidance of immunological supervision [12].

The genetic mutations most frequently found in pre-cancer lesions which precede infection from EBV in the oncogenic process include allelic deletion on the 3p and 9p chromosomes, with deactivation of the tumour suppressor genes RASSF1A – 3p21.3 – and p16/CDKN2A – 9p21.3 –.

In correspondence with the region next to the p16/CDKN2A locus a miRNA has been identified, mir-31, constantly down-regulated in NPC, in the same way as occurs in progression of ovarian, prostate and mammary tumours.

Induction of NPC by the EBV has been associated with coexistence of genetic factors, especially the presence of haplotypes HLA-NS1N2, BW17-AW19, BW17 Ablank, as well as environmental factors such as diet rich in salt water fish or contamination of food and soil with phorbol esters.

The primitive site of the tumour is generally represented by the Rosenmuller fossa, even if it is difficult to detect it at this stage. As the matter of fact it tends to spread rapidly usually at the level of the upper lateral cervical lymph nodes, which show hard consistency and are unmoveable on the superficial and deep levels.

Occasionally phenomenon may become extrinsic to the nasal, auricular and neurological area through tumour invasion of the adjacent anatomical structures.

Diagnostic definition is typically histological, where the neoplasia may show up under three different forms:
1. well differentiated and keratinising squamous form;
2. non-keratinising form;
3. poorly differentiated form with abundant lymphocyte infiltration.

4.4 Epithelioma of the salivary glands

Infection from EBV may also be associated with manifestations of tumours other than NPC, such as epithelioma of the salivary glands, a rare carcinoma manifestation, which is endemic to southern China and the Inuit population of the Arctic [13].

This neoplasia presents with close histological similarities with non-differentiated non-keratinised nasopharyngeal carcinoma and is expressed mainly at the level of the parotid gland with lymph node infiltrations.

LMP1 protein can often be found in epithelioma of the salivary glands associated with EBV, with characteristics of mutation sometimes overlapping with those identifiable in nasopharyngeal carcinoma, such as C-terminal mutations, which appear to promote cell proliferation and increase the oncogenic strength of EBV [14].

4.5 Hodgkin's lymphoma

Hodgkin's lymphoma appears initially in the lymph node regions of the cervical cephalic area and markedly with cervical lymphadenopathy associated with fever, loss of weight, heavy sweating, widespread itching and asthenia. Diagnosis of this disease is based on the presence in the histological material of multi-nuclear giant Reed-Sternberg cells.

Association of the neoplasia with EBV infection can be found in approximately 40% of cases, where it is generally possible to detect high titres of antibodies against EBV, as well as the presence of the virus and related antigens in the Reed-Sternberg cells [15].

Treatment involves chemotherapy, radiotherapy or both, depending on the stage of the disease.

4.6 Laryngeal carcinoma

Various studies carried out by different authors have put forward the hypothesis of a probable pathogenic involvement of EBV in determining squamous cell laryngeal carcinoma.

Muderris et al. [19] in 2013, starting from this assumption, analysed EBV DNA by a molecular method, RT-PCR, in tumour tissues of patients afflicted with laryngeal carcinoma, to identify the effective causal role of the virus and co-pathogenic correlations between the EBV infection and living habits, use of tobacco or alcohol, glottic, supraglottic, sub-glottic site of the tumour and level of neoplastic differentiation, good, moderate or poor. The authors concluded that, in spite of the methods used, there was no clearly demonstrated correlation between EBV infection and squamous cell laryngeal carcinoma.

It is difficult to base diagnosis of neoplastic manifestations associated with EBV infection solely on clinical evidence, since they are not always clearly shown or anyway clearly distinguishable from other pathologies.

It is this context that places particular importance on histological and laboratory diagnosis, by demonstration of the virus, the viral antigen or viral DNA and serum antibodies.

The virus can be isolated from the saliva, from peripheral blood or from lymph node tissue, making use of its capacity for immortalising cultivated human lymphocytes.

The viral nuclear antigen, indicator of virus latency, may be found in lymph node tissue, in the nasopharyngeal carcinoma tissues and sometimes also in peripheral blood.

During the acute stage of the infectious disease, approximately 1% of the lymphocytes in peripheral blood contains an EBNA, searching for which in tissues by means of immunofluorescent technique is hindered by subjective interpretation and lack of availability of antibodies that are monospecific to the different components of the antigen complex. Such a wide interval is essentially attributable to factors of variability, such as geographical and ethnic differences, among them also the lack of precise, standardised codification of the important biological parameters, especially regarding the correlation between positive EBER and presence of OSCC. Other markers, though, offer greater probative indications, such as LMP1 and EBNA2 [16].

Even though the ororhinopharyngeal area acts as a residence and transmission site for EBV, cervical cephalic tumours associated with EBV are relatively rare, even if they are often mistakenly diagnosed, inasmuch as they express symptoms that may simulate those pertaining to non-oncological diseases.

The oncogenic role of EBV in associated tumours is of a cofactor nature that requires other genetic mutations and can be associated with other infections, such as malaria and HPV.

Infection from EBV is in any case a critical oncogenic event that is correlated to rapid tumour progression and to the metastatic phenotype.

The standard techniques for detecting EBV, however, are not always reliable because the expression of the EBV gene may vary in different settings. Incomplete viral associations are often reported, but may reflect the oncogenic effects of EBV in a micro-environment or loss of EBV during tumour development.

Epigenetic re-programming induced by EBV provides a mechanism to "strike and manage" the virus, where maintenance of oncogenic phenotypes occurs without the virus or of the viral expression of the gene.
Further studies will continue to define the contributions of EBV to initiation and progression of oral tumours and provide biomarkers associated with the virus for diagnosis, prognosis and therapeutic treatment.

5. HIV infection and ENT related disease

The HIV, which is responsible for AIDS is a retrovirus of the Lentivirus genus, belonging to the family of the Retroviridae. There are two distinct strains: HIV-1 and HIV-2, the former being ubiquitous and responsible for the typical form of AIDS, while the latter is widespread mostly in western Africa and in Asia and is responsible for a more attenuated form of the disease. Transmission of the virus occurs in 3 ways: sexual, bloodborne and vertical mother-to-child.
Sexual transmission occurs mainly through anal relations, either homo or hetero, a method considered more at risk since the anal epithelium is more fragile and more easily traumatised by the passage of the virus through micro-lacerations. Vaginal and oral relations are less risky.
Blood is a very important way of transmission, especially in more developed countries. This can happen through transfusions of infected blood and its derivatives (a method that is today drastically reduced owing to screening tests on blood collections) or between drug addicts who use injectable drugs involving promiscuous use of infected syringes.
Mother-to-child vertical transmission occurs usually at the moment of birth, when the baby comes into contact with its mother's blood. However, the possibility has also been demonstrated for transmission of the virus through the placental barrier during the first and second trimester of pregnancy. Another transmission possibility arises in the breastfeeding stage, since the virus is present in the colostrum and maternal milk.
The HIV target cells are lymphocyte CD4+ T helpers. The bond between virus and cell occurs through the surface protein gp120 of the CD4s. After binding the virus penetrates into the cell and its RNA is replicated through reverse transcriptase into double stranded DNA which is then integrated into the DNA of the guest cell. At this point the infected cell can immediately replicate the virus or remain in a latent stage of non-infection for a highly variable period.
The consequence of the viral infection is progressive depletion of the CD4+ cells, which leads to corresponding and proportional immunodeficiency which leaves the infected subject more easily predisposed to common infections and opportunistic infections, as well as being more susceptible to developing tumours.
At the start of the 1980s, when AIDS was described and the virus identified, the most typical manifestations of AIDS were the infections from opportunistic germs, above all pulmonary infections from Pneumocystis carinii, appearance in non-immunocompromised subjects of rare tumours, such as KS, and HIV encephalopathy or "AIDS-dementia complex". Since the 1990s and the introduction of antiretroviral drugs and the employment of therapeutic strategies involving the association of two or more of these antiviral drugs (the so-called HAART), clinical manifestations have changed, with paucisymptomatic forms appearing, but quite typical in HIV positive subjects, while the more typical forms of AIDS have become less frequent [1 2]. Nevertheless, recognition of these various pathologies, involving especially the head and neck area, is highly important, as it constitutes a form of monitoring the development of the disease. To make the natural history of HIV infection more complex, side effects of antiviral treatment may sometimes come into play, that is the so-called IRIS, an atypical reaction to infections after employment of antiretroviral therapy, with recovery of the CD4+ count [3]. This is a paradoxical worsening of the clinical picture in response to opportunistic infections. It is likely that during HIV infection with lowering of the CD4+ count the subject is colonised by slightly invasive opportunistic germs. After immune reconstitution, with increase in CD4+ and decrease in HIV, the immune system recognises the antigens of the opportunistic micro-organisms and activates the inflammatory reaction, which is really responsible for tissue damage and eventually worsening of the clinical picture. This manifestation benefits from anti-inflammatory and cortisone treatment.
The research was conducted on PubMed database. MeSH terms have been used, as well as free words, as shown in Box 1.
Using this research syntax, 2770 bibliographical citations were identified (research updated to 21.06.2017).
The term HIV, including its two serotypes HIV-1 and HIV-2 was introduced in 1988. The term AIDS was introduced in 1983.
Global bibliographical research thus obtained was filtered for "review" and "systematic review", giving overall 504 bibliographical citations (a figure which did not alter by filtering also for publication period after 1988).
Publications concerning case reports and therapeutic aspects were excluded from this first selection; works concerning generic aspects of HIV infections and not focused

Box 1. Research Strategy
Research Methodology

((“HIV Infections”[Mesh] OR “HIV”[Mesh]) OR (((((((“acquired immunodeficiency syndrome”[Title/Abstract] OR “acquired immune deficiency syndrome”[Title/Abstract]) OR “human immunodeficiency virus”[Title/Abstract]) OR “t cell lymphotropic virus type iii”[Title/Abstract]) OR “lymphadenopathy associated virus”[Title/Abstract]) OR “aids infection”[Title/Abstract]) OR “hiv infection”[Title/Abstract]) OR “hiv infections”[Title/Abstract])) AND ((((“Otorhinolaryngologic Diseases”[Mesh] OR “Otolaryngology”[Mesh]) OR “Head and Neck Neoplasms”[Mesh]) OR ((((((((((((((((((((((((“otorhinolaryngologic disease”[Title/Abstract] OR “otorhinolaryngologic diseases”[Title/Abstract]) OR “cholesteatoma”[Title/Abstract]) OR (“hearing disorder”[Title/Abstract] OR “hearing disorders”[Title/Abstract])) OR “herpes zoster oticus”[Title/Abstract]) OR “labyrinth diseases”[Title/Abstract]) OR “otitis”[Title/Abstract]) OR “otomycosis”[Title/Abstract]) OR “otosclerosis”[Title/Abstract]) OR “retrocochlear”[Title/Abstract]) OR “susac syndrome”[Title/Abstract]) OR “tympanic membrane perforation”[Title/Abstract]) OR “laryngeal”[Title/Abstract]) OR “laryngitis”[Title/Abstract]) OR (“vocal cord disorders”[Title/Abstract] OR “vocal cord dysfunction”[Title/Abstract])) OR “epistaxis”[Title/Abstract]) OR “nasal obstruction”[Title/Abstract]) OR “nasal polyps”[Title/Abstract]) OR (“nasal septal perforation”[Title/Abstract] OR “nasal septal perforations”[Title/Abstract])) OR (“paranasal sinus diseases”[Title/Abstract] OR “paranasal sinus disorders”[Title/Abstract])) OR “rhinitis”[Title/Abstract]) OR “rhinoscleroma”[Title/Abstract]) OR (“pharyngeal disease”[Title/Abstract] OR “pharyngeal diseases”[Title/Abstract] OR “pharyngeal disorder”[Title/Abstract] OR “pharyngeal disorders”[Title/Abstract])) OR (“deglutition disorder”[Title/Abstract] OR “deglutition disorders”[Title/Abstract])) OR “lemierre syndrome”[Title/Abstract]) OR (“nasopharyngeal disease”[Title/Abstract] OR “nasopharyngeal diseases”[Title/Abstract] OR “nasopharyngeal disorders”[Title/Abstract])) OR “pharyngitis”[Title/Abstract]) OR “velopharyngeal insufficiency”[Title/Abstract])) OR ((((((((((((((((“head and neck neoplasm”[Title/Abstract] OR “head and neck neoplasms”[Title/Abstract]) OR (“esophageal neoplasm”[Title/Abstract] OR “esophageal neoplasms”[Title/Abstract])) OR (“facial neoplasm”[Title/Abstract] OR “facial neoplasms”[Title/Abstract])) OR (“mouth neoplasm”[Title/Abstract] OR “mouth neoplasms”[Title/Abstract])) OR (“gingival neoplasm”[Title/Abstract] OR “gingival neoplasms”[Title/Abstract])) OR “oral leukoplakia”[Title/Abstract]) OR (“lip neoplasm”[Title/Abstract] OR “lip neoplasms”[Title/Abstract])) OR (“palatal neoplasm”[Title/Abstract] OR “palatal neoplasms”[Title/Abstract])) OR (“salivary gland neoplasm”[Title/Abstract] OR “salivary gland neoplasms”[Title/Abstract])) OR (“tongue neoplasm”[Title/Abstract] OR “tongue neoplasms”[Title/Abstract])) OR (“otorhinolaryngologic neoplasm”[Title/Abstract] OR “otorhinolaryngologic neoplasms”[Title/Abstract])) OR (“ear neoplasm”[Title/Abstract] OR “ear neoplasms”[Title/Abstract])) OR (“laryngeal neoplasm”[Title/Abstract] OR “laryngeal neoplasms”[Title/Abstract])) OR (“nose neoplasm”[Title/Abstract] OR “nose neoplasms”[Title/Abstract])) OR (“pharyngeal neoplasm”[Title/Abstract] OR “pharyngeal neoplasms”[Title/Abstract])) OR (“parathyroid neoplasm”[Title/Abstract] OR “parathyroid neoplasms”[Title/Abstract])) OR (“thyroid neoplasm”[Title/Abstract] OR “thyroid neoplasms”[Title/Abstract])) OR (“tracheal neoplasm”[Title/Abstract] OR “tracheal neoplasms”[Title/Abstract])))

only on aspects of interest in otorhinolaryngology, as well as descriptive works unsupported by adequate statistics, were also excluded. For this work, 123 publications were considered valid, which cannot all be cited for publishing reasons.

Research was also conducted on grey literature (conventions, official congress reports, communications etc.) This research generated extrapolations from the records of the XVIth National Convention for Updating of the Association of Italian Hospital Otorhinolaryngologists (A.O.O.I.), the Internal Guidelines of the Integrated University Health Organisation of Udine drafted by the S.O.C. Clinic of Infectious Diseases [4] and a chapter of the Official Report of the XCIInd National Congress of the Italian Society of Otorhinolaryngologists and Cervical-Facial Surgeons.

5.1 General aspects

After contagion, the virus may remain silent for a considerable time, or appear as an acute infection (primary infection) with non-specific symptoms and signs however. The HIV primary infection picture is characterised by: widespread lymphadenopathies with rear lateral cervical areas the most affected, high temperature, asthenia, lethargy, night sweating, pharyngitis, maculopapular rash, ulceration of the mucous membrane, loss of weight and diarrhea [5 6]. Given that the clinical picture is non-specific, recognition of the HIV infection may prove to be difficult, but persistent high temperature with no apparent reason should lead the clinician to confirm or not the HIV infection through serological investigations.

Subsequent true clinical manifestations of HIV and of AIDS are strictly correlated to blood levels of lymphocyte T helpers and are more serious according to how low the CD4+ count is [7]. The more serious clinical forms appear when the CD4+ count falls below 200/mmc, for example: Pneumocystis carinii pneumonia, Toxoplasmosis, multifocal leukoencephalopathy, infections from atypical mycobacteria, molluscum contagiosum and bacillary angiomatosis. In the more advanced stage of HIV disease (full blown AIDS), when the CD4+ count is lower than 50/mmc, patients are at risk of: Pseudomonas pneumonia, Cytomegalovirus retinitis, central nervous system lymphoma, disseminated aspergillosis and histoplasmosis.

The CDC, control organs of the US public health system, has drawn up a classification, shown in Table 5.I, which includes crossing the CD4+ count with clinical manifestations to define various clinical categories of progressive seriousness [8].

The spread of HIV may be considered endemic in sub-Saharan African regions and in the 1990s a considerable increase was seen in Southeast Asia [14]. Since the mid-1980s the method of transmission of HIV has undergone a considerable change: the proportion of consumers of narcotics by injection has fallen from 76.2% in 1985 to 5.3% in 2012, while cases attributable to sexual transmission have risen. In particular, the cases attributable to heterosexual

transmission have risen from 1.7% in 1985 to 42.7% in 2012 and cases attributable to homosexual transmission between males over the same period have risen from 6.3% to 37.9%. In Italy in 2012 3,853 new diagnoses of HIV infection were reported, with incidence of 6.5 new cases per 100,000 residents (lowest incidence, of 0.6%, was seen in Calabria, while the highest, 10.5%, was in Lombardy), which has been substantially a stable incidence over recent years. The most representative age group is that of 25-34 year-old (36.1% of total cases, with incidence of 17.1/100,000). The male sex is more affected, with a M/F ratio of 3.8 in 2012 [4].

At the start of the 1980s HIV infection appeared mainly as AIDS and was burdened with high mortality from disseminated opportunistic, viral, bacterial, mycotic and parasitic infections, as well as from rapid development of neoplastic disease. After the introduction of antiretroviral therapy, especially HAART, the natural history of the disease changed. Apart from the possibility of encountering asymptomatic HIV positive subjects, clinical pictures are very often paucisymptomatic and the serious manifestations may be seen many years after contagion. If on the one hand improved prognosis and increased survival can be seen, on the other hand in HIV+ subjects, immunodepressed anyway, an increase can be seen in incidence of neoplastic diseases, also those non-AIDS correlated [9].

This development of the natural history of HIV disease has meant that today's Otorhinolaryngologist plays a role of primary importance in supporting the Infectious Disease Specialists and Internists in monitoring afflicted patients and in making early identification of the first signs and symptoms of the disease, because the onset of the disease often appears, in fact, in the head and neck area, with percentages that easily reach 80% of cases [5 10].

5.2 Clinical ENT manifestations from HIV

Oral cavity and oropharynx

Lesions in the oral cavity and, by anatomical continuity, of the oropharynx constitute the most frequent and widespread diseases that may be found in HIV+ subjects, so much so that they play a crucial role in early diagnosis and monitoring of disease from HIV. The amount of attention paid to clinical manifestations in the oral cavity during HIV infection is borne out by the abundance of scientific literature published over the years and in various geographical areas [11-18].

Although the various oral diseases described are found everywhere, there are certain geographical differences between emerging countries and developed or industrialised countries. Oral candidiasis remains the most common opportunistic infection seen on the whole continent. Oral KS prevails in Africa and in Latin America, while histoplasmosis and penicilliosis have been found in patients with advanced disease in Thailand. Disease of the salivary glands associated with HIV has high prevalence in Africa and in Latin America, especially in the paediatric population [19].

In Europe and the USA, after the introduction of HAART, a fall was seen of 10-50% in oral manifestations, especially candidiasis. In the USA and in the United Kingdom, however, an increase was reported for oral verrucas, while in both the USA and in Europe a growing trend has been observed in disease of the salivary glands correlated with HIV [20].

Although it is undisputed that oral manifestations have decreased, particularly in more developed countries, they remain observable in HIV+ subjects. The re-emergence of these lesions leads to suspicion of the failure of the thera-

Table 5.I. CDC classification for diseases from HIV.

	Clinical categories		
no. of CD4+	**A: asymptomatic, acute HIV infection**	**B: symptomatic infection, non A - non C * conditions**	**C: symptomatic, conditions indicating AIDS**
1: > 500 cell/mmc	A1	B1	C1
2: 200-499 cell/mmc	A2	B2	C2
3: < 200 cell/mmc	A3	B3	C3

** Clinical conditions characterising category B:*

- bacillary angiomatosis;
- oropharyngeal candidiasis;
- vulvovaginal candidiasis, persistent, frequent or poorly responsive to treatment;
- cervical dysplasia (moderate or severe/carcinoma in situ);
- constitutional symptoms e.g. persistent high temperature (38.5°C) or diarrhea > 1 month;
- oral hairy leukoplakia
- multimetameric or recurring herpes zoster
- idiopathic thrombocytopenic purpura
-listeriosis
-pelvic inflammatory pathology, especially if complicated by tube-ovarian abscesses
-peripheral neuropathy

py; they have, however, retained their predictive validity, but on the other hand, there are more sophisticated and easier to use diagnostic technologies available, so their diagnostic function is reduced.

Some oral manifestations during HIV infection tend to appear with different incidence between the two sexes, as reported by an ad hoc review [21]. The studies taken into consideration showed differences by geographical location, category of risk of transmission of the virus and stage of the disease. Overall, in at least 15% of women infected with HIV presenting with oral lesions, candidiasis remained the most frequent lesion. In women it is less common to find oral Kaposi sarcoma and oral hairy leukoplakia.

There now follows description of the various oral and oropharyngeal lesions found in HIV+ subjects.

Oral ulcers. A large part of the oral ulcers found in HIV+ subjects are caused by Herpesviridae [5 18 22 23].

Primary infection from Herpes simplex virus (HSV), usually type 1, presents with fever, lymphadenopathy, gingivitis and painful oral lesions originating as blisters which give rise to ulcerous lesions when they burst. The recurrent form may affect the lips or intraoral mucosa. In the first case the blisters develop into ulcerous-crusted lesions. Intraoral lesions are confined to small groups, mainly to the keratinised mucosa (gums, hard palate, dorsal surface of the tongue). After the blisters have burst the ulcers are painful and in HIV+ subjects can last for as long as several weeks.

Re-activation of the Varicella-zoster virus may present with a prodrome of dental pain, preceding the eruption of the typical ulcerous-crusted lesions in the area of one or more branches of the trigeminal nerve.

CMV ulcers are similar to aphthous lesions and appear in subjects with CMV disseminated infection. Diagnosis is based on microscope investigation with immunohistochemistry with the discovery of typical intranuclear or intracytoplasmic inclusions.

Recurrent aphthous ulcers are not more frequent in HIV+ subjects compared to the rest of the population, but they are more serious and more prolonged. Unlike HSV these ulcers involve the non-keratinised oral mucosa, such as the labial and buccal mucosa and the lateral margins of the tongue. Aphthous ulcers, encircled by an erythematous ring with pseudomembranous base can even reach 1 cm in diameter. The dimension and persistence of the ulcer often requires bioptic examination to exclude malignant lesions or opportunistic infections [18].

Oral hairy leukoplakia (OHL). This lesion is characterised by corrugated, asymptomatic, non-removable white patches which typically localise on the sides of the tongue but may also affect the ventral face of the tongue, the floor of the mouth and other parts of the oral mucosa [5 18]. OHL is less common in women than in men and is rare in young children. This lesion is typical of HIV+ subjects, even if it is not exclusive, since it is described in immunodepressed subjects for other reasons and sometimes in immunocompetent subjects [24 25]. Although the presence of OHL is not always an indication of rapid progression towards full-blown AIDS in the absence of antiviral treatment, its frequency of appearance is proportional to the level of deterioration of the CD4+ count [18 24].

The EBV gamma-herpesvirus has been recognised as an etiological agent of OHL [26] and evidence of its presence in lesions is needed in order to confirm diagnosis [18].

From a histological point of view, OHL presents with hyperkeratosis, with thickening of the stratum spinosum (acanthosis), with groups of globular koilocyte-like keratinocytes, with the absence of atypia or other signs of dysplasia and without inflammatory cell infiltrate in the epithelium or adjacent connective tissues. From an ultrastructural point of view the surface globular keratocytes contain numerous nucleocapsids of the herpesvirus [27].

Usually in permissive herpesvirus infections (that is, when the cell allows complete viral replication), abundant viral replication leads to cell lysis. In OHL a new state of EBV infection has been found, with concomitant expression of replicant and transforming proteins, which together contribute to development of the lesion and induce many of the histological characteristics of OHL, such as acanthosis and hyperproliferation. Unlike other infections of permissive herpesvirus, expression of EBV transforming proteins inside the OHL tissue, permissively infected, enables survival of the epithelial cells and may improve viral replication [28].

Oral candidiasis. This disease remains one of the most frequent manifestations of otorhinolaryngological interest in HIV+ subjects, even after the introduction of antiretroviral drugs. In a study of 122 patients with advanced AIDS, extensive oral mycotic colonisation was found in 81.1% of cases, a third of which were symptomatic, and in 25.3% resistance to antimycotic treatment was also found [29].

Candidiasis may appear in four different forms: pseudomembranous, erythematous, hyperplastic and angular cheilitis [5 18].

Pseudomembranous candidiasis is characterised by a removable white patch, formed from a mixture of fungal hyphae, desquamated epithelium and inflammatory cells. This form may affect every part of the oral and oropharyngeal mucosa.

Erythematous candidiasis appears as small reddish de-

papillated areas on the palate and dorsal surface of the tongue. Identifying this form is important as a sign of progress in HIV infection.
Hyperplastic candidiasis is rarer and appears as white non-removable plaque and may be confused with an OHL; unlike this however the mycotic plaque is homogeneous and without corrugation.
Angular cheilitis appears as a fissured hyperemic lesion at the level of the mucosa of the labial folds and may be monolateral or bilateral.
The most common symptoms are oral soreness and dysgeusia. Oral candidiasis may often spread to the pharynx and esophagus, which is an indication of worsening of the HIV infection. In this case the predominant symptoms are dysphagia and retrosternal chest pain. Oral candidiasis is quite common also in HIV+ young children.
The most common etiological agent is Candida albicans, even if other species have been isolated, such as Candidakrusei and Candidadubliniensis [30]; Candida albicans is a normal saprophyte of the oral cavity. Various studies have been conducted to understand the activation of Candida virulence during HIV infection. Data collected up to now indicates that immune factors, both systemic and local, are important for control of infections from opportunistic micro-organisms [31 32]. The most recent data [32] indicates that the sub-class Th17 of the T-helpers plays an important role in controlling the virulence of the fungus. Selective loss of these leucocytes with progress of the HIV infection causes decay of containment of the fungus on the oral epithelium and enables Candida a. to express its pathogenic potential. In addition, HIV infection seems to generate a selective, favourable environment for overexpression of potential virulence of the fungus, especially favouring production of particular enzymes such as aspartyl-proteinase. These enzymes can degrade critical defensive components of the host as defensive complement and epithelial protein, like histatin 5 and E-cadherin. It seems that this strengthening of virulence of Candida is partly induced by the bond between the fungus and HIV or to a protein induced by HIV, such as the GP160 found in the mantle of the virus.
Other mycoses. Sometimes in the advanced stage of HIV infections, certain forms of invasive mycoses may appear on the oro-facial tissues, such as histoplasmosis, cryptococcosis, aspergillosis and zygomycosis. Oral lesions are typically chronic, but not specific and may present nodular or ulcerative lesions [33].
Periodontal diseases. In HIV+ subjects unusual periodontal diseases may be observed, which may also be very serious. The two forms most described are: linear gingival erythema and necrotising ulcerative periodontitis [18 30 34].
Linear gingival erythema (also known as gingivitis from HIV) is constituted from a red band affecting the gingival margin and may affect both adults and young children. It does not seem to be linked to an inflammatory process and is frequently not associated with accumulated plaque. Rather than a real gingivitis it seems this hyperemia is caused by vasoactive action of cytokines.
Necrotising ulcerative periodontitis (also known as periodontitis from HIV) is a more serious lesion; it causes pain, gingival bleeding, rapid extensive destruction of gingiva, periodontal tissue and the alveolar bone, with consequent loss of teeth.
Oral verrucous lesions. In recent years an increase in oral verrucas has been observed in HIV+ subjects, in spite of the introduction of HAART treatment [35]. It is now clear that HPV infection is associated with multi-form hyperplastic nodular or papular oral lesions in these patients. Verrucas may appear as exophytic lesions similar to cauliflowers or as pointed lesions, or again as a wide, flat form including the papillomas, the verruca vulgaris and the focal epithelial hyperplasia (Heck's disease). Focal epithelial hyperplasia affects young children mostly and has been observed in various countries spread around the world, including Italy, but mainly among North, Central and South American natives and their descendants. Clinically it presents with multiple papules and plaque, flat, asymptomatic pink coloured, soft, smooth or rough surface and of dimensions between a few millimeters and a few centimeters. The site is in the oral mucosa: cheeks, tongue, gingiva, but above all, the mucous side of the lower lip. Condyloma acuminatum, a disease which is typically transmitted sexually in the context of non HIV+ subjects, has also increased considerably. These lesions are all associated with different types of HPV, in particular serotypes 13, 32 and 7 (this serotype is primarily associated with butcher's warts) [28]. Various other serotypes have then been isolated from time to time.
Hypertrophy of the lymphoid rhino-oropharyngeal tissue. An initial sign of HIV infection may be hypertrophy of the lymphoid tissue of Waldeyer's ring. These tissues constitute one of the main sites of replication of the virus and its presence characterises certain histopathological aspects, such as florid follicular hyperplasia, follicular lysis, presence of multi-nucleated giant cells which probably originate from dendritic cells, seats of intense viral replication [35 36]. From a symptomatological point of view the patient may complain of obstruction of airways, pharyngitis, fever. Increase of lymphoid mass may simulate a neoplastic picture, so a biopsy is often required. Immunohistochemical investigations carried out on lymphoid tissue taken from HIV+ subjects have revealed the pres-

ence of protein of viral derivation and absence of other micro-organisms.

Nasal and sinusal manifestations

Allergic rhinitis. Exacerbation of allergic rhinitis appears in HIV+ subjects twice as frequently as in non-infected subjects [5 37]. It is probable that, during HIV infection, imbalance is created between sub-classes of T-helpers Th1 and Th2 in favour of the latter group [38]. The Th1 may be implicated in control of infections, while the Th2 may help plasma cell production of IgE and would therefore be responsible for the allergies. Atopic subjects with current asthma usually present less serious depletion of CD4+, with a count no lower than 200 cells/mmc, while for allergic rhinitis there is no correlation with depletion of CD4+ [39].

Sinusitis. Acute recurrent sinusitis and chronic sinusitis are very common in HIV infected subjects, with percentages reaching even 68% [40]. The clinical picture of sinusitis in HIV+ subjects does not differ from that of the common population. In infected subjects, various factors may contribute to the onset of rhinosinusitis: decay of cell mediated and humoral immune function, increase in allergic reactivity, decrease of mucociliary function [41].

In the initial stages of the disease, while the HIV+ subject's defences are still only slightly compromised, rhinosinusitis is sustained by the common bacteria (Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenzae) which afflict the general population. When the immune defence is compromised, that is, when the CD4+ count falls below 200/mmc, patients become more susceptible to opportunistic infections [42]. In these cases, carrying out imaging investigation (CT and NMR) is especially important in assessing the level of invasion of adjacent structures: orbit and anterior cranial fossa. For this reason, rhinosinusitis in HIV+ subjects must be promptly and thoroughly treated with suitable antimicrobic therapy and if necessary also surgically, by means of FESS.

Other rhinosinusal infections. a) Leishmaniasis. This is a parasitosis sustained by protozoa of the Leishmania genus and is transmitted by the bite of the female sandfly. Although this disease is endemic to particular geographical areas, it may be found in almost the whole world (except the Antarctic and Oceania) and interest in it has increased due to its appearance in immunodepressed subjects, such as HIV+ subjects. In humans there are three distinct clinical forms of Leishmaniasis: cutaneous, muco-cutaneous and visceral. It is the muco-cutaneous form that is of special interest to the otorhinolaryngologist [43]. The muco-cutaneous form is caused by the extension of cutaneous lesions to the mucosa, by contact or by hematic or lymphatic means. The nasal mucosa is more affected, followed by oral, pharyngeal and laryngeal involvement respectively. Initially, nasal involvement appears as a common rhinitis with nasal obstruction and epistaxis. Necrotic phenomena take over subsequently, with destruction of the septum and nasal deformation. The disease may progress to affect other muco-cutaneous tissues with ulcerations and serious mutilation of the face. b) Histoplasmosis. As already mentioned in the section on disease of the oral cavity, this is a mycosis caused by Histoplasma capsulatum. Disseminated histoplasmosis is very widespread in South America and can be found in 70-90% of cases of full blown AIDS [44 45]. It may appear both on a cutaneous and a visceral level, affecting the mucous membranes of the oral cavity, pharynx and larynx. Nasal localisation is rare, but it has been described as a first sign of disease in subjects with AIDS, presenting as septal ulceration [44]. Although rare, nasal localisation requires differentiated diagnosis from other manifestations of HIV infection, such as Leishmaniasis and nasal lymphoma or with granulomatous diseases, such as Wegener's granulomatosis [45]. Diagnosis is both histological and microbiological. c) Infection from Acanthamoeba. This is an opportunistic parasitic infection which, in rare cases, can give rise to rhinosinusitis in immunocompromised subjects, such as in cases of advanced HIV infection [46 47]. This form is almost always fatal. Presenting as a destructive lesion, differential diagnosis must be made mainly from the neoplastic forms.

Laryngeal manifestations

At the level of the larynx, in HIV+ subjects no lesions are found that are typical or very suggestive of HIV infection, such as, for example, OHL for the oral cavity. Various diseases, opportunistic infections or rare neoplasias have been described, such as, for example: tuberculosis, histoplasmosis, lymphoma, Kaposi's sarcoma, candidiasis, aspergillosis, actinomycosis [5 48]. The symptomatological picture is that linked to anatomo-functional impairment of the larynx (dysphonia, inspiratory dyspnea, dysphagia, odynophagia, ab ingestis phenomena). Differential diagnosis must be made principally with squamous cell carcinoma. Diagnostic investigations are the routine ones: laryngoscopy with biopsy and imaging (CT and/or NMR). In HIV+ subjects there is special interest in laryngeal tuberculosis, inasmuch as this is a rare disease and its discovery may make the clinician suspicious and lead him/her to continue with serological investigations to confirm or not the HIV infection. The tubercular lesion usually appears as a whitish lesion with a wide base, exophytic,

without erythema or edema, rarely presenting ulcerated or erythematous.

Cutaneous manifestations

Occasionally dermatological manifestations during HIV infections can be the first sign of an asymptomatic disease, but indication of advanced immune deficiency; these may be signs of opportunistic infections or neoplasias. The variety of symptoms and signs of cutaneous lesions which may involve the head and neck area are a consequence of progression of HIV-correlated immune deficiency and therefore a clinical parameter of monitoring of the underlying disease [49].

Bacillary angiomatosis. This disease, which was only described from 1983 on and especially in HIV+ subjects, is the most frequent cutaneous manifestation, second only to Kaposi's sarcoma, associated with immune deficiency from HIV, then also described in other forms of immunodepression. The etiological agent is Rochalimaea or Bartonella henselae [50]. This disease is characterised by protruding cutaneous lesions, reddish, berry shaped, often surrounded by a squamous collar. If they are traumatised, the lesions bleed abundantly. Frequently there are lymphadenopathies present, especially in the lateral cervical areas. Other signs and symptoms consist of: fever, anemia, pulmonary nodules, pleural exudate, ascites, bacteremia, endocarditis, encephalopathy, involvement of muscles, bones, liver or spleen. This disease responds well to antibiotic treatment with macrolides or doxycycline.

Seborrheic dermatitis. Diffuse seborrheic dermatitis is very frequent in HIV+ patients, usually affecting the face and scalp, but it may sometimes appear at the level of the outer ear [5]. Treatment is based on the use of cortisone creams or containing ketoconazole, but the characteristic in HIV+ subjects is to be impervious to treatments. The areas involved by seborrheic dermatitis must in any case be cleaned, because they are often subject to superinfections.

Other cutaneous manifestations. Bacterial infections of the skin, especially by Staphylococcus aureus, are very common in subjects infected by HIV. These infections appear as impetigo, folliculitis or cutaneous abscesses. Systemic infections from Cryptococcus, Histoplasma capsulatum and intracellular Mycobacterium avium may all present with cutaneous lesions [5].

Cutaneous tumours, in particular Kaposi sarcoma, will be dealt with in the section dealing with head and neck masses.

Otologic and audio-vestibular manifestations

Damage to the auditory apparatus may derive from recurrent common infections or from opportunistic infections (middle and outer ear), from possible ototoxic action of drugs or from action of HIV itself on the central nervous system (inner ear and vestibular system) [51 52].

Disease of external ear. Incidence of external otitis is not increased in the population afflicted with AIDS. Most of the external ear infections found in HIV+ subjects are caused by common micro-organisms and are resolved with standard treatment [53]. The etiological agents in order of frequency are: Pseudomonas aeruginosa, Proteus and Aspergillus species. The clinical picture is the typical one: intense otalgia with hearing loss and blocked ears, purulent otorrhea, edema and hyperemia of the EAC.

It should not be forgotten, however, that HIV+ subjects, since they are immunodepressed, as are diabetic subjects, may encounter malignant external otitis and osteomyelitis of the cranial base, which can often prove fatal.

A few cases of Kaposi sarcoma have been described also involving the outer ear.

Disease of the middle ear. Patients afflicted with HIV can present acute and chronic otitis media, both effusive and cholesteatomatous, with an incidence no different from that of the non-infected population [5 54]. However, HIV+ subjects are more predisposed to recurrence and to longer course of the infection.

A particular form of chronic otitis media found in HIV+ subjects is that sustained by Pneumocystis carinii, which exploits the middle ear as a possible extrapulmonary seat [55 56].

This type of chronic otitis, mono- or bilateral, presents with persistent polypoid formation, sometimes destructive of the bone tissue, perforation of the tympanic membrane, otorrhea, otalgia and transmissive or mixed hearing loss. It may become complicated with invasion of the middle cranial fossa. Diagnosis requires histological examination. Treatment is mainly based of the use of cotrimossazole.

Sensorineural hearing loss. Sensorineural hearing loss can be a consequence of an infection directly involving the inner ear (viral forms) or the central nervous system [9]. Perceptive hearing loss in HIV subjects varies considerably on the basis of case studies and the stage of the disease. It is probable that a greater percentage is found among HIV+ subjects that report their hearing disturbance. A group of 30 HIV positive subjects in stages II and III of the disease were evaluated by means of vestibular tests, as well as with conventional tonal and high frequency audiometry. Results have shown normal traditional audiometry, while high frequency audiometry has shown a deficit in 23% of the subjects.

The micro-organisms that can determine sensorineural hearing loss are: Pneumocystis carinii, Candida albicans, Staphy-

lococcus aureus, Mycobacterium tuberculosis, Toxoplasma gondii, Cryptococcus neoformans, Treponema pallidum, herpesviruses such as HSV, VZV, CMV, and HIV itself.

The Varicella-zoster virus is responsible for the Ramsay-Hunt syndrome (intense otalgia, hearing loss, paralysis of the facial nerve and vertigo) and this picture can sometimes be the first manifestation of HIV infection.

Meningitis from cryptococcus is a rather insidious infection, accompanied by headache, alteration of the mental state and convulsions. It may be associated to sudden or progressive hearing loss.

Subjects with HIV infection in course, who have contracted Treponema pallidum, present rapid worsening of neurosyphilis which is often accompanied by hearing loss [5].

HIV itself has been implicated in directly causing neurological damage. So-called HIV encephalopathy or AIDS dementia complex, are known to be a set of symptoms and neurological signs whose main clinical component is development of progressive dementia. Apart from dementia, patients with HIV encephalopathy can present motor alterations, uncertain gait, lack of balance and difficulty in effecting rapid alternating movements. HIV has been demonstrated in the brain and in the cerebrospinal fluid of infected individuals, with or without neuropsychiatric anomalies. However, the virus does not directly infect the neuronal cells, but those of the perivascular macrophage line and the microglia cells: monocytes that are already infected in the blood can migrate to the brain, where they resettle as macrophages, or, like macrophages, they can directly infect inside the brain. Sensorineural hearing loss in infected subjects can be included among HIV-correlated neurological diseases.

Sensorineural hearing loss can be an indirect sign of neoplastic manifestations (lymphoma, Kaposi sarcoma) which involve the central nervous system.

The iatrogenic role played by antiretroviral drugs on the auditory apparatus is uncertain, in a recent review no significant association was found between antiretroviral treatment and hearing loss [57]. However, some drugs used for treating infections afflicting HIV+ subjects may have ototoxic effects, one of these being aminoglycoside.

Vestibular disorders. With HIV infection in course, the vestibular system can be also involved just as the central nervous system and the auditory apparatus. Initially, the data reported in the literature were not unequivocal, which was probably due to the type of population investigated. HIV positive subjects but not declared as vertiginous, when subjected to oculomotor and electronystagmography investigation, presented oculomotor disorders in 27% of cases, but with caloric test normal. Yet subjects who complained of subjective vertigo in various stages of the disease on the CDC classification [8], presented substantially stable peripheral disorder in the various classes (33.3% in class A, 50% in classes B and C), while in the advanced forms of disease (classes B and C) central vestibular damage was much more evident [57 59]. Another study conducted on asymptomatic HIV+ subjects from a vestibular point of view, in various stages of the disease compared with non-infected subjects and investigated using posturography and electronystagmography, detected involvement of the whole vestibular system, both peripheral and central, in the early as well as in the advanced stages of the disease, while not detecting characteristic and specific vestibular alterations in subjects with AIDS [60]. A recent systemic review [61] detected peripheral vestibular involvement in at least 50% of patients afflicted with AIDS and central vestibular involvement in an even higher percentage. Post mortem studies have documented involvement of the whole vestibular system. Opportunistic infections such as otoneurosyphilis and encephalitis cause secondary vestibular disorders, resulting in objective vertigo and imbalance.

Thyroidal manifestations

The most common thyroidal hormone alteration found in HIV+ subjects, including the initial stage of the disease, is the lowering of blood levels of reverse-T3 (rT3) and an increase in thyroglobulin, while free T3 remains unaltered; in the advanced stages of the disease free T3 is also lower [62 63]. After immune reconstitution following HAART, dysregulation of the autoimmune response may appear, which may in turn encourage the onset of Basedow-Graves disease in 1-2% of patients [63]. The thyroid may also be the seat of opportunistic infections which usually lead to subacute thyroiditis.

Head and neck masses

Parotid disease. HIV+ subjects can be afflicted with a typical acquired form of cystic disease: lymphoepithelial parotid cyst (Fig. 5.1), which presents with increased mono- or bilateral volume of the parotids, usually asymptomatic, but which may sometimes generate pain [5 18 64]. Lymphoepithelial cyst is a benign form and seems to originate from the peri- and intraparotid lymph nodes. Lymphoid hyperplasia is probably encouraged by an increase in CD8 lymphocytes as a response of the host to infection. Lymphoid hyperplasia may cause obstruction of the salivary ducts, squamous metaplasia and formation of cysts. This manifestation, which may affect both adults and young children, is closely correlated to diffuse lymphadenopathy and is practically pathognomonic with

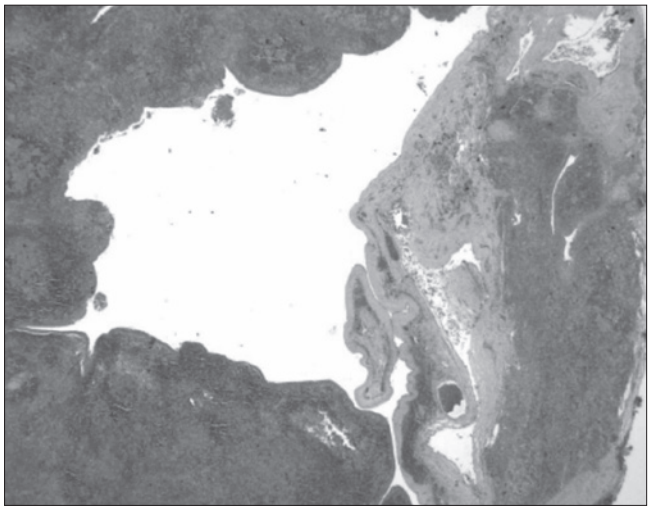

Fig. 5.1. Histological section of lymphoepithelial cysts of the parotid from HIV.

HIV infection. Diagnosis is based principally on needle aspiration cytology, backed up with imaging investigation (Ultrasound, CT and NMR). In advanced forms xerostomia may be present. When enlargement of the parotid becomes important, with noticeable cosmetic alteration and symptomatology (pain and xerostomia), a therapeutic option may be taken into consideration involving: surgery (preservative superficial parotidectomy), sclerosant injections, radiotherapy.

Other tumoral masses of the parotid. A study in the US detected a higher risk of incidence of lymphoepithelial carcinomas and squamous cell carcinomas in the parotid in subjects afflicted with AIDS, probably because of activation of EBV, a virus known to be potentially oncogenic, which localises also in the salivary glands [99].

Head and neck tumours. Certain tumoral diseases are strictly correlated with AIDS, such as KS, which will be considered later. For other tumours, defined as non-AIDS, that is those commonly found in the general population, data taken from the Scientific Literature published over the years is not completely unequivocal. The common trend is that HIV+ subjects are more exposed than the general population to developing any kind of neoplasia, including those of the head and neck area (oral cavity, oropharynx and larynx) [66 67]. The forms of tumour most frequently found are lymphomas and tumours strictly correlated with viral infections or with known carcinogenic substances such as cigarette smoke. Just 10 years after the emergence of AIDS, an association was reported between the infectious state of HIV and increased risk of HNSCC [68]. Subsequently, the correlation between HIV and HNSCC looked uncertain, but it seemed more evident that tumours appeared at a younger age in HIV+ subjects [69].

Thanks to the possibility of treatment offered by HAART, HIV+ subjects lived longer and could be observed over longer periods. In spite of HAART treatment, these subjects were more exposed to tumours of the head and neck with these characteristics: younger age groups afflicted, stage of disease already advanced on presentation, clinical course of disease more aggressive, high consumption of tobacco in afflicted subjects [70].

As well as consumption of alcohol and tobacco, HPV infection, particularly serotype 16, with possibility of genetic modifications, constitutes a risk factor for HNSCC in HIV+ subjects. Cell proliferation may be increased by viral interference with the tumour-suppressor protein (p53, Rb) [71]. Over the last two decades, HPV has taken on a front-line role in diagnosis of oropharyngeal carcinomas in the general population, since its discovery makes the tumour more susceptible to radiotherapy, with a better prognosis. HPV is very widespread in HIV+ subjects, which makes the risk of head and neck tumours correlated with this virus decidedly higher, with values ranging from 1.5 to 6, according to the case studies [72 73].

The nasopharyngeal carcinoma is another tumour associated with viral infection, sustained by EBV. A study in the US showed risk of incidence of around 2.4 higher in HIV+ subjects than in non-infected subjects, both for the keratinising form and the non-keratinising form [65]. A study conducted in South Africa, where HIV is endemic, moreover, gave the opposite result, inasmuch as malignant tumour was detected in a markedly higher percentage in HIV negative subjects, while in HIV+ subjects simple lymph node hyperplasia was prevalent [74].

A recent retrospective review, conducted in Nigeria, a sub-Saharan state where HIV is endemic, on 119 patients (47 HIV+ and 72 HIV negative) biopsied for oropharyngeal neoplastic disease, showed that in HIV negative subjects SCC were prevalent, while in HIV+ subjects lymphomas prevailed, both non-Hodgkin and Hodgkin, with statistically significant differences [75].

From the data derived so far, the following summary can be made:

- HIV+ subjects are more predisposed in general to display tumoral disease, not only rare forms typical of AIDS, but also those non-AIDS;
- among these tumours, the ones that are effectively more frequently found, at least in the head and neck area, are lymphomas, which will be discussed further on;
- the SCCs do not seem to be more frequent in HIV+ subjects than in the general population, even though

they are more susceptible to activation of potentially oncogenic viruses. Other co-factors are probably necessary to determine the onset of a tumour, proof of which comes from the emergence of the fact that exposure to tobacco smoke and alcohol constitutes a serious risk factor for HIV+ subjects. Once a HNSCC has arisen, though, it proves more aggressive and with a worse prognosis, probably taking advantage from immune deficiency of HIV+ subjects.

Cutaneous carcinomas. Patients infected by HIV are more predisposed than the general population to developing basal cell and squamous cell carcinomas of the cutis [5 76]. Subjects with clear skin and excessive exposure to the sun have a higher risk of developing cutaneous carcinomas. Squamous cell carcinomas seem to have a particular habit of developing in the head and neck area and appearing in the more advanced stages of HIV disease, while basal cell carcinomas develop mainly at the level of the trunk and may appear in all the stages of HIV infection. As in the general population, protein p53 is overexpressed. Treatment consists of surgical excision.

Kaposi Sarcoma (KS). This is an angioproliferative disorder involving spindle cells, neoangiogenesis, edema and inflammation. There are 4 distinct variations: classic, African endemic, associated with immunodepression or with transplant, associated with AIDS. The last form, unlike the other three, is very frequent in the head and neck area. KS is a tumour which behaves uncertainly, since some of the characteristics of malignancy are missing [77].

The angioproliferative disorder typical of KS is caused by the infection of mesenchymal stem cells by Herpesvirus HHV-8 [18 28 71 77]. It was previously believed that a pathogenic role was sustained by increased concentration of glucocorticoids in the blood and saliva of HIV+ subjects [78]. This theory derived from the observation that AIDS-associated SK cells present a high level of receptor protein for glucocorticoids and that the growth of cells in cultivation was stimulated by the glucocorticoids above all in the presence of growth factors such as oncostatin-M. According to the Authors, this pathogenesis was not in conflict with the co-existence of HHV-8 infection. However, the theory was not taken up nor confirmed by subsequent studies, leaving the leading role in the etiopathogenesis of KS to HHV-8 infection. This virus is widespread all over the world, but is extremely frequently found in homosexual males. However, HHV-8 is present in the oral epithelium and is abundant in saliva, which today constitutes the main source of the virus, and it was considered possible that transmission occurs through this biological fluid. The oropharynx facilitates viral replication of HHV-8 and constitutes a sort of container for this virus. The oral cavity at the moment constitutes a major source of HHV-8 infection.

From the pathogenic point of view, it is not clear if the KS derives from monoclonal proliferation of cells infected by HHV-8 or from non-neoplastic oligoclonal expression of infected cells. The most likely theory is a halfway stage: the KS originates as a polyclonal expansion and then follows a monoclonal development [79].

From the point of view of molecular pathogenesis, the tat (trans-activator of transcription) of the HIV stimulates growth and re-activation of latent infection of HHV-8, which in turn promotes proliferation of endothelial cells [77]. The HHV-8 virus participates in cell growth, in induction of apoptosis, in angiogenesis and in immune modulation. This virus contributes to production of the proteins that directly inhibit adaptive and innate immunity of the host.

From a clinical point of view, KS may present with 10 variations: patches, plaque, nodular, lymphadenopathic, exophytic, infiltrative, telangiectatic, ecchymotic, keloidal and cavernous or lymphangioma-like [77 80].

Oral Kaposi sarcoma (OKS) usually presents with patches in its early stages and in nodular form in the late stage, with possible formations of plaques in the intermediate stage. The most frequently involved sub-sites are: hard and soft palate, gingiva and back of the tongue; it may present with a single or multiple localisation [30 81]. The symptomatology is variable, from almost asymptomatic forms to more serious forms with pain, bleeding, difficulty in chewing, dysphagia and, in more progressive forms, with the involvement of other areas, there may be dysphonia, dyspnea and serious cosmetic disfigurements [82]. The clinical course is also highly varied, with the possibility of mild forms with gradual progression or fulminating forms. Rapidly progressive facial lymphedema associated with extensive, advanced OKS is an indication of the worst prognosis. OKS may regress with antiretroviral therapy or present with a re-activation as part of the IRIS. Oral lesions from AIDS-associated KS are better controlled with HAART rather than with systemic chemotherapy [83].

OKS may also arise at a paediatric age and in particular is very common in young children in the sub-Saharan area, while life expectancy of HIV+ young children with KS is short [84].

A staging for OKS has been proposed, as shown in Table 5.II [85], and considers the extension of the lesion in relation to the site of onset (T), involvement of one or more sites (L), the presence of symptoms or signs (S), in order to establish a stage of disease (1, 2 or 3) to be associated with a specific choice of treatment.

Differential diagnosis of OKS must be made with: atrophic candidiasis, erythroplakia, pyogenic granuloma, bac-

illary angiomatosis, median rhomboid glossitis, hemangioma and lymphoma.

Lymphomas. NHL is recognised as an AIDS-defining condition [71]. Clinical manifestations of the lymphoma in AIDS patients include soft tissue masses, with or without ulceration and tissue necrosis and the oral sites most affected are the gingival, palatal and alveolar mucosa. NHL is often diagnosed in an advanced stage and in around 50% of patients it is already affecting bone marrow. Risk of developing NHL is uniformly distributed in the various distinct groups according to risk of transmission. Risk of developing a NHL is estimated to be 1.6% per annum of HIV infection [71]. The risk, calculated as 19%, is also present in subjects undergoing HAART, given that antiretroviral therapy does not appear sufficient to prevent this malignancy [86].

The spectrum of AIDS-correlated NHL includes: systemic lymphoma, primitive lymphoma of the central nervous system and two rare entities, primary effusion lymphoma and plasmablastic lymphoma of the oral cavity [86]. The primary effusion lymphoma has been associated with HHV-8, while plasmablastic lymphoma has been associated with EBV. This latter virus has also been associated with the aggressive Burkitt lymphoma, with the large cell lymphoma and with the immunoblastic lymphoma. EBV infection, as already stated in the previous section, causes a decrease in production of protein p53, a known tumour suppressor [71].

Lymphoblastic lymphoma, which on account of its typical oral localisation is of particular interest to the otorhinolaryngologist, is a more aggressive variation of the diffuse large B-cell lymphoma [87]. This lesion may present with a purple-red, painful mass involving the gingival mucosa. Characteristically it responds quickly to treatment, but presents high percentage of recurrence with very poor prognosis.

HL is the most common non-AIDS-defining malignant neoplasia in HIV+ subjects and presents with progressive incidence. HL is more common among subjects who are users of injection drugs than the other risk groups. HIV+ patients with HL have a higher frequency of infection from EBV than HIV negative subjects [71]. HL is diagnosed in advanced stage (stage 3 or 4) in approximately 75% of HIV+ subjects, who usually present non-specific symptoms (high temperature, night sweating, loss of weight).

Table 5.II. Proposed staging of oral KS according to JB Epstein.

Area affected by tumour *
T1: < ¼ of the oral site
T2: > ¼ and < ½ of the oral site
T3: > ½ of the oral site
Localisation of the tumour
L1: single oral site
L2: multiple oral sites
L3: single oral site and other extra-oral sites requiring treatment
L4: multiple oral sites and extra-oral sites requiring treatment
Symptoms/signs of tumour
S0: no symptom
S1: macular lesion
S2: detected mass
S3: mass interfering with function or causing pain or ulcerous mass
Treatment on the basis of the OKS stage
Stage 1: T1 or T2; L1 or L2; each S; local treatment
Stage 2: T3; L1 or L2; S3; regional treatment
Stage 3: each T, L3 or L4; systemic treatment

** Oral sites: hard palate, soft palate, tonsillar lodge, back of the tongue, lateral margins of the tongue, gingiva, buccal mucosa, floor of the mouth, ventral surface of the tongue; specify right or left and the gingival site related to the site of the tooth*

ENT manifestations at paediatric age

A large part of the HIV correlated manifestations that can be seen in adults can also be found in young children, some manifestations, however, are typical of the paediatric age. In young children, the main method of transmission is vertical: mother-to-child; there are some rare transmissions by sexual means from possible sexual abuse or through contaminated blood or hematic derivatives [5]. Manifestations of HIV in the ENT area are present in approximately 40% of the infected young children population [88].

HIV+ young children are much more susceptible than adults to bacterial and viral infections and may present recurrent and persistent otitis media, rhinosinusitis with chronic rhinorrhoea and parotitis [89].

Oral candidiasis is the most common form of mucous cutaneous HIV in the paediatric population, involving around 60-75% of symptomatic paediatric patients. Involvement of the oesophagus by Candida is less frequent, but more serious, since it causes odynophagia and halts growth, especially in breast-feeding children and very young children. Other oral manifestations include herpetic lesions of the oral mucosa and palatal petechiae caused by thrombocytopenia.

Otitis media is particularly frequent and due not only to common germs (above all pneumococcus), but also to more unusual organisms, such as Staphylococcus epidermidis, species of Enterococcus, Escherichia coli, Pseudomonas aeruginosa.

HIV+ young children, as well as being more susceptible to pathologies of the middle ear, present more easily with

sensorineural hearing loss [90]. A prospective study in the USA assessed prevalence of hearing loss in HIV+ and HIV exposed but not infected young children compared to the general paediatric population [91]. Hearing loss was found in 20% of HIV+ young children and in 10.5% of young children exposed but not infected. After adjustment for level of education of the care givers, the odds ratio for hearing loss in young children with associated HIV infection was 2.13% (CI 95%: 0.95-4.76; p = 0.07). Perinatal HIV infection represents a definite risk factor of hearing loss in the paediatric population.

Rhinosinusitis is certainly one of the most common problems in patients with AIDS and there is a correlation between their immune state and gravity and aggressiveness of rhinosinusitis [88]. In some rare cases a lymphoma that develops in a rhinosinusal site may appear as acute rhinosinusitis, rather than chronic, because of its rapid growth [5 88].

Masses in the head and neck area are very common in the HIV+ paediatric population, the same as in the adult population, and in particular lateral cervical adenopathies and parotid hypertrophy are frequently found, presenting in approximately 30% of infected young children. Needle aspiration cytological examination often reveals a picture of benign lymphoepithelial cysts. In cases of lateral cervical adenopathies histological findings are often required to distinguish a lymphoma from a secondary infection.

5.3 Overview of treatment

As already stated at the beginning, it is not part of the objectives of this review to give a systematic treatise on the treatment of otorhinolaryngological manifestations during the course of HIV infections.

Here just the basic principles of treatment are reported, which lay down three main lines: specific treatment of HIV infection with antiretroviral drugs; treatment of infections; treatment of neoplasia.

Treatment of infections in HIV+ subjects does not differ substantially from that of non-infected subjects, however, since the infections are often recurrent and protracted, antimicrobial treatment often has to be aggressive and protracted. In view of the frequency of opportunistic infections and antibiotic resistance, culture examination with antibiograms is almost always required. Antibiotic resistance is a common phenomenon in bacterial infection, but some mycetes can also present resistance to antimycotics in subjects with HIV. Apart from systemic treatment, a topic treatment may be associated, as in the case of otitis externa and media or in the case of viral lesions of the oral cavity.

Therapeutic protocols in cases of head and neck neoplasia are applied in principle also to HIV+ subjects. Surgical treatment, where indicated, does not create problems of a technical nature, but rather greater post-operative attention has to be paid, to prevent infection of the surgical site. The main problems can arise when a patient has to undergo chemoradiotherapy, a treatment that is known to be in itself a potential depressor of the immune system. The oncologist therefore has to choose antineoplastic drugs with caution and rely on collaboration with a specialist in infections, to associate an antiretroviral therapy.

Antiretroviral therapy has to take into consideration all the clinical aspects of the patient, starting from the stage of the HIV disease and the presence of associated syndromes (encephalopathy, nephropathy etc.) or pre-existing syndromes (e.g. chronic viral or exotoxin-induced hepatopathy). Given the complexity of the treatment strategy, which has to take into account all these variables, it is recommended to refer to the specific guidelines [4].

Here, a brief overview will be given of antiretroviral drugs. These drugs are divided into three main categories: inhibitors of viral reverse transcriptase, drugs which inhibit viral proteases and agents which interfere with entry of the virus. Reverse transcriptase inhibitors include both nucleoside analogues (zidovudine, didanosine, zalcitabine, stavudine, lamivudine, emtricitabine and abacavir) and non-nucleoside reverse transcriptase inhibitors (nevirapine, delavirdine and efavirenz). Reverse transcriptase inhibitors block the replication cycle of the virus at the point of DNA RNA-dependent synthesis, that is, during the stage of reverse transcription.

Protease inhibitors include: saquinavir, ritonavir, tipranavir, lopinavir/ritonavir, fosamprenavir, atazanavir and darunavir. These drugs act in the final stage of the HIV replication cycle, to inhibit viral protease, which is an enzyme allowing new viral particles to mature and become infected in turn.

Fusion inhibitors are agents which prevent the entry of the virus, such as enfuvirtide and maraviroc. Enfuvirtide acts in the stage when the virus binds with the cell that it will then infect. It is a synthetic peptide, derived from transmembrane protein gp41 of the HIV virus. It can bind to viral protein gp41 and prevent it from binding with the cell, thereby blocking its entry. This molecule does not act against HIV-2.

Just to give an example, one HAART plan is ATRIPLA, which consists of: efavirenz 600 mg + emtricitabine 200 mg + tenofovir 275 mg (a reverse transcriptase analogue, which, instead of inhibiting the enzyme, occupies

its site of activity and therefore prevents synthesis of DNA).
In conclusion, ENT manifestations with HIV infection in course, even after the introduction of HAART, are very common and they often even constitute the first warnings. Some forms are almost typical of HIV infection, such as oral hairy leukoplakia or oral Kaposi sarcoma. Finding these lesions should immediately arouse the suspicions of the otorhinolaryngologist, who must then send the patient to an ID specialist for further investigation and treatment. Also finding common neoplasia, but in unusual sites, such as a lymphoma affecting the gingival mucosa, should constitute an alarm bell. Common infections (otitis and rhinosinusitis, oral candidiasis), even if they are recurrent, need not arouse the suspicion of the otorhinolaryngologist, but protraction of the infection and recurrence, together with a careful anamnesis in identifying a possible risk group, may lead to a correct diagnosis.

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6. Infections from typical and atypical mycobacteria

6.1. ENT infections from typical mycobacteria

TB currently constitutes an emerging risk in industrialised countries and was indicated by the WHO as a serious problem of public health on a world level as long ago as 1993. In Italy incidence of TB in recent years, however, has been lower than 10 cases/100.000 inhabitants, a threshold below which the disease is considered by the WHO as low incidence. Worldwide though it is still one of the main causes of morbidity and mortality, with 10.4 million new cases and 1.8 million deaths in 2015.
After a marked decrease up to the middle of the 1980s, determined by introduction and improvement of anti-tuberculosis chemotherapy, incidence of the disease has again increased in relation to the demographic increase, immune deficiency (mainly due to HIV infection, but also to anti-neoplastic chemotherapy treatments and immunosuppressive treatments in transplant patients), precarious living conditions (poverty, migratory phenomena) and the developing resistance to antibiotics [1-3].
Apart from increased incidence, it has also been possible to see, over the last few decades, evolution in clinical manifestations. In effect, the tubercular localisations in the cervical facial area, which in the past were usually secondary to pulmonary localisations, are currently often being seen as primary forms (first clinical expression of the disease), both at a lymph node level and at the level of the upper airways, and they are today among the most frequent extrapulmonary localisations of the disease [2].
The most frequent localisation at the head and neck level is that of the lymph nodes, which constitutes 70-90% of tubercular peripheral adenopathies and 95% of the cases of relevance to ENT; among the other cervical facial localisations the most frequent are those at the level of the larynx and middle ear, while nasal, rhino-oropharyngeal and salivary cases constitute less than 1% of the total [1 3-5].
Lastly, it is currently rarer to observe forms of tubercular disease with acute onset and rapidly progressing development, while it is more frequent to meet pictures of an insidious onset and slowly progressing development [2].
Extrapulmonary TB is caused by MTBC, which includes Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis BCG and Mycobacterium africanum [6 7]. Mycobacterium bovis, the etiological agent of bovine TB, is still an important etiological agent of TB in humans also, especially in rural zones, where consumption of non-pasteurised milk is still high [8]. In our country the most frequent pathogen agent in cause is Mycobacterium tuberculosis.
Local inoculation of the germ at the level of the upper airways occurs by inhalation of bacillifer dust (starting from the outside environment but also from an endogenous pulmonary source) or from lesions from scratching, while inoculation by hematic or lymphatic means may start from a misunderstood source (aspect of primary localisation) or from an already known source (pulmonary by hematogenous means, mucous by lymphatic means). In tubercular cervical adenopathies, the primitive source is often rhinopharyngeal and diffusion is by lymphatic means.
The rarities of the oro-rhino-sinus localisations might be determined by related resistance to this mucosa, caused by mucociliary clearance and richness of the lymphatic tissue.
At an anatomopathological level, penetration of the mycobacterium initially determines an increase of polymorphonuclear neutrophils, subsequently replaced by macrophages and lymphocytes. This stage corresponds clinically with the appearance of mucous hyperplasia. Subsequently, the typical lesion is represented by a central zone of giant cells (Langhans cells), a middle zone of epithelioid cells containing the mycobacterium and a peripheral zone with lymphocytes and fibroblasts. This stage corresponds clinically with the appearance of reddish-brownish fine granulations, sometimes joining to

form an exophytic pseudo-tumorous mass, pedunculated or sessile, of squashy consistency (tuberculoma). Lastly, a central caseous necrosis appears, due to disgregation of macrophages and bacilli, which may clinically present with lesions that may be ulcerous and superficial or deep and indolent. The caseous necrosis zones may calcify or even merge, to give a clinical picture of cold abscess.
The cervical facial clinical manifestations are represented by:

Cervical lymphadenopathies
These typically affect young subjects, more often of the female sex, the opposite to what is seen in pulmonary forms.
They are generally primitive forms (even if it cannot be excluded that in certain cases there are secondary forms with primitive nasopharyngeal or already healed oropharyngeal lesions) of submandibular or jugular carotid localisation, or more rarely supraclavicular; frequently the lesion is monolateral, sometimes also affecting only one or two lymph nodes [2].
On a clinical level the adenopathy is initially almost always mobile and indolent; there is a progressive increase in volume, with hardening and adherence to the lower levels, while the cutis takes on a violet colour; there is rapid development towards colliquation and fistulization is rare (10%) [2].
Impairment of the general condition, with high temperature and night sweating is not constant, 20-40% depending on the studies [2].

Laryngeal (and hypopharyngeal) localisation
This was the most widespread laryngeal pathology at the beginning of the 20th century; its incidence then gradually decreased, to increase again over the last few decades. It presents clinically with non-specific signs, but its infectiousness requires rapid diagnosis and medical treatment.
It is more frequent in the male sex [2] and contagion usually occurs by airborne means from an infected subject.
As an exception, it constitutes the only manifestation of the disease, much more frequently it is in the presence of a pulmonary lesion at the same time [1].
The symptoms are non-specific, according to the localisation of the lesion (dysphonia, dyspnea, dysphagia, haemoptysis, coughing, otalgia), while systemic symptoms are not constant.
Objectively a diffuse hyperemia is often present, with granulomatous or polypoidal lesions of the vocal chords [4], but the exophytic aspect of the laryngeal tubercular lesions does not permit differential macroscopic diagnosis of the carcinoma [1]; however, there are certain characters that should cause suspicion of this etiology, such as the presence of multiple lesions, often of a destructive nature (especially against the free side of the epiglottis), frequent immobility of one or both of the vocal chords because of the involvement of the cricoarytenoid articulation, the rare subglottal involvement and that of the satellite cervical adenopathies.

Auricular localisation
Tubercular otitis is a rare disease, constituting only less than 1% of the chronic diseases of the ear [2]; it is often diagnosed extremely late, because of its low prevalence and its insidious, non-specific clinical signs [10].
Inoculation of the bacillus at the level of the middle ear may arise through a previous tympanic perforation, in a retrograde manner starting from the nasopharynx, or by hematogenic means starting from another endogenous source [10 11].
The same anatomopathological alterations occur at the auricular level as at the other areas and development of the tubercular granuloma towards the central necrosis explains the more typical and classic manifestation of tubercular otitis, that of the tympanum with multiple perforations [12]. The disease can develop towards osteitis, with the appearance of complications such as petrositis, paralysis of the VII cranial nerve and labyrinthitis [3].
Clinically, tubercular otitis is characterised by a classic triad, consisting of multiple tympanic perforations, serous otorrhea and facial paralysis. Otorrhea is usually serous, not associated with pain, except in a small percentage of cases, and usually otalgia is a sign of extension of the infectious process to the mastoid cavities [11 13]. Facial paralysis often appears early in the course of the disease [11], sometimes with spontaneous recovery and sometimes recurrent. Other clinical signs can appear in connection with the loco-regional extension of the disease (petrositis with diplopia, involvement of the inner ear with vertigo and sensorineural hearing loss. intracranial dissemination with tubercular abscess, tuberculoma, encephalopathy, meningitis) [3 14].
The classic otoscopic picture (multiple microperforations) is not frequent; more often a sero-mucinous otitis is found, or a fibroadhesive otitis, or a granulomatous otitis, where the granulations hide a total or subtotal perforation at the merging point of the microperforations [4 9 11 12].
Tubercular disease at the auricular level must be suspected in the presence of facial paralysis associated with non-colesteatomatous chronic otitis media [3] and, in general, all forms of otorrhea without pain, persisting for months and non responsive to common antibiotic treatments.

Nasal and nasopharyngeal localisation

At the nasal and nasopharyngeal level, too, the symptomatology caused by the tubercular localisations is non-specific, including mucopurulent and sanguinous rhinorrhea, nasal obstruction, epistaxis, tubal symptoms [4]. Objectively, there may also be the presence of crustiness, diffuse mucosal hyperemia with reddish-brownish fine granulations (nasal lupus), sometimes ulcerations; there may also be present an exophytic pseudotumorous neoformation, peduncated or sessile, squashy (tuberculoma). Most often the sites involved are the lateral walls of the nasal fossa and the anterior portion of the septum. The paranasal sinuses can also be involved in exceptional cases, with bone erosion and possible complications (meningitis, invasion of the hypophyse, cutaneous invasion) [1]. Differential diagnosis must be made with neoplastic lesions and with Wegener granulomatosis [9].

Nasopharyngeal localisation is more frequent than that of the nasal fossa and it may be misunderstood and discovered by chance following the appearance of cervical adenopathies.

In this case too it may be possible to find hyperemia and diffuse hyperplasia of the mucosa or a pseudotumorous mass or an ulceration.

Oral and oropharyngeal localisation

Oral and oropharyngeal localisations are rarer [2] and these may be primary or secondary lesions to an extrathoracic dissemination of a pulmonary focus [1].

In the oral cavity the disease may appear as indolent ulceration at the level of the gingiva or palate or floor of the mouth [13] or as a lesion of the lateral margin of the tongue [1].

At the oropharyngeal level, the lesions appear more often on the palatine tonsils with tonsillar hypertrophy or ulceration or exophytic lesion, associated with extremely non-specific symptoms (dysphagia, odynophagia) [2].

Salivary localisation

Involvement of the salivary glands is exceptional [1 2]; lesions may be associated with oral and dental lesions or may appear because of hematic or lymphatic dissemination. In primary forms the clinical picture is that of suppurative sialadenitis or of a tumour-like lesion, while secondary forms are usually associated with pulmonary TB [15].

Diagnosis of extrapulmonary TB creates difficulty due to the diversity of the clinical manifestations that can mimic other pathologies (Wegener's disease, lymphoma, carcinoma, amyloidosis, sarcoidosis, histiocytosis, tertiary syphilis, fungal infections, etc.), to the difficulty of taking samples in some sites, to the sometimes paucibacillary nature of the samples taken (e.g. at the auricular level) and to the time needed for the growth of mycobacteria in cultivation medium [3 6 16 17].

Microscopic examination of the material permits identification of the bacilli, after suitable staining which highlights alcohol-acid resistance (the most classic is Ziehl Neelsen staining) [3]; a negative result cannot be interpreted as definite negativity, while a positive result allows diagnosis of mycobacterial infection but not of tuberculosis.

For culture examinations solid egg-based media are classically used, of the Lowenstein-Jensen type, which require times of 3-6 months for the colonies of mycobacteria to be appreciated.

Today combination of a solid medium with a liquid one enables diagnostic times to be shortened to 1-2 weeks [3], but the use of egg-based medium is still necessary, because it enables growth of some strains of mycobacteria which do not develop in other media [18]. Positive reading of the medium must anyway be confirmed by verification of alcohol-acid resistance of the microscopic preparation.

If the culture is positive, the next step is identification of the mycobacterium with different types of tests (molecular tests, chromatography, radiometric tests).

Methods of molecular analysis (Polymerase chain reaction, PCR), today enable notable reductions in diagnostic delay caused by long growing times of a mycobacterium in cultivation; through techniques of gene amplification it is possible to obtain in a few hours millions of copies of nucleic acid, to enable quick, sensitive and specific detection of mycobacteria (greater sensitivity than with microscopic examination), as well as differentiation between the various types of mycobacteria, which may prove essential in optimising treatment, since Mycobacterium bovis and Mycobacterium bovis BCG are intrinsically resistant to pyrazinamide, one of the main, first choice antitubercular drugs [7 8]. These molecular techniques, however, do not make it possible to obtain an antibiogram, for which culture examination is necessary anyway [3].

Image diagnostics enables highlighting of important findings, none of them pathognomonic, though.

In the case of auricular localisation, CT images reveal a non-specific picture of chronic otitis media with soft tissue in the cavity of the middle and mastoid ear, which can be either eburnean or pneumatised, and signs of osseous lysis may appear subsequently [11]. NMR does not provide advantages, even if it shows up increase in signal (after administration of a means of contrast) at the level of the facial nerve, in cases of paralysis of that nerve.

At the oral, oropharyngeal and nasopharyngeal level, CT and NMR can be helpful in differential diagnosis, because they show the absence of an invasive character of this le-

sion, while at the laryngeal level it is important (to suspect TB rather than a carcinoma) to find in the images diffuse bilateral lesions without destruction of the laryngeal architecture, absence of significant signs of infiltration, preservation of the thyro-hyoid-epiglottic space and paraglottal spaces [19].

Regarding the cervical localisation, imaging methods consist of US (for superficial adenopathies) and CT (especially for deep adenopathies), which enable verification of the increase in dimensions and the structural alterations of the adenopathies. For culture diagnostics, fine-needle aspiration is used, which presents less risk of secondary fistulisation than bioptic sample [20], but if the cytological result is doubtful and/or the bacteriological examination is negative, a lymphadenectomy is necessary.

Antituberculosis vaccination in Italy is currently obligatory only in highly selective cases: new-born babies and young children below the age of 5 years with tuberculin test negative, who are living with or have close contact with persons afflicted by TB in the contagious stage, or health workers, medical students, trainee nurses or anyone else with tuberculin test negative and working in health environments with high risk of exposure to multiresistant strains or who work in health environments at high risk and cannot (in the event of cutireaction) be subjected to preventive therapy on account of clinical contraindications to the use of specific drugs [20].

Antituberculosis treatment may be supported by a discreet number of drugs, but there are currently reported a number of cases of multiresistant TBs (approximately 400,000 cases, according to the Ministry of Health).

Antituberculosis drugs are distinguished on the basis of antimycobacterial strength, evidence of effectiveness, use in clinical practice and pharmacological classification in order of the following succession according to the WHO:

a) First-line drugs: rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin. Rifampicin and isoniazid are those of greatest importance, with bactericide effect also at intracellular level, ethambutol is a bacteriostatic for both intra- and extracellular bacilli and streptomycin has a bactericide effect only on extracellular ones;
b) Second line drugs:
   1) injectable antituberculosis drugs: amikacin, kanamycin, capreomycin;
   2) fluoroquinolones: moxifloxacin, levofloxacin;
   3) oral bacteriostatic drugs (in order of effectiveness): ethionamide, prothionamide, cycloserine, terizidone or PAS;
   4) minor antituberculosis drugs: linezolid, clofazimine, amoxicillin/clavulanic acid, imipenem/cilastatin-meropenem, clarithromycin, thiacetazone.

The guidelines lay down induction therapy with 4 or 5 antibiotics (isoniazid + rifampicin + pirazinamide + ethambutol + streptomycin) for a period of 2-3 months (treatment to be reset as soon as the results of an antibiogram are available), with a further period of 5-6 months (after culture exam becomes negative) with isoniazid + rifampicin with or without ethambutol [3].

Surgery, which is fundamental in almost all sites to enable biopsy and therefore microscopic examination, presents only a few indications in treatment of TB.

It is only at the auricular level that this takes on a primary role, because in the majority of cases it is performed before the diagnosis of tuberculous disease, because of the presence of a chronic otitis media resistant to classic medical treatment or because of suspected cholesteatoma; therefore, it is the surgical treatment itself that enables TB diagnosis [3]. On the other hand, when the diagnosis is already known, the role of surgery is controversial, even though according to some it may help healing anyway [11]. Surgery remains important, however, in cases of complications or as a second intention for repairing sequels [3].

At the laryngeal level it is only as an exception that surgical treatment becomes necessary, for acute or rapidly worsening dyspnea (tracheostomy or unblocking).

In the cervical site, surgical operation is not indicated except in the event of failure of medical therapy, in the event of development during treatment or in the event of lymph node residues at the end of the same, and it should be performed in the most conservative manner possible, even if sometimes phenomena of adherence may make it inevitable to sacrifice the inner jugular vein or the sternocleidomastoid muscle.

6.2 ENT infections from atypical mycobacteria

For many years only three species of mycobacteria were considered pathogens in humans: tubercolosis, bovis and leprae, but shortly after the discovery of the tuberculosis bacillus (Koch, 1882) other bacilli were identified in human secretions, which were in some way similar to the previous ones. In 1954 Timper and Runyon [21], created the first collection of NTM isolated from humans and published a fundamental work explaining the relationship between disease and properties of the causative bacteria, without any further reference to pathogenicity in laboratory animals. Their classification system has remained in use under the name of “Runyon scheme”.

Therefore, the definition of non tuberculous or atypical mycobacterium is applied to mycobacteria belonging to the group of Mycobacterium tuberculosis complex (tuberculosis, bovis, africanum) and of Mycobacterium leprae [22].

NTM are microorganisms present in all geographical areas which behave as ubiquitous saprophytes, since they can survive in the most disparate conditions; indeed they have been isolated in the soil, in water, in vegetation and in foods, such as meat and eggs. They infect pets and wild animals and may also be present in the normal pharyngeal flora of humans.

More than 170 species are known [24] but only 20 of these are capable of acquiring pathogenic strength for humans [25].

They are distinguished from tuberculous mycobacteria by their bacteriological and epidemiological characteristics and their different clinical presentation. Furthermore, clinical experience has by now demonstrated that treatment of the two forms calls for substantially different treatment measures. That is why they were called "atypical mycobacteria", a term which has now been replaced by NTM or MOTT (Table 6.I).

Proposed NTM classifications are based on phenotypes or on genotypes. Classification based on phenotypes uses analysis of cultural characters (growth speed, pigmentation of the colonies, growth temperature) and on biochemical characters (especially metabolic activity).

Classification based on genotypes developed as a result of identification within the genome of preserved regions that regulate essential functions, which have undergone less variation during evolution.

Today atypical pathogenic mycobacteria are classified into 2 main groups: those with rapid growth, which grow in culture in one week, and those with slow growth, which take several weeks to grow.

The rapid growth group includes Mycobacterium fortuitum complex, Mycobacterium abscessus, Mycobacterium chelonae.

The slow growth group includes, among others: Mycobacterium avium complex, Mycobacterium marinum, Mycobacterium kansasii, Mycobacterium scrofulaceum, Mycobacterium gordonae, Mycobacterium ulcerans, Mycobacterium malmoense, Mycobacterium hamophilum, Mycobacterium xenopi (Table 6.II).

Infection occurs through inhalation, direct contact or inoculation, ingestion. Infections are therefore of environmental origin, but cases have been reported of nosocomial transmission through contaminated instruments. Interhuman contagion is non-existent and in those rare familiar cases there is always a common source present [27]. It is also very rare to find contagion from animal to human. Factors capable of favouring onset of disease are: the dose of infection, long term colonisation, impaired immune response, predisposing alterations at the level of the organ involved and concomitant pathologies [26 27].

Frequencies of atypical mycobacteriosis vary from country to country: in Italia this corresponds to 2-7% of all forms sustained by mycobacteria, while the greatest number of cases have been recorded in Japan (up to 20%). The spread of AIDS has caused a general increase in the number of patients afflicted with these infections, with strong prevalence for infections sustained by Mycobacterium avium.

Intracellular complex. In humans the species that are most frequently responsible for mycobacteriosis are: avium/intracellulare, kansasi, xenopi, fortuitum and haemophilum.

Atypical mycobacteria cause 4 main clinical syndromes in humans:

- respiratory disease;
- superficial lymphadenitis;

Table 6.I. Distinguishing characteristics between tuberculous and non tuberculous mycobacteria.

	Tuberculous mycobacterium	Non tuberculous mycobacterium
Habitat	Obligatory parasite	Ubiquitous (water-soil-animals-food)
Virulence	Pathogen	Opportunistic
Contagion	Human/human	Environmental
Epidemiology	Familiar TB contacts	Absence of TB contacts
Site	Supraclavicular, posterior cervical	Angulo-mandibular upper anterior cervical
Evolution	Slow	Faster
Cutis involvement	Infrequent	Always presenting evolution
Systemic symptoms	Low-grade fever, asthenia	Absent
PPD-T	Positive	Negative or weakly positive (between 5 and 10 mm)
QuantiFERON	Positive	Negative
Frequency	Infrequent	Infrequent but 10 times more frequent than TB
Sensitivity to anti-TB antibiotics	Good	Absent or poor

Table 6.II. Classification of mycobacteria pathogenic for humans (from AT Cruz. Non Tuberculous mycobacterial skin and soft tissue infections in children. Up to Date 2017, www.uptodate.com).

Mycobacterium tuberculosis complex
M. tuberculosis
M. bovis
M. africanum
M. microti
M. canetti
M. leprae
Rapid growth non tuberculous mycobacteria
M. fortuitum complex
M. fortuitum
M. peregrinum
M. porcinum
M. chelonae
M. abscessus
M. abscessus subspecie sabscessus
M. M. abscessus subspecies bolletii
M. M. abscessus subspecies massiliense
M. smegmatis
M. mucogenicum
Slow growth non tuberculous mycobacteria
Photochromogens
M. kansasii
M. marinum
Scotochromogens
M. gordonae
M. scrofulaceum
Nonchromogens
M. fortuitumcomplex
M. avium
M. intracellulare
M. chimaera
M. terrae complex
M. ulcerans
M. xenopi
M. simiae
M. malmoense
M. szulgai
M. asiaticum
M. haemophilum

- disseminated disease;
- infections of the cutis and soft tissues.

Pulmonary forms have greater incidence in adult age, while some extra-pulmonary and lymphoglandular forms clearly predominate in immunocompetent subjects in pediatric age. Extra-pulmonary mycobacterioses affecting the cervical facial area include descriptions of manifestations affecting the cutis and soft tissues, of the thyroid, of the parotid [30 31], of the middle ear [32-35] and of the paranasal sinuses, and of the lymphatic glands.

In this paper we deal exclusively with the pathologies of a cervical-facial presentation and therefore of the superficial lymphadenitis of the cervical-facial area and infections of the cutis and soft tissues.

Infections of the cutis and soft tissues

Most of the infections of the cutis and soft tissues fromatypical mycobacteria are caused by Mycobacterium fortuitum, Mycobacterium abscessus and Mycobacterium chelonae among the rapid growth mycobacteria and by Mycobacterium marinum and Mycobacterium ulcerans among the slow growth types.

The common entrance door is represented by cutaneous abrasions and penetrating trauma, including piercing, tattoos, acupuncture, injections and surgical procedures. It involves typically infections resulting from trauma occurring in the swimming pool or wounds in water [37 38]. They may affect various areas of the body, including, although less commonly, the cervical-facial area. High incidence is also reported in subjects undergoing plastic surgery, including operations on the face, with possible highly disfiguring consequences [39].

The period of incubation is variable, from a little less than 1 week to periods of 4 months for rapid growth mycobacteria, up to 9 months for slow growth types; commonly periods of 3-4 weeks [40].

Cutaneous lesions from atypical mycobacterial infections are polymorphic and may appear as ulcers, plaque, nodules, papular erythema, folliculitis and this variability may contribute to delay in diagnosis. They often start, however, as an area infiltrated with slow growth, which starts to ulcerate after a few weeks and, unlike infections from pyogenic bacteria, most of the time the lesions are indolent (with the exception of Mycobacterium haemophilum), without signs of local or systemic phlogosis and satellite lymphadenitis is rare. On the other hand, infections from rapid growth mycobacteria may also determine localised cellulitis and/or abscesses with local pictures almost indistinguishable from other bacterial infections (e.g. from Staphylococcus aureus or from Pseudomonas aeruginosa) [41].

In immunocompromised patients, cutaneous lesions may be disseminated and/or represent the first manifestation of disseminated disease [63].

Infections of the cutis and soft tissues from atypical mycobacteria may become complicated by penetration of the infection into the deeper tissues, causing pictures of chronic myositis, tenosynovitis and osteomyelitis.

Clinical suspicion of infection of the cutis and soft tissues from mycobacteria may be given greater value by certain anamnestic data, such as exposure to water, for example swimming pool or bathing, or history of penetrating trauma or surgical procedures; another indication could come

from lack of response to common anti-staphylococcus and/or anti-streptococcus antibiotics, if administered, and negative culture test for common germs, if carried out; just as support for the diagnosis may derive from positivity to Mantoux intradermo reaction, even though that does not help to distinguish between tuberculous and non tuberculous causes, while it should be borne in mind that negativity to that test does not exclude the diagnosis [43].

A definite diagnosis requires isolation of atypical mycobacteria in culture from material obtained through biopsy, either needle aspiration or drainage. The culture is essential for distinguishing between tuberculous and non tuberculous (atypical) forms, for diagnosis of species of atypical mycobacteria causing infection, when possible and to perform any anti-mycobacteriogram for susceptibility to drugs commonly used in treatment.

Correct clinical significance must then always be assigned to the cultural isolation, since it may simply be contamination, considering the fact that atypical mycobacteria are ubiquitous in the environment and that their pathogenicity is variable, so that the quantity of isolate and multiplicity or not of positive samples must also be given consideration [44].

A histological exam is not always nullifying, since in this type of infection there are generally no pathognomonic aspects. It may actually show granuloma of the dermis, occasionally with colliquation, abscessed forms, acute or non-specific chronic infections. Also, positivity to Ziehl-Neelsen staining shows in a minority of cases, therefore negativity does not exclude the diagnosis [43].

Histological tests and microbiological tests generally become necessary for differential diagnosis between infections of the cutis and soft tissues by atypical mycobacteria towards another series of conditions caused by infection, including cutaneous Leishmaniasis, tuberculosis, sporotrichosis, nocardiosis and, in immunocompromised patients, also aspergillosis, cryptococcosis and histoplasmosis.

Treatment of infections of the cutis and soft tissues from atypical mycobacteria generally requires a combination of medical and surgical treatment [44].

Draining of abscesses, removal of devices or fitted units, surgical debridement in cases of infection involving deeper or more complicated tissues are essential procedures for successful treatment.

Empirical medical treatment, while awaiting culture isolation of species requires a macrolide (azithromycin or clarithromycin) associated with one of the following antibiotics: a fluoroquinolone (generally ciprofloxacin), doxycycline or trimethoprim-sulfametoxazole.

Nevertheless, the empirical regimen should be assessed case by case, on the basis of various host-dependent factors and on the basis of the suspected species of mycobacterium, and it should therefore always be agreed with the infection specialist.

In the more severe forms of infection, a treatment regimen may also be recommended with antibiotics administered parenterally (such as amikacin, cefotixime, meropenem).

Once the cultural isolation has been obtained, a combined treatment can be set up, with at least two drugs to which the isolated species is known to be susceptible [47].

The duration of the medical treatment depends on the clinical response and should be continued for at least 1-2 months after complete resolution of the symptoms. Follow-up during the treatment is recommended at monthly intervals, to check the correct assumption of the drugs and tolerance to them, as well as improvement of the cutaneous lesion, in other words progressive response to the treatment, or not.

If clinical improvement has not been achieved in 4-6 weeks of treatment, it should be checked that the treatment is being observed and if there are potential causes of malabsorption, then an alternative diagnosis should be taken into consideration and surgical procedure should also be assessed, also for the purposes of collecting optimal material for histological and microbiological examination; besides that the addition may also be considered of an anti-streptococcal and anti-staphylococcal antibiotic, for the possibility of over-infection from pyogens.

Lymphadenitis

NTM lymphadenitis represents by far the most frequent type of manifestation in the head and neck area and affects patients in the first years of life (< 5 years of age), although it may also be found in adults, where, however, the tuberculous form is more frequent [48].

There are difficulties in assessing the true incidence of this disease, due to lack of notification that occurs in many countries and the dispersion of the cases in relation to low incidence [11], which in any case varies between 0.6 and 1.6% per 100,000 young children [54 55].

Overall incidence of the disease increased noticeably in the 1980s and 1990s, in parallel with the increase in cases of AIDS [49 50]. Recent data has revealed that in the last few decades incidence of NTM disease has increased in immunocompetent individuals, even if it is not clear whether that represents a true increase or reflects greater diagnostic attention and/or is due to improvements in diagnostic methods [50-53].

The entry route of the germ is not always detectable with certainty, but it is often suspected to be at the base of the localisation of the disease (for example the oropharyngeal

mucosa for cervical lymphadenitis or abrasions of the cutis for cutaneous infections).
It is easy to understand how, because of the natural tendency for babies to put anything into their mouths and the fact that the germ is ubiquitous, it is easy for the oral cavity to become contaminated and from there, through small lesions of the oral mucosa just with the teething process, it can reach the draining lymph nodes in that area [57 58].
This would explain greater incidence in the 1 to 5 year-olds and the typical localisation at the angulo-mandibular, submental or lateral upper cervical level, while in the localisations anterior to the tragus in the parotids, the entry door might be the conjunctival mucosa.
It should be underlined, however, that, despite the elevated incidence of possible contacts, only a tiny number of patients contract the disease. For this situation to arise, it would appear indispensable that there is a combination of favourable factors connected with the host, such as transitory immune deficiency, or connected with the infecting agent, such as the particular bacterial load or virulence of the germ [59]. However, the majority of young children with localised disease do not present immune deficiency different from those presenting disseminated forms where immune compromise is present [54].
Generally, lymphadenitis from atypical mycobacteria appear as unilateral lymphadenopathy, hard-elastic, < 4 cm in diameter, slowly increasing in volume (several weeks). The overlying cutis gradually changes colour from rosy to violet, becomes thinner until it resembles a parchment and suppurates through the formation of a fistulous passage, which may be spontaneous or from a direct trauma. This is typically known as cold abscess, since the cutis does not feel hot nor erythematous, unlike what occurs in the pyogenic forms. There may or may not be high temperature or other associated systemic symptoms, but these little patients never have systemic manifestations or localised in other sites. If prescribed, the common antibiotics against streptococcus or staphylococcus give no results [28 45].
Diagnosis of lymphadenitis from atypical mycobacteria cannot be made only on a clinical basis, especially at the onset of disease [48]. Clinical suspicion may be helped by the lack of response to anti-streptococcal and/or anti-staphylococcal antibiotic treatment, the laboratory parameters which are not indicative of acute bacterial infection and the lengthy period of lymph node tumefaction [45 60].
Penn set out 4 clinical stages connected with progression of the infection with infiltrations of the cutis [61]. In the first stage there is a minimum of cutaneous alterations which presents adherent and with increased vascularisation. In the second, slight hyperemia begins, with infiltration and initial lymph node colliquation. In the third, the infection progresses with the formation of a colliquated central area covered by thin violet cutis, of consistency like papyrus, before reaching the fourth, where fistulisation appears, with external suppuration (Table 6.III, Fig. 6.1).
The tuberculin skin test (TST) may help, inasmuch as the derivates of purified protein are a heterogeneous group consisting of more than 200 mycobacterial peptides, some of which having expression both of tuberculous and non tuberculous mycobacteria. Therefore, positivity to this test may result in both cases. In fact, some studies report positivity ranging from 30-60% [54 55]. The QuantiFERON test, however will always be negative, since it is specific only in cases of infection from tuberculous mycobacterium. Therefore, by associating positivity, even if weak, of the TST, with negativity of QuantiFERON, a NTM infection may be suspected.
Today it is no longer possible to perform the two step Mantoux test, because the derivates obtained from various strains of NTM are no longer available.
The diagnosis is therefore not easy in the absence of specific exams; one has to work exclusively on the basis of clinical history and objective examination, aided by hematic exams and cutaneous tests and going into differential diagnosis with other sub-acute or chronic lymphadenitis, such as disease from cat scratches, toxoplasmosis or even with neoplasias, especially lymphomas.
There are, therefore criteria which arouse the suspicion of a lymphadenopathy from non tuberculous mycobacteria [62]:

- monolateral cervical lymph node swelling, persisting longer than 2 weeks;
- good general condition, absence of fever, no signs of acute suppurative infection;
- age included between 0-5 years;
- TST weakly positive, with infiltrate > 5 mm and < 10 mm;
- IGRA negative;
- US findings compatible with necrosis and internal col-

Table 6.III. Clinical stages of progression of lymphadenitis from NTM, Penn's Classification (from Penn et al., 2011 [61], mod.).

Stage I	Painless, firm Adherent to over lying skin Increased vasculary
Stage II	Fluctuance
Stage III	Skin changes – violaceous coloration Thinning of skin, parchment-like changes, shiny appearance
Stage IV	Fistulization

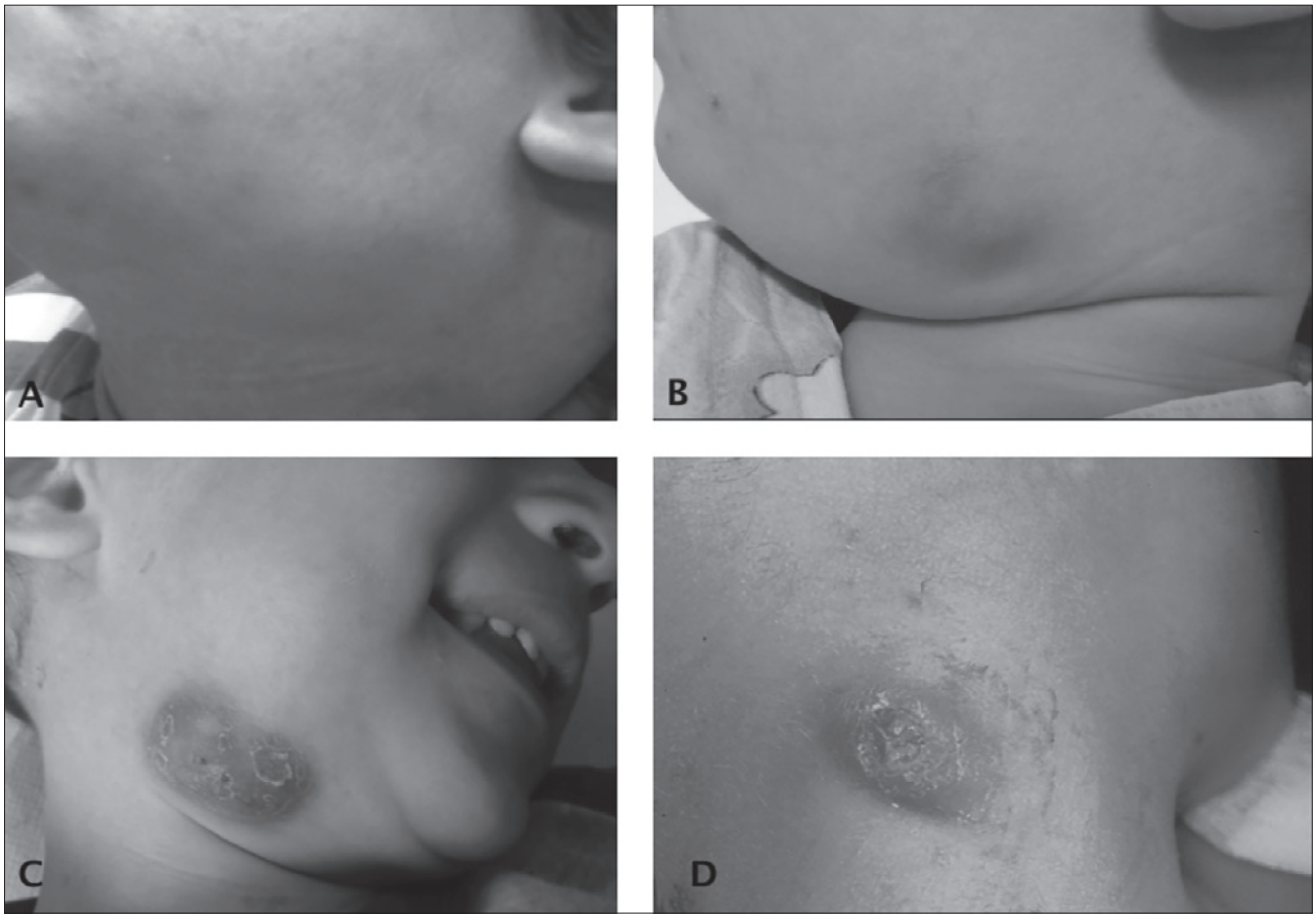

Fig. 6.1. A: I stage B: II stage C: III stage D: IV stage according to the classification of Penn.

liquation and findings which exclude malignant disease at imaging diagnostics;
- negativity of serological investigations, indicative of acute infection from EBV, CMV, adenovirus, bartonella, toxoplasma;
- clinical evolution with slow progression of inflammation with cutaneous involvement and subsequent fistulisation.

Radiological exams are not always necessary, but may be helpful in differential diagnosis with other pathologies affecting the cervical facial lymph nodes.

The US shows lymph nodes initially hypoechoic and in a more advanced stage with colliquated appearance and with edema of surrounding soft tissues.

Chest radiography is useful for differential diagnosis with tuberculous or neoplastic etiology.

If performed, CT shows lymph nodes with reinforced periphery, with central hypodensity [63].

Definitive etiological diagnosis will only be obtained with isolation of the pathogen germ from culture examination. This investigation can only be performed, however, after surgical removal of the lymph node tissue or after aspiration, or spontaneous discharge of colliquated material. Moreover, culture isolation of the germ requires lengthy response times (they are considered negative if growth does not occur in 8 weeks) and in a percentage of cases it results negative with sensitivity varying from 50 to 70%. In a case study of 477 patients, Lincoln reports 122 as negative [64], while Schaad reports 46 negative cases out of 82 patients [65] and Margileth 56 negatives out of 110 [66]. Methods of molecular biology may be helpful, with gene amplification in PCR for microbacteria; this exam gives a rapid answer (48 h) of positivity to mycobacteria, distinguishing between tuberculous and non tuberculous [67].

Then, in many cases, even a simple anatomo-pathological finding of a chronic granulomatous necrotising inflammation, associated with a clinical history and a suggestive objective exam may prove useful for purposes of diagnosis.

Penn showed that we can have 4 categories of diagnosis, ranging from certain to presumed, based on culture and

histological exams, on cutaneous tests or only on clinical findings [61] (Table 6.IV).

Lastly, it should not be forgotten that, once the diagnosis is made, it is indispensable to perform HIV tests, to show up a possible picture of opportunistic infection in immune deficiency; moreover, the HIV test is also included in the exams for differential diagnosis of superficial lymphadenitis of the cervical facial area, as will be seen in the following paragraph.

Differential diagnosis of lymphadenitis of the cervical facial area includes infectious and non infectious causes. Among the infectious causes, apart from the tuberculous forms, attention should be drawn to pyogenic lymphadenitis from streptococcus or staphylococcus, disease from cat scratches from Bartonella henselae, toxoplasmosis, viral infections such as EBV and CMV, HIV infection (both as expression of a misinterpreted picture of seropositivity and as a syndrome part of acute HIV infection). Among the non infectious causes, benign cysts (cysts of the thyroglossal duct) should be mentioned and malignant lymphadenitis [55 56].

Treatment

Three options are possible for treatment:

- surgical, involving complete removal of the lymph node package or incision and curettage of the same;
- medical, with antimycobacterial antibiotic treatment for an extended period from 6 to 12 months;
- simple observation.

The optimal treatment is still subject to debate. The majority of Authors agree that complete removal of the infected lymph nodes is considered the "gold standard" [70-77] on account of its higher and more rapid percentage of full recovery (> 92%).

This recommendation is preferred for various reasons:

a) incidence of full recovery with good aesthetic results is higher than medical treatment and if the surgery is undertaken at an early stage, iatrogenic risks are reduced;
b) surgery avoids toxic effects and the costs of lengthy antibiotic treatment;
c) surgical operation makes material available for culture that can confirm diagnosis.

The only random study is that of Lindeboom, who subdivided 100 patients with laterocervical lymphadenopathies from non tuberculous mycobacteria into 2 groups: 50 were treated surgically and 50 only with medical treatment.

Assessment was made of 2 parameters: 1) recovery rates, understood as regression of swelling and normalisation of the cutaneous lesion in 75% of cases and absence of recurrence after 6 months; 2) complications of surgical treatment and the secondary adverse effects to the antibiotic medical treatment. The surgical approach shows a success index of 96%, compared to the 66% of the antibiotic treatment. The Author concluded that surgical removal is a more effective treatment than antibiotics. The latter is only recommended in cases where surgical excision is not possible, because of the extent of the disease and consequently the risks of lesion of the facial nerve are very high [77].

From the point of view of surgical technique, removal of the package of lymph nodes may be attributed to a selective functional emptying of the level, or the levels, affected in a single piece, together with the associated cutaneous lesion, if present. However, the type of operation does not seem to affect the rate of recurrence, as long as all the tissue that is macroscopically affected is removed [78]. Surgical incision and curettage are performed and recommended when the lesion is too extensive for complete removal of the diseased lymph nodes. This option, though, does present high incidence of recurrence, which requires further surgical procedures [79] and in a percentage of 90% causes chronic fistulisation [61 57]. The percentage of recovery with surgical removal is 90%, as compared to less than 20% after incision and curettage [80].

With simple observation, progressive involvement of the cutis can be seen, which becomes violet in colour, the consistency of papyrus and with subsequent fistulisation in a period of 3-8 weeks. Total resolution occurs in 9-12 months in 71% of cases [80]. Harris agrees with a surgical approach in superficial localisations with cutaneous

Table 6.IV. Categories of diagnosis according to Penn (from Hogan et al., 2005 [71], mod.).

Diagnostic categories for cervicofacial NTM infection	
Category	**Criteria**
I	Culture positive for NTM
II	"Typical clinical picture"
	AFB found on smear or special stain of tissue
	Culture negative
III	"Typical clinical picture"
	Positive TST(PPD)
	AFB not found
	Culture negative
IV	"Typical clinical picture"
	Histology compatible with NTM infection
	AFB not found
	Culture negative

NTM: non tuberculous mycobacteria; AFB: acid-fast bacilli; PPD: purified protein derivative

involvement and points out that in 4 patients with localisation at the level of the deep lymph nodes, full, spontaneous resolution occurred in a period of between 5 and 12 months, possibly because the caseous necrosis causes a fall in ph and creates a hypoxic environment, which prevents multiplication of the bacteria beyond the natural mechanical barrier of the muscle layer. The same Author also suggests a waiting approach when localisation is particularly extensive, with consequent increase of iatrogenic risks connected with a surgical operation [73].

Several factors can impede attempts at complete surgical removal of the lesion, making this procedure particularly difficult. First and foremost is the nature itself of the lesion, being tissue which infiltrates and incorporates the nearby structures. Also, the secondary cutaneous involvement, not permitting a linear incision, makes the same choice obligatory as that in relation to the extent.

When removal is extensive by necessity, there may be tension in the sutures which gives way to loosening and to dyschromia, with greater possibility of keloid formation, which is already quite frequent in pediatric age. Therefore, considering the progressive cutaneous involvement, the earlier the operation is done the better the aesthetic results.

However, the most invalidating complication is linked to the potential damage to the facial nerve and especially to the marginal branch, which is often found to be incorporated into the granulomatous tissue when the lesion is affecting the mandibular angle area [61 70 71 75]. A percentage of lesions to the marginal nerve is reported which varies from 24% of Gonzales [15] to 5% of Claesson [81]. The damage is almost always temporary and functional recovery over time is greater than 90%. In laterocervical localisations, lesion of the spinal nerve is rarer because of the dimensions of the same and because of less infiltrations of surrounding tissue.

For this reason, in lesions localised in the vicinity of the facial nerve or particularly extensive, medical treatment can be performed with double antibiotic (clarithromycin and rifampicin) for a period that may vary between 6 and 12 months, postponing treatment of any residual fistula or cutaneous scarring outcomes to a surgical approach once the active infection has been resolved [56].

No neurological damage has been reported in cases treated only with incision and drainage [81].

Careful research in the literature of the last 10 years on the argument is suggested, in order to give clarity to the management of these rare diseases in young children. After typing into the PubMed search engine the words “Non tubercolous mycobacteria” and “cervical lymphadenitis” 13 articles were taken into consideration concerning management of adenitis from NTM (period 2008-2017).

The 13 publications looked at [54 56 74 76 82-88] included a total of 691 young children with an average age of 34,15 months. The clinical characteristics of these young children are summarised in Table 6.V.

The prevalent localisation was the submandibular area, followed by the laterocervical and the areas of the parotids and pre-auricular. Only a small percentage showed multiple localisations and other areas of the neck and 1% a bilateral localisation. Reddening of the overlying area was present in 25% of cases. It was not reported in the majority of the works taken under examination whether these patients had previously undergone antibiotic treatment inactive on the NTM before the definitive diagnosis. The interval between the start of the symptomatology and the diagnosis was highly variable (from 1 to 30 months). In 445 patients the species isolated was Mycobacterium avium/intracellulare, Mycobacterium haemophilum was second in order of frequency (50 cases); significant findings were also recorded for Mycobacterium ulcerans, lentiflavum, marinum and kansasi.

In the majority of cases surgical therapy of removal of the infected lymph node or the package of lymph nodes was the treatment that was chosen (Table 6.VI). Only in 25% of cases (170 patients) was it decided to proceed with anti-

Table 6.V.

Total patients	691	
Sex	Males	286 (41.4%)
	Females	405 (58.6%)
Mean age	34.15 months	
Localisation	Submandibular	323
	Latero-cervical	249
	Parotid	47
	Pre-auricular	44
	Others	28
Mycobacteria	M. avium/intracellulare	445
	M. haemophilum	50
	M. lentiflavum	17
	M. ulcerans	28
	M. marinum	6
	M. kansasi	6
	M. malmoense	4
	Others	31

Table 6.VI.

Radical lymphadenectomy	356
Drainage/debulking	54
Needle aspiration	10
Antibiotic treatment	77
Antibiotic and surgical treatment	101
No treatment	93

biotic treatment alone or a period of observation. Surgical treatment was reported in 12 out of the 13 works studied. Lymphadenectomy was carried out on 356 patients. The operation was almost always performed as early as possible, so as to try to avoid spontaneous fistulisation, an event which led to a worse aesthetic result when it occurred. An operation of incision and drainage or needle aspiration of the purulent secretions was reported in 64 patients. The complications described mainly concern paralysis of the facial nerve, almost always transitory, when it was decided to proceed with lymphadenectomy, while in the procedure of incision and drainage the predominant among the collateral effects were permanent fistulisation and aesthetic damage. Antimycobacterial antibiotic treatment was carried out in 77 cases as an exclusive treatment and in 101 cases in association to surgical treatment.

The antibiotic used was clarithromycin, almost always associated with rifabutin, ethambutol, rifampicin and ciprofloxacin. Doses were different in the various studies examined (for example clarithromycin was administered in doses varying between 15 and 30 mg/kg/die). Duration of treatment was also highly variable, between 1 and 10 months This heterogeneity of treatment obviously prevents comparative analysis between the various protocols. The observational approach of this disease is reported in the study by Zeharia, which includes 92 young children, to which one further patient has to be added from another study. The results reported show that 71% of the little patients have had full resolution after six months, a percentage which rose to 100% after one year. The conclusion of this study is that observation of the patient without any medical or surgical treatment being set may effectively be an alternative in management of NTM cervical lymphadenitis in immunocompetent young children. It should be pointed out, however, that 91.3% of patients presented a spontaneous fistulisation between the third and eighth week of observation and at a distance of two years it was still possible to note a scar in the area of the neck where it had occurred. The limit of this work is that it is an observational study without comparison with other treatments.

In conclusion, where the common, routine antibiotics fail in treatment of chronic cervical lymphadenitis in young children below the age of 5 years, diagnosis should be taken into consideration for adenitis from non tuberculous mycobacteria. Once a tuberculous form has been excluded with specific cutaneous tests for the NTM, with tests for tuberculin and immunoanalytical techniques (IGRA), histological and microbiological exams, a treatment programme has to be decided according to an individual approach towards each patient.

Antibiotic treatment has demonstrated fair effectiveness both alone and in association with surgical therapy, but compared to an operation for total removal of the lymph node, the results appear statistically inferior and substantially equal to those in which it was not proceeded with any treatment. The reasons remain equally unknown as to why in some patients exclusive antibiotic treatment leads to definitive resolution of the disease, while others have to undergo surgical therapy. The therapeutic scheme must be targeted on the basis of specific isolation, but empirically, while awaiting the culture result, it consists of, as far as a young child is concerned, a scheme of three drugs with a macrolide (azithromycin or clarithromycin) in combination with ethambutol and rifampicin; while in the young and in adults classic anti-tuberculosis treatment should be started, with the addition of a macrolide, inasmuch as it is a common tuberculous etiology [63]. Lindeboom reported full resolution in 66% of patients treated for 2 months with exclusive antibiotic treatment with clarithromycin-rifabutin, but the percentages appear overlapping, though, with those of the observational study of Zeharia.

The optimal length of treatment for lymphadenitis from atypical mycobacteria is not known and patients are treated up to symptomatological resolution, commonly from three to six months.

Response to treatment must be monitored clinically at monthly intervals, to ensure that the patient is assuming correctly and tolerates the treatment well [63].

There is no standard definition of treatment failure in the case of lymphadenitis from atypical mycobacteria, but it may be said that treatment has failed in the event of failed symptomatological resolution within six months and/or there is clinical worsening or progression of disease during treatment. In these cases, it is necessary to verify correct assumption of the treatment and/or observance of it and, if confirmed, susceptibility to the drugs has to be verified using an anti-mycobacteriogram, when possible (not possible on slow growth atypical mycobacteria), and in particular resistance to clarithromycin has to be tested; the options available will then be two, either surgical operation or modification of the therapeutic scheme [47 89].

Case study of the Pediatric Hospital, "Bambino Gesù", IRCCS, Rome

From 2008 to 2017 in the ORL unit of the Pediatric Hospital of "Bambino Gesù", 73 patients were treated, with chronic granulomatous cervical lymphadenopathy from suspected infection from non tuberculous mycobacteria. According to the Penn classification, only 45 patients (25 F – 20 M) were considered with certain diagnosis given positivity to culture exam (62%). 28 patients (38%) were excluded with presumed clinical diagnosis who pre-

sented with only some of the reported characteristics, but who showed negative to culture exams on removed material. Ages were between 12 months and 16 years, with a peak of incidence between 3 and 4 years (Fig. 6.2). No significant progressive increase of incidence was noted for the years under consideration.

In 10 cases a pre-operative diagnosis was possible, after having carried out a culture exam on pus samples from the lesion, while in the remaining 36 the clinical diagnosis was confirmed on biological material removed None of the patients had immune deficiency. Of the 45 cases, 42 were caused by the Mycobacterium avium complex, while there were single cases for Mycobacterium malmoense, xenopi and abscessus.

The main localisation was at the upper latero-cervical level, followed by that of the mandibular angle. There were very frequent multiple localisations (Table 6.VII).

Because of the diagnostic difficulties shown previously, only 9 patients reached our observation in clinical stage 1; 4 patients reached stage 4, but two of those as a result of an unsuitable therapeutic approach as a consequence of an excision biopsy or of a partial removal of the lymph node packet that was involved. All the other patients were in stage 2 or 3.

42 patients underwent surgical operation with a specific treatment with clarithromycin (15 mg/kg) and rifampicin (10 mg/kg) in the stage of preparation for the surgical operation for a variable period of approximately 30 days, to achieve reduction of the local bacterial load and/or reduction of the inflamed cutaneous area. 1 case, aged 2 years, with parotid localisation is still in antibiotic treatment after 6 months, having had various episodes of spontaneous suppuration, which did not permit a surgical approach, while 2 patients were lost at follow-up.

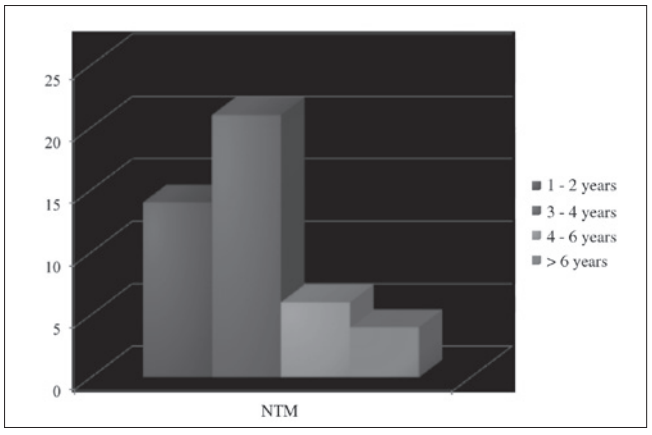

Fig. 6.2. Age distribution of children affected from cervical Non Tuberculous Mycobacteria infection recorded at Bambino Gesù Hospital in the period 2008-2017.

Tabella 6.VII. Cervical localisation of Non Tubercoulous Mycobateria infections recorded at Bambino Gesù Hospital in the period 2008-2017.

Total Non Tuberculous Mycobacteria infections	N = 45
Upper latero-cervical level	18
Mandibular angle	14
Parotid	2
Submandibular	1
Multiple sites	10
Latero-cervical level + parotid	5
Latero-cervical level + mandibular angle	2
Latero-cervical level + mandibular angle + parotid	1
Mandibular angle + parotid + Submandibular	1
Parotid + Submandibular	1

In the latero-cervical localisation, selective drainage was always carried out at the level affected, including the area of cutaneous infiltration. In the angulo-mandibular localisations, only in 4 cases was it not necessary to remove the gland, while in the parotid localisations a superficial parotidectomy was always carried out.

In 50% of the cases of parotid and angulo-mandibular localisation, there was a deficiency in the marginal branch of the facial nerve, but in only 2 cases of angulo-mandibular localisation this deficit has not been recovered within the period of 12 months.

The surgical scar showed a tendency to enlargement and to keloid only in cases in which initial cutaneous infiltration was greater than 3 cm.

If a follow-up of 12 months is considered, there were no recurrences of lymph node disease.

In conclusion, infections from NTM represent an emerging disease in which, in pediatric age, lymph node involvement is prevalent, unfortunately this kind of etiology is often misconstrued, causing delays in diagnosis or inappropriate medical and surgical treatment. In agreement with the majority of Authors, it is felt that surgical operation is the treatment to choose in these diseases, which should be done as early as possible. The difficulties lie precisely in its early diagnosis; in fact, only a culture exam will give a certain diagnosis, but this can only be done with biological material and only after several weeks of culture.

In the meantime, the lymph node lesion will have progressed its clinical stage with progressive extension and cutaneous involvement. This places the surgical operation more at risk of iatrogenic lesions of the facial nerve, with the need for more extensive removal of the affected

cutaneous area, with the association of possibly greater aesthetic damage.
If the disease is particularly extensive, the only solution is lengthy antibiotic treatment associated with a possible curettage of the lesion. This option, though, leads to worse aesthetic outcomes and greater collateral effects linked to the medical treatment.
However, the patients should be considered case by case, while seeking to succeed in shortening the length of the disease and reducing morbidity to a minimum.

7. Non-specific granulomatous lymphadenitis

The lymphatic system consists of a network of lymphatic tissue, transport ducts and lymphoid cells. It plays a double role, ensuring maintenance of balance of extravascular fluids and representing a system of immune barrier. The second function is ensured by the lymphoid cells and lymphatic tissue organised into structures called lymph nodes (about 600 in the human body), mainly located at the level of the roots of the limbs, in the neck and in the vicinity of the main blood vessels.
The lymph nodes can be reached through the blood or, especially, through the lymphatic ducts, by etiological agents which induce reaction processes. When a pathogenic element enters the organism, it is captured by cells holding antigens (dendritic cells and macrophages) which activate an initial immune response; these cells pass through the lymphatic system up to the nearest lymph node, where they interact with T-cells and activate them to trigger the following stages of immune response.
These activations of lymph node immune response (lymphadenitis) may represent the local reaction to afflictions restricted to the tributary territory or may be part of a disease picture that is not strictly localised but may rather have a multi-station nature. This is why a lymph node biopsy may be useful, not only for investigating the disease of the lymph node, but also to obtain more general diagnostic indications. Depending on the disease, there may be various lymph node stations involved and therefore also the site of the lymphadenopathy can often prove useful for diagnostic purposes.
Intensity and characteristics of the lymph node alterations depend on the type of etiological agent causing them, its manner of action and the subject's reactivity. In general, hyperplastic phenomena of lymphatic tissue or a granulomatous aspect are the most prevalent, but there are various morphological pictures. A "granulomatous lymphadenitis" diagnosis is of a histological type and is based on the finding of granulomas in the lymph nodes, in other words aggregates of histiocytes similar to epithelial cells (epithelioid cells); these granulomas may contain a central necrosis, sometimes radially enclosed by palisading spindled histiocytes, encircled by small lymphocytes and plasma cells. This histological picture can be seen when substances are present which the histiocytes cannot digest or in the presence of a T-mediated immune response. The morphological picture of the granulomatous forms is varied (Table 7.I). Possible findings may be: hyperplasia of the lymphatic tissue with dissemination of epithelioid cells, isolated or in small groups (Piringer-Kuchinka lymphadenitis), correlated with infectious mononucleosis and toxoplasmosis; granulomatous lymphadenitis featuring outbreaks of epithelioid cells and plurinuclear giant cells with or without caseous necrosis, linkable with tuberculosis, brucellosis, sarcoidosis, berylliosis, histoplasmosis, etc.; necrotizing granulomatous lymphadenitis with infiltration by granulocytes correlated with cat-scratch disease, tularemia, lymphogranuloma venereum, yersiniosis, etc.; necrotizing granulomatous lymphadenitis without infiltration by granulocytes, correlated with disease of an unknown cause, such as KFD.
Cervical lymph nodes are frequently involved are and clinically more manifest than other lymph node stations. This is why the otorhinolaryngologist is often called on in the diagnostic stage of a lymphadenopathy of which the nature has yet to be established.
Granulomatous lymphadenitis with cervical localisation may be separated into non-infectious forms (which are not included in this treatise) and infectious forms. The latter may sometimes be separated into suppurative forms (from tularemia or "cat scratch" disease) and non-suppurative forms (from tuberculosis, atypical mycobacteria, brucellosis, toxoplasmosis, mononucleosis). Worth of mention are also granulomatous forms from unknown causes such as KD and KFD, which may have a cervical presentation in the initial stage, entering differential diagnosis with the previous ones (Table 7.II).
Lymphadenopathy is the term currently used to describe the condition in which one or more lymph nodes become abnormal in dimensions, consistency and mobility. The normal dimension of a lymph node is generally indicated as < 1 cm, even though various criteria of normal dimensions are given, depending on the anatomical region and on the age range.
A lymphadenopathy may be either the expression of a localised disease or a systemic one. In about 75% of cases lymphadenopathies present in localized form, about 50% of which involving the cervical-facial area. In the remaining 25% of cases the lymphadenopathy is pathognomonic of an underlying systemic disease.
Achieving a diagnostic picture of a cervical lymphade-

Table 7.I. Histological pictures and correlated diseases.

Morphological picture	Characteristics	Correlated diseases
Piringer-Kuchinka lymphadenitis	Hyperplasia of the lymphatic tissue with dissemination of epithelioid cells isolated or in small groups	Infectious mononucleosis Toxoplasmosis
Granulomatous lymphadenitis with giant cells without caseous necrosis	Outbreaks of epithelioid cells and plurinuclear giant cells without caseous necrosis	Sarcoidosis Crohn's disease Berylliosis Neoplasia
Granulomatous lymphadenitis with giant cells with caseous necrosis	Outbreaks of epithelioid cells and plurinuclear giant cells with caseous necrosis	Tuberculosis Brucellosis Histoplasmosis
Necrotising granulomatous lymphadenitis with infiltration by granulocytes	Granulomas of epithelioid cells with necrotic haemorrhagic areas with granulocytes	Cat-scratch disease Tularemia Lymphogranuloma venereum Yersiniosis
Necrotising granulomatous lymphadenitis without infiltration by granulocytes	Granulomas of epithelioid cells with necrotic haemorrhagic areas without granulocytes	Unknown cause KFD KD

nopathy often proves very demanding. Possible different diagnoses vary with the age of the patient: malignant diseases are more common in the elderly, while reactive and infectious diseases are prevalent in the first decades.

The presence of lymphadenopathies in some anatomical regions appears to correlate with greater risk of malignant disease: at the supraclavicular level the incidence of malignant lymph node disease is 90% in subjects aged > 40 years and 25% in subjects in lower age groups [1].

Granulomatous lymphadenopathies, in the picture of cervical lymph node disease, represent an estimated overall share of between 6% and 15% [2,3]. In a series of 251 diagnostic cervical lymphadenectomies, incidence of granulomatous forms proves highly influenced by the age of the patients: 23.1% in the age group 0-20 years, 9.8% between 21 and 45 years, 3.1% between 45 and 65 years, 3.8% in subjects > 66 years [3].

Diagnostic approach to a cervical adenopathy is based on:

- history;
- assessment of accompanying signs and symptoms;
- clinical assessment;
- laboratory diagnostics;
- imaging;
- cyto-histopathological study.

Full, accurate collection of case history data is required, in order to determine the etiology of a lymphadenopathy. Age, onset modalities, duration of symptoms, concomitant diseases as well as context and circumstances in which the lymphadenopathy was diagnosed are equally highly valuable information. Apart from that, also history of contact with animals, assumption of certain foods or drugs, environmental risks and history of recent infections or immune disorders of any kind can give further help in forming a diagnostic picture. Where granulomatous lymphadenitis is present, the accompanying symptomatology consists in most cases of high fever, feeling generally unwell, arthralgia and localised or generalized cutaneous manifestations.

Where cervical lymphadenopathy is present, each patient must undergo a full, systematic inspection, aimed at identification of every adenopathy that can be felt to be present. Identification of a localised rather than a systemic adenopathy is fundamental in forming a diagnostic picture. If, according to convention, the pathological dimension

Table 7.II. Granulomatous lymphadenites of a possible cervical localization.

Non infectious	Infectious suppurative	Infectious non-suppurative	Unknown cause
Sarcoidosis	Tularemia	TB	KD
Sarcoid-like	Cat-scratch disease	Atypical mycobacteria	KFD
Berylliosis		Brucellosis	
		Toxoplasmosis	
		Mononucleosis	

of a cervical lymph node usually indicated as > 1 cm, it should not be forgotten that in areas such as the supraclavicular one the cut-off is equivalent to 0.5 cm. When exploring the lymph nodes, the presence of pain and tension are signs that are scarcely specific, mainly correlated with an underlying inflammatory situation. Consistency of a lymph node involved in an acute inflammation tends to be increased and is associated with local tension which is more or less marked; a chronic inflammation leads to fibrosis, with consequent hardening of the node. The presence of merging lymph nodes may be a sign either of a benign disease (sarcoidosis, mycobacterial infection, etc.) or malignant one (carcinoma metastasis, lymphoma, etc.). Preserved mobility is usually expression of an infectious or immune type of lymph node disease.

In any case, a correct differential diagnosis is based on analysis of all the possible causes of a cervical lymphadenopathy, in accordance with the "CHICAGO" acronym [4]:

- C = cancers;
- H = hypersensitivity syndromes;
- I = infections;
- C = connective tissue disorders;
- A = atypical lymphoproliferative disorders;
- G = granulomatous;
- O = others.

Laboratory diagnostics include performance of a hemochromocytometric study. The presence of anemia, a lymphocytosis rather than a lymphocytopenia, atypical lymphocyte, high values of ESR and CRP or of IgM antibodies may all be very helpful in diagnosis.

In imaging diagnostics the first choice exam in assessing a cervical adenopathy is echography, keeping CT and MRI in reserve for cases of suspect concomitant involvement of the chest and abdomen or for cases of suspect neoplasia.

To make data collected with echography exams more sensitive, certain algorithms have been proposed, such as the Solbiati index, which uses the ratio between longitudinal length and transverse diameter to distinguish cases presumably associated with benign diseases (ratio > = 2) from those that are potentially malignant (ratio < 2).

Tissue diagnostics represents the gold standard in the study of lymphadenopathies.

FNAC is a simple, safe procedure, featuring elevated sensitivity and specificity in diagnosis of lymph node metastasis and providing good accuracy in diagnosing granulomatous lymphadenopathy, reactive hyperplasia, infections and lymphomas. Immunohistochemistry, flow cytometry and US-guided FNAC are other methods permitting implementation of the potential of diagnostics.

Percutaneous image-guided core needle biopsy is a method which enables collection of a greater quantity of sample tissue and therefore can provide a diagnosis in cases of failed diagnosis with FNAC. Core needle biopsy is considered a good alternative to performance of a diagnostic lymphadenectomy.

The literature shows 6 criteria considered significant in forecasting the usefulness of performing a lymph node biopsy:

- age > 40 years;
- lack of tenderness;
- dimensions of lymph node;
- generalised itchiness;
- supraclavicular localisation;
- hardened consistency of lymph node.

The presence of one or more of the above-mentioned criteria, as well as difficulty in forming a diagnosis on the basis of results of FNAC and/or core needle biopsy, are indicators for performing a lymph node biopsy.

In support of the already amply mentioned diversity, Figure 7.1 shows decisional algorithms for cervical lymphadenopathy diagnostics in adults [1]; Figures 7.2 and 7.3 show decisional algorithms for cervical lymphadenopathy diagnostics in young children [5].

The following is a presentation of the most common causes of granulomatous lymphadenopathies of an infectious nature. Regarding secondary forms of infections from typical and atypical mycobacteria and from HIV, please refer to the relevant chapters.

7.1 Tularemia

Tularemia is an infection sustained from a Gram-negative coccus, Francisella (Pasteurella) tularensis, a parasite of some wild rodents, which may be transmitted to humans through contact with infected animals or by means of bites from ectoparasites (ticks, etc.).

Human infection appears as ulceration at the point of penetration and associated with satellite lymph node reaction. Depending on the area of penetration they have differing clinical forms: oculoglandular (penetration through the conjunctiva), ganglionic ulcer (penetration through the cutis). The disease can also be contracted by inhalation (pulmonary form) or by ingestion of contaminated materials (typhoid form). Apart from the lymph nodes, the disease can spread through hematic routes and generalise in various organs and tissues, where formation of granulomatous nodules can be seen, which progress into purulent colliquation. If not treated adequately, the disease may cause death of the patient or present frequent recurrence, due to the intracellular nature of the parasite, which multiplies sheltered from antibody activity inside histoid cells (monocytes) [6].

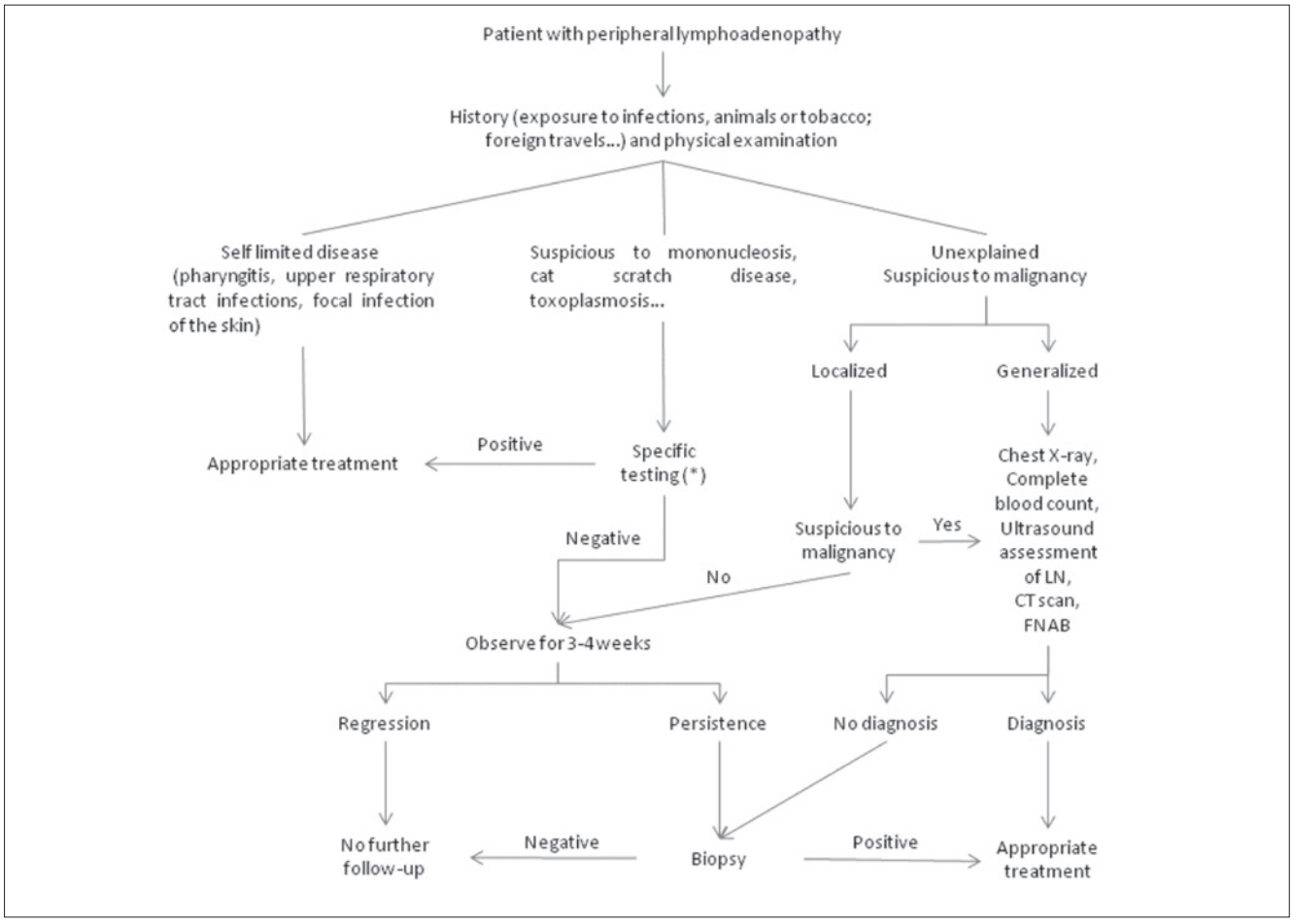

Fig. 7.1. Algorithm for the diagnosis and evaluation of patients with peripheral lymphadenopathy (from Mohseni et al., 2014 [1], mod.).

In potentially exposed subjects (generally hunters, cooks, leather workers), the diagnosis is based on cultural isolation of the parasite in biological materials (expectorate, blood, exudates). For diagnostic purposes, it helps to look for antibodies through agglutination of killed bacteria suspension (positive starting from the 9th-10th day of infection) and intradermal reaction with bacterial lysate (positive starting from the 10th-15th day of infection).
The infection may also be treated successfully using treatment with fluoroquinolones, aminoglycosides or tetracycline. Francisella tularensis is insensitive to penicillin and sulfonamides [7].

7.2 Cat-scratch disease

Cat-scratch disease is an infection sustained from a Gram negative bacillus of the Rochalimaea hensenlae species, a parasite of the endothelia and the reticulo histiocytary system. The natural reservoir of the bacterium is unknown and there is no arthropod transmitter. Transmission of the infection occurs through transcutaneous inoculation, usually through sharp pointed objects or by animals (generally cat scratches).
The clinical form may vary. The most common morbid form is a chronic granulomatous lymphadenopathy; in typical form it starts with formation of a papule at the point of inoculation, which arises after an incubation period of 4-6 days. Within a few days this papule develops into a pustule, accompanied by a consistent regional adenopathy and, in 30-40% of cases, high fever. The infection may also develop into atypical forms that are more difficult to diagnose (granulomatous conjunctivitis with preauricular adenopathy, tonsillitis, cerebral arteritis, pneumonia with hilar adenopathy…). In cases of acquired immune deficiency (such as in HIV infection or immunosuppressive therapy) angiomatous manifestations are possible, both cutaneous (in differential diagnosis with KS) or hepatic, as well as feverish recurrent forms with bacteriemia [8-10].

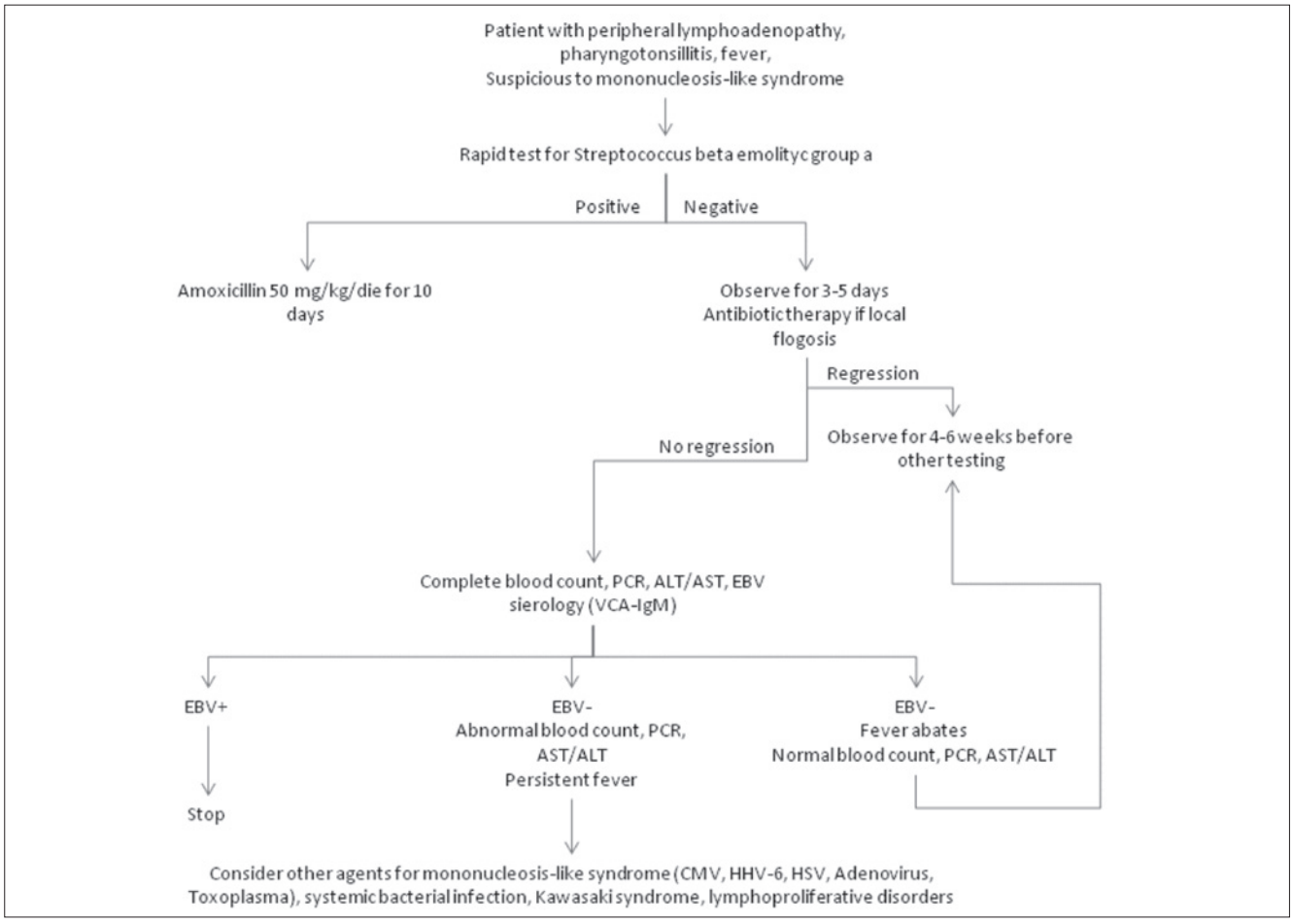

Fig. 7.2. Algorithm for the diagnosis and evaluation of pediatric patients with peripheral lymphadenopathy suspicious to mononucleosis-like syndrome (from Chiappini et al., 2015 [5], mod.).

Diagnosis is formed by isolation of the microorganism or by demonstration of specific serum antibodies.

The infection is resolved mainly in a spontaneous manner, but over periods that are not short (2-4 months). Antibiotics, aminoglycosides, rifampicin and a combination of sulfonamides and trimethoprim are useful in reducing the duration of the disease [9 10].

7.3 Brucellosis

Brucellosis is an infection sustained by a Gram negative coccus bacillus family, of which only three species are pathogens for humans (Brucella melitensis, Brucella abortus, Brucella suis), transmittable to humans by means of ingestion of unpasteurised milk or fresh dairy products, or through direct contact with infected animals (Brucellae are natural pathogens for various kinds of mammals, in which they cause subacute scarcely symptomatic infections and are eliminated with milk and urine, as well as with the embryonic tissue when the infection induces abortion). When the infection occurs by oral means, the appearance of cervical adenopathies is more frequent than what can be seen after infection arising from contact with animals (83 *vs* 63%) [11].

Human infection takes a much slower course, preceded by a long period of incubation and accompanied by fever, with quite varied progress (interspersed with periods of apyrexia), well preserved general condition, splenomegaly and articular pain. Once they have passed the cutaneous and/or mucous barrier, Brucellae have tropism with histiocytary cells, inside of which they resist the phagocytes and multiply to form granulomas of tubercular appearance within the regional lymph nodes and organs that are rich in histiocytary cells (liver, spleen, bone marrow).

In potentially exposed subjects (generally agricultural workers and veterinary surgeons), the diagnosis is based on cultural isolation of the parasite in biological material

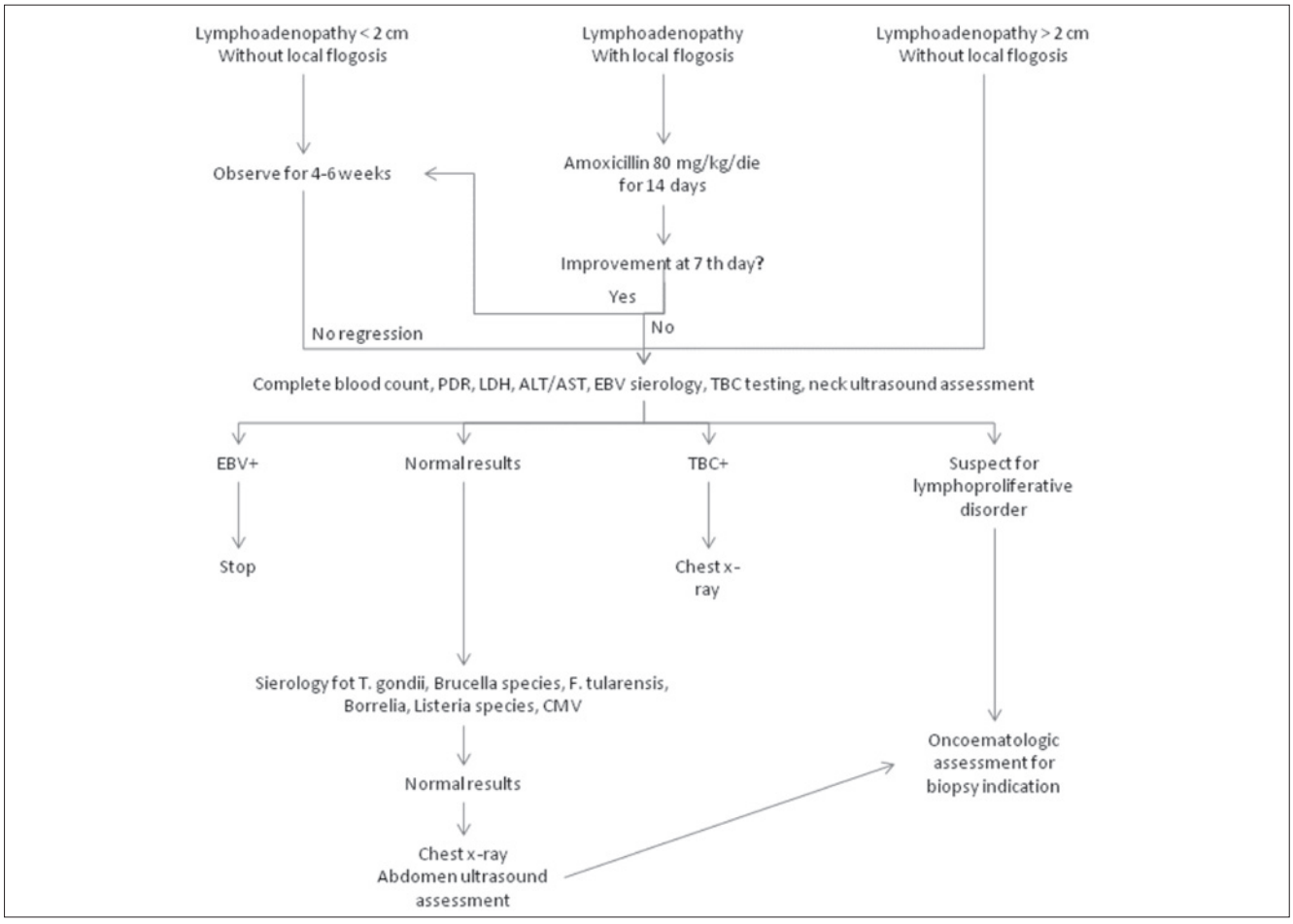

Fig. 7.3. Algorithm for the diagnosis and evaluation of pediatric patients with peripheral lymphadenopathy without local flogosis (from Chiappini et al., 2015 [5], mod.).

(blood or, in the event of negative blood culture, by means of examining bone marrow samples from sternal puncture). Given that brucellosis has a long incubation period, at the time of appearance of the first symptoms there may already be an elevated presence of antibodies. For the purpose of diagnosis therefore, it is possible to look for antibodies by means of agglutination tests, generally associated, for differential diagnosis, to that of Salmonella, because of overlapping symptoms in the initial stage 12.
Eradication of the infection can be achieved by treatment with tetracycline; other active antibiotics are chloramphenicol, streptomycin, rifampicin and the sulfonamides. Prolonged treatment is often necessary, probably because of the intracellular localisation of the Brucellae.

7.4 Toxoplasmosis

Toxoplasmosis is an infection sustained by a family of parasitic endocellular protozoi, of which only the Toxoplasma gondii species is a pathogen for humans. Its spread is cosmopolitan and in the Italian population prevalence of seropositivity is extremely high, ranging from 40 to over 80%. Seropositivity increases with age, indicating continuity of risk of contagion. The life span of Toxoplasma gondii involves a sporogonic stage in the final host and an asexual stage in intermediate hosts, including in humans. The non-sporulated oocyst is formed in the intestinal epithelium of infected cats and expelled through the faeces; sporulation occurs after expulsion. If ingested by a human, it releases sporozoites, which penetrate the intestinal epithelium and pass by blood and lymphatic routes to reach, in the form of merozoites, the organs of the reticular endothelial system and especially the lymph nodes. A human can also be infected by ingestion of meat contaminated by cysts and, in a totally exceptional manner, by transfusion of infected blood. Maternal foetal transmission occurs exclusively during the parasitic stage of initial infection [8 13].

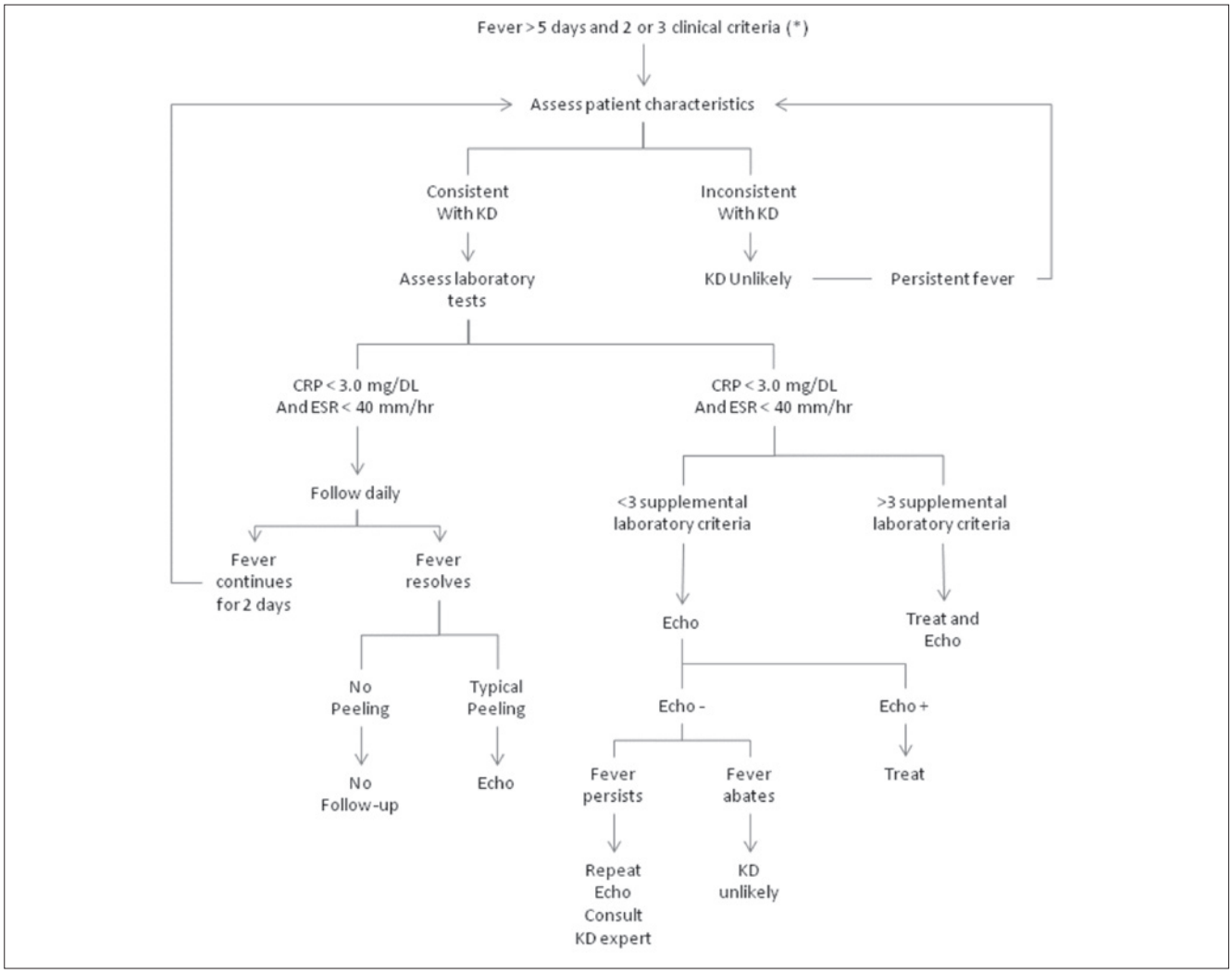

Fig. 7.4. Evaluation of suspected incomplete Kawasaki disease Clinical criteria are: changes in extremities (acute: erythema of palms, soles; edema of hands, feet; subacute: periungual peeling of fingers, toes in weeks 2 and 3), polymorphous exanthem, bilateral bulbar conjunctival injection without exudate, changes in lips and oral cavity (erythema, lips cracking, strawberry tongue, diffuse injection of oral and pharyngeal mucosae), cervical lymphadenopathy (>1.5 cm diameter, usually unilateral) (from Newburger et al., 2004 [17], mod.).

Toxoplasma infection in most cases has an asymptomatic course; it rarely makes itself evident but mostly in the form of mononucleosis-like feverish lymphadenitis [14]. There are very rare forms with polyvisceral involvement and hemorrhagic forms similar to rickettsiosis. The connatal forms have greater importance, more serious in the first three-month period (inasmuch as it is often abortive), more often subclinical if the infection occurs after the first three months of pregnancy. In the latter case the clinical manifestations vary widely, from just the serological stigma of infection down to serious diseases (so called Sabin's Tetrad, which includes endocranial calcification, hydrocephalus, chorioretinitis, convulsions). Chorioretinitis may also set in later, up to the fifth year of life. In cases of acquired immune deficiency (from HIV or immunosuppressive), there is the possibility of meningoencephalitis from Toxoplasma gondii, caused by uncontrolled replication of the protozoi, with necrosis of the surrounding cerebral tissue as far as coma and death, and ocular forms (chorioretinitis).

Diagnosis is based on direct search for the parasite or its components and on serological investigations to identify past contact through searching for specific antibodies. Particular problems arise in forming an early diagnosis of certainty of foetal infection, based on searching for IgM antibodies in funicular blood

samples taken from echo-guided funicular centesis. On birth, in consideration of the transplacental transfer of maternal IgG, it is based on the search for Ig of the M class. In an immune compromised host, serological diagnosis can prove difficult because of reduced antibody production.
Treatment is based essentially on the use of pyrimethamine and of sulfonamides. Treatment with spiramycin alone is used in prevention of connatal forms, when seroconversion has been demonstrated in the course of pregnancy [15].

7.5 Infectious mononucleosis

Mononucleosis is an infection sustained by the Epstein Barr DNA virus. The anatomical site of the initial infection is represented by the oropharyngeal epithelium, where the virus replicates and can give rise to a persistent infection which may even last for several years, during which the infected subject, even if asymptomatic, acts as a source of infection, by eliminating the virus through saliva.
Infection from EBV is very widespread in all populations and is generally contracted in childhood, when it is normally asymptomatic. First infection in youth or adult age, on the other hand, appears more frequently in an acute mode, characterised by fever, pharyngitis, lymphadenitis and splenomegaly, together with alterations of hepatic functions. In about 50% of cases the disease is accompanied by the presence of a malodorous, whitish grey exudate from the palatine tonsils. In some cases, it may be accompanied by various types of complication, from the appearance of morbilliform exanthems localised in the trunk to icterus, down to neurological complications [16].
Looking for heterophile antibodies (Paul Bunnel Davidsohn reaction) is an excellent tool for diagnosis. In cases of uncertainty antibodies can be looked for in comparison to various antigens of the virus, either non-structural (early) or structural.
Mononucleosis generally requires only treatment of symptomatic support. Fever and pharyngitis benefit from use of paracetamol. Although they can accelerate recovery from pharyngitis, glucocorticoids are only used for reducing size of the pharynx swelling [17]. There is no evidence of significant benefit from clinical practice of treatment with acyclovir [18]. The patient must stop practising violent sports for 6-8 weeks to avoid the possible, even if infrequent, occurrence of rupture of the spleen. Recovery from mononucleosis is often gradual, with an alternating condition of feeling unwell lasting some time.

7.6 Kawasaki disease (KD)

KD is a self-limited acute vasculitis, of unknown etiology, which mainly strikes subjects in infancy and childhood.
The disease may present either in endemic or epidemic form and strikes mainly, but not exclusively, subjects of Japanese race. The etiology is unknown, even though its clinical and epidemiological characteristics strongly suggest an infectious cause.
KD is characterised by fever, bilateral non exudative conjunctivitis, widespread erythema of the oral and labial mucosa, modifications of the extremities, rash and cervical adenopathies. Aneurisms or ecstasy of the coronary arteries develop in 15-25% of untreated subjects and may cause cardiac ischemia or sudden death. Today KD has replaced rheumatic disease as the primary cause of acquired cardiac disease in infancy [19].
Diagnosis is based on the presence of fever persisting for at least 5 days, accompanied by at least 4 of the 5 main clinical signs:

- modification of extremities;
- polymorphous exanthem;
- bilateral non-exudative bulbar conjunctivitis;
- widespread erythema of oral and labial mucosa;
- cervical adenopathies.

These clinical signs are not usually present simultaneously, so careful supervision is often necessary in order to form a diagnosis.
In the presence of 4 of the 5 main criteria, the diagnosis may be made on the fourth day of the disease; patients with fever persisting for 5 days but with less than 4 diagnostic criteria, may be diagnosed when coronary disease is shown by echocardiogram or angiography.
A diagnostic algorithm for these "atypical" or "incomplete" cases of KD is shown in Figure 7.4 [19].
Fever in KD is typically remittent, with peaks of temperature from 39 to 40°C. Without suitable treatment, fever will persist for about 11 days, although it may last even for 3-4 weeks. If treated, the fever is usually resolved in 2-3 days.
Laterocervical adenopathy is the least common of the main diagnostic criteria. This is typically unilateral and confined to the region of the anterior cervical triangle. The classic criteria of inclusion require the presence of one or more lymph nodes with diameter > 1.5 cm. The lymph nodes are mostly hard, not fluctuating and not painful, the overlying cutis is generally not reddened. Appearance of an adenopathy often represents one of the early symptoms of KD.
A sub-population of KD has recently been shown, which is assessed at around 9% of observed cases and characterised by presentation limited to fever and cervical adenop-

athy: these are subjects characterised by slightly higher age, who reach diagnosis later and with higher inflammatory markers, but without showing less favourable prognostic factors [20].
In general, from a histopathological viewpoint, lymph nodes are characterised by widespread thrombotic arteriolitis and necrotic phenomena.
Treatment of KD carriers in acute stage aims at prevention of onset of coronary disease and is based on early administration (by the 7-10th day of disease) of high doses of acetylsalicylic acid, associated with intravenous administration of immunoglobulin. The role of steroids and Pentoxifylline is subject to discussion.

7.7 Kikuci-Fujimoto disease (KFD)
KFD or necrotising granulomatous lymphadenitis, is a disease of unknown cause, very probably secondary to a hyper immune reaction induced by different antigenic stimuli or an autoimmune process in which apoptosis plays a determining role [21].
KFD preferably strikes subjects aged < 40 years, prevalently of the female gender. It has low incidence, estimated between 0.5 and 5% of all cases of adenopathies evaluated histologically.
The disease is characterised by an acute or sub-acute beginning and usually develops over 2-3 weeks. Lymphadenopathy is the most common initial symptom and involves the cervical and supraclavicular lymph nodes in almost all cases (74-90%) [22].
The adenopathies typically have dimensions no greater than 3 cm, hard consistency, rarely painful to the touch.
Generalised lymphadenopathy is an infrequent occurrence (< 5% of cases); extranodal involvement is rare, but may involve kidneys, liver, gastrointestinal tract, thyroid, parathyroid, adrenals and bone marrow.
After lymphadenopathy, the most frequent initial symptom is fever, present in 30-50% of afflicted subjects.
Other symptoms, such as arthralgia, tiredness, skin rash and hepatosplenomegaly may be present.
ESR level and anaemia are the most common laboratory findings.
Diagnosis is typically histological. KFD is characterised by areas of focal paracortical necrosis, encircled by histiocytic aggregates. Three different histological profiles have been described, which appear to correspond to evolutionary or sequential stages, to different etiological agents or also to differences in the inflammatory response in the patients: proliferative, necrotising and xanthomatous.
There is no specific treatment: signs and symptoms tend to regress spontaneously within 4 months. The rare patients with severe or persistent symptoms may benefit from treatment with corticosteroids.
Long term follow-up is recommended, due to the possibility of relapse (up to 3% of cases) and the possibility of developing a systemic lupus erythematosus.

8. Emerging pediatric ENT infectious diseases

8.1 Rhinosinusitis
"*Acute bacterial otitis media and acute bacterial sinusitis are the most common complications of viral upper respiratory infections and are probably the most common indications for the prescription of antimicrobial agents*": this first paragraph of the *Clinical Practice Guideline* on treatment of sinusitis, drafted by the American Academy of Pediatrics [1], makes it clear how much attention is being paid today to rhinosinusal phlogistic disease in pediatric age.
In this age group, infectious rhinitis, for which prevalence is constantly increasing both in countries with highest rates of industrialization and in those with a predominantly agricultural economy, is more easily capable of influencing complications to the detriment of the paranasal sinuses and, in the first three years of life, of the "paranasal sinus with modified function", which is represented by the middle ear.
CT scan and NMR images acquired with inflammation of the upper airways in course show that involvement of the paranasal sinuses by a viral inflammation of the upper airways, which was in the past underestimated due to incomplete or non existent development of the sinuses in early infancy [2], is almost constant and therefore justifies the more correct terminology of rhinosinusitis.
Although it has long been considered a disease affecting especially adult age groups, rhinosinusitis finds extremely fertile ground in children, influenced by the greater frequency of viral forms that they can contract at school age. The number of infections in childhood is distinctly higher (from seven to ten episodes of viral rhinitis) than what is seen in adult ages (from two to five episodes) [3 4]: a percentage of these viral infections, estimated between 0.5 and 2 per cent, is complicated by a bacterial superinfection [3 5]. According to the National Center for Disease statistics, rhinosinusitis represents one of the most frequent, if not the most frequent, diseases in the United States of America: the document estimates that a percentage between 5 and 10 per cent of children afflicted by URI will develop an acute rhinosinusitis, which not infrequently may progress into a chronic disease. Bhat-

tacharyya [6] published data in 2009 from the US National Health Interview Survey, referring to the years between 1997 and 2006, from which there emerges an annual prevalence of acute and chronic rhinosinusitis equivalent to15.2% of the population; it is therefore understandable that this problem is of extreme importance in terms of health policies. In a study from a few years ago, Ray estimated the cost of treatment for chronic rhinosinusitis in the USA to amount to 5.8 billion dollars in 1996, 31% of which (1.8 billion) for care administered to patients aged below 12 years.

Not all paranasal sinuses are well developed at birth. Radiological exams and especially tomography show that the maxillary sinuses and ethmoidal chambers already become pneumatized at the 3rd-4th month of embryonic life and, even though they are of small dimensions, they are the only sinuses already present at birth [7]. The ethmoid expands rapidly in the first seven years of life and completes its development at around 15-16 years; the maxillary sinuses also develop very rapidly and are almost complete in the first ten years, reaching their final dimensions by expanding downwards after growth of the second set of teeth, with progressive pneumatization of the alveolar process, which brings the floor of the maxillary sinuses, in adult age, to a position 4-5 mm lower than the floor of the nasal fossa. The frontal sinuses are not initially distinguishable from the anterior ethmoidal cells and they start a slow growth process from the age of one year, until they become radiologically evident in 30% of children around the sixth year of life, reaching full development at around 19 years. At birth, the sphenoid is little more than an extension of the sphenoethmoidal recess; from the age of seven years it starts to develop progressively towards the rear and in 85% of children its pneumatization is visible on CT at the age of 8 years [7]. In a long term prospective epidemiological study, which would be rejected today by any Ethics Committee, Maresh and Washburn [8] followed, from 1925, the case histories of one hundred healthy children, subjecting them to four flat radiographies, one for each season of the year, and detecting quite a high percentage (30%) of "diseased" antra in the first six years of life, a value which halved (15%) from six to twelve years. It was not the dimensions of the sinuses, but the season that influenced radiological evidence of an episode of catarrh.

Another large scale prospective study by Bagetsch [9] studied a pediatric population of 24,000 children and detected that in the first five years of life there is a highly significant difference in the prevalence of URI in children who attend nursery school compared with children who are late in entering the community (72 against 27%). Certain considerations can be derived from these and other epidemiological studies:

- prevalence of rhinosinusitis in the first years of life is distinctly higher in children who attend nursery school than in those who enter the community later;
- in temperate climates the frequency of rhinosinusal inflammation is greater in cold seasons (autumn and winter);
- there is a progressive decrease in prevalence of acute rhinosinusitis after 6-8 years, correlated with maturity of the immune defense systems.

Although it is commonly accepted that incidence of acute rhinosinusitis in pediatric age is very high, there is still a persistent uncertainty about the real entity of this datum, since clinical diagnosis of children is often underestimated because of overlapping with other common manifestations of diseases, such as viral rhinitis, rhino-adenoiditis or allergic rhinitis, such that, in 2012, an EPOS [10] study group considered it impossible to differentiate on clinical criteria a chronic rhinosinusitis process compared with a rhino-adenoiditis of early infancy. It is therefore understandable that data reported in the literature is understated compared to the true incidence of disease, probably because the family pediatrician considers rhinosinusitis a pathological process with more serious clinical evidence and excludes the milder forms from a possible diagnosis, even though they represent 80-90% of the total [11].

In pediatric age, greater incidence of infections affecting the URI and the significant role played by the adenoidal vegetation in determining a condition of dysventilation and stagnation of bacterial biofilm lead to easier obstruction of the sinusal ostia, smaller in dimensions than in adult age, and explain the greater frequency of detection of diseases affecting the sinuses in the pediatric age. The beginning of an inflammatory process is almost always represented by a viral rhinitis using inflammation of the mucosa to induce an edema with related congestion of the sinusal ostium; the consequence is less ventilation of the sinuses, to which is added natural reduced ciliary activity of the respiratory epithelium, which is also secondary to the virosis. Subsequent evolution is represented by stasis and stagnation of secretions in the sinusal chambers; the inflammatory process, which started in the first instance with a stage characterised only by accumulation of mucous secretions, then evolves towards bacterial colonisation of germs both from the nose and the rhinopharynx, taking on the typical purulent characteristics.

The bacterial biofilms and exotoxins they produce play, in association, an important pathogenic role; the former use a matrix consisting of polysaccharides, nucleic acids and protein to ensure bacterial aggregation and lasting colonisation;

while the latter determine damage to the mucosa, with related assistance for spreading the process of infection. With regard to the role played by the adenoids in encouraging inflammation of the paranasal sinuses and especially in helping them to recur and/or to evolve toward a chronic disease, most authors agree in considering adenoid volume as a less significant factor than bacterial load [12 13], even though some authors stress the correlation between the level of lymphatic hypertrophy and the disease of the maxillary sinus, which is found in 34% of patients with significant adenoid volume and in only 13% of patients with normotrophic adenoids [14].
In early infancy the inflammatory process involves more or less widely all the paranasal sinuses, as witness to the predisposing role played by immune immaturity; in later infancy, on the other side, the sinusal involvement is usually just on one side, as a consequence of ostiomeatal obstruction caused, as in adults, by an anatomical disease element, such as septal deviation, concha bullosa or a non-diagnosed unilateral choanal atresia.
From a clinical point of view, forms of rhinosinusitis are considered acute, sub-acute and chronic on the basis of the duration of their symptomatology; this means indication of acute forms as manifestations lasting 10 and 30 days, sub-acute forms as those with clinical manifestations persisting for over 4 weeks but not longer than 12 and chronic forms as those manifesting symptomologies for more than 3 months.
Intensity of clinical expression permits distinction between mild, moderate and severe forms.

Acute Rhinosinusitis (ARS)
Differential diagnosis between a common cold and ARS may prove difficult in pediatric age, because of scarce compliance of young patients with examinations, however minimally invasive they may be; an adenoiditis may present clinically with the same signs and symptoms as a rhinosinusitis: purulent nasal secretion, anterior and posterior, and coughing both day and night; moreover the two processes may often overlap: in a study conducted by Marseglia et al. [15] on 287 children with signs and symptoms of rhinosinusitis lasting longer than ten days, endoscopic nasal examination confirmed the presence of purulent-like secretion of the middle meatus (indication of rhinosinusitis) in 89.2% of cases, which was isolated in 80.8% and associated with adenoiditis in 19.2% of cases; in 7% of the children the catarrhal secretion wetted only the nasopharyngeal lymphatic tissue (adenoiditis without sinusitis). Involvement of both structures was more frequent in the younger group of patients (0-5 years of age), while the isolated inflammation of the sinuses without signs of adenoiditis thypcal of the older patients. In the light of what has just been stated, a review of acute rhinosinusitis in children by Brook [16] proposes the use, as a parameter for making a correct diagnosis, of the presence of two major criteria or of one major criterium and two minor ones, lasting at least 10 days (Table 8.I).
The beginning and the clinical course in children may have different characteristics from each other: in some patients it appears as a continuum, compared to the initial rhinitic process and differentiation is difficult, except for persistence of a rhinorrhea of more than ten days, as for a viral coryza.
In other cases, however, a beginning may be seen with severe manifestations that right from the start make the seriousness of the clinical picture evident (high fever > 38.5 degrees centigrade, with purulent rhinorrhea). It is not unusual, however, that after an initially mild symptomatology, rapid worsening of the inflammatory picture may be observed, with the onset of greater symptoms accompanied by restlessness and behavioural changes.
Traditional flat x-rays are not suitable for evaluating patients with acute rhinosinusitis and even CT, which also represents the most suitable examination for diagnosis of rhinosinusitis, is not suitable for diagnosis of forms without complications, if it is considered that 80% of patients with a banal episode of URI present with radiological anomalies, which are absolutely not significant in a clinical sense, while in the presence of signs and symptoms suggesting complicated forms, such as persistent, worsening headache, presence of focal neurologic deficit, orbital edema or an ocular motility disorder it is indispensable to rely on a complete documentation by acquiring CT images, which are required also for the purposes of surgical planning.
The guideline document issued by EPOS in 2012 [10] also excludes ultrasound diagnostics as a possible backup in the diagnosis of an acute rhinosinusitic process and confirms the use of magnetic resonance in studying patients with individual complications.
Even though the spontaneous healing rate for acute rhi-

Table 8.I. ARS diagnostic criteria.

Major criteria	Minor criteria
Facial pain	Headache
Congestion of facial soft tissues	Halitosis
Blocked nose	Asthenia
Rinorrhea	Pain in the dental arch
Hyposmia-anosmia	Cough
Fever	Otalgia, auricular fullness
Purulent nasal secretion	

nosinusitis is around 50-60%, extended antibiotic treatment is recommended for at least 10-14 days and in any case for 7 days after symptomatological remission, in order to achieve faster healing and avoid suppurative complications. Empirical antibiotic treatment (not based on microbiological identification of the germ) represents the conventional approach in treatment of acute rhinosinusitis, which is easily understandable if one considers the difficulty of proceeding with obtaining suitable material for cultural examination contained inside the paranasal sinuses. Studies conducted on patients in adult age groups show a significant correlation between cultures resulting from material obtained at the meatal level and that present in the maxillary cavity; but similar studies have not yet been conducted on pediatric patients, even though an analogy with what has been observed in adults may be presumed.

For mild or moderate forms without complications and in children below the age of 2 years, assumption of amoxicillin is recommended, or alternatively second-generation cephalosporin, while administration of macrolides should be reserved for patients with known allergy to beta-lactams. In patients with severe forms or having risk factors (entry into community, recent antibiotic treatment), or in the event of failed response to amoxicillin within 48-72 hours, treatment should be commenced with high dosage of amoxicillin and clavulanic acid.

Treatment with levofloxacin, which was once reserved only for adults, is used today also in pediatric ages, when severity of the clinical picture recommends it.

Nasal irrigation with hypertonic solution (3%) and especially use of nasal topical steroids, particularly in patients with allergic rhinitis, is emphasised in every guideline, in order to facilitate decongestion of the nasal mucosa and improve drainage at the OMC level.

A study from 2015 by Ragab [17] shows that it is possible to achieve the same clinical and laboratory result in patients subjected to treatment with amoxicillin and patients subjected to nasal irrigation with saline solution of 0.9%; Tugrul [18] achieves the same results using fluticasone propionate and abundant irrigation with saline solution and proposes this associative scheme as a first line therapy in ARS in children. Therefore, the consideration of the EPOS 2012 study group [10] is held in suspension as far as its chapter devoted to treatment of ARS is concerned: "*antibiotic therapy seems to accelerate resolution of ARS in children but whether an acceleration of improvement of the symptoms with antibiotics in these children is worth the increased risk of antimicrobial resistance remains to be determined*".

Surgical treatment, in the acute stage, should be reserved for selected cases, especially for forms not responding to medical treatment, with severe symptomologies or when there is a high risk of intracranial or orbital complications. The management protocol for acute rhinosinusitis in pediatric age is represented in Figure 8.1.

The complications of an ARS, frequent especially in immunocompromised patients, may involve the orbit (optic neuritis, periorbital cellulitis, orbital abscess), the central nervous system (meningitis, subdural and epineural empyema, cerebral abscess, venous sinus thrombosis) or the bone (maxillary osteitis, frontal osteitis). Complications affecting the orbit are the most common, representing 80% of the total, caused by propagation of the infection through the dehiscence of the lamina papyracea, and their frequency is greater in pediatric age than in adults because of easier ostiomeatal obstruction and the delicacy of the bone structures involved. They are classified in accordance with the scheme suggested by Chandler [19 20] into:

1. preseptal cellulitis (Fig. 8.2);
2. orbital cellulitis;
3. subperiosteal abscess;
4. orbital abscess (Fig. 8.3);
5. cavernous sinus thrombosis.

Initial treatment in these cases consists of intravenous therapy with high dosages of antibiotics and cortisones; failure to improve in the first 48 hours influences recourse to ESS, which is first choice in a case of orbital abscess, with chemosis, proptosis, limitations to ocular motility and reduction of visus.

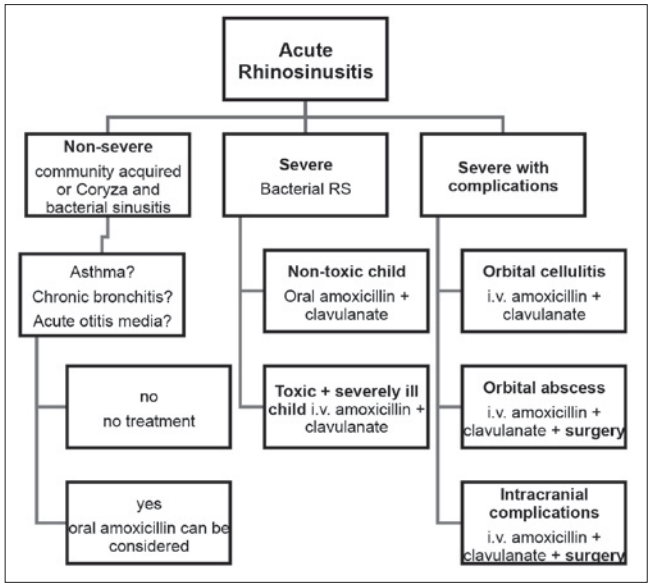

Fig. 8.1. Decisional algorithm evidence based scheme for therapy in children with acute rhinosinusitis (from Clement, 2007 [2], mod.).

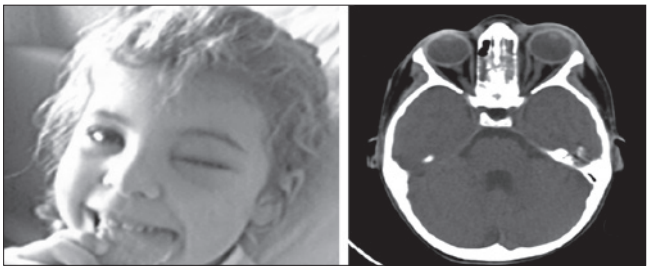

Fig. 8.2. Preseptal orbital cellulitis.

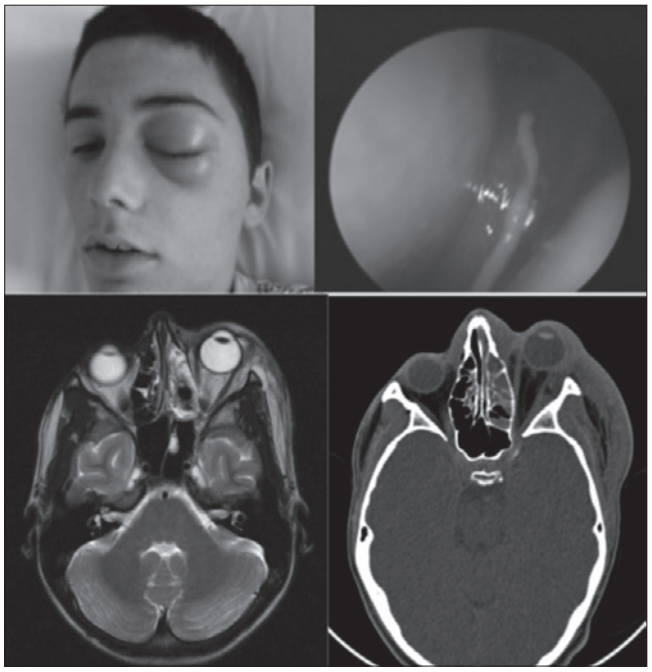

Fig. 8.3. Orbital abscess in ethmoid-maxillary sinusitis.

Chronic Rhinosinusitis (CRS)

Even more than the acute forms, diagnosis of CRS in pediatric age is made more difficult by the overlapping of symptoms compared with other common diseases capable of causing nasal obstruction in this age group, such as adenoid vegetations, chronic forms of adenoiditis, allergic rhinitis [10].

Diagnosis of CRS is suggested by the presence, for a period of time in excess of twelve weeks without remission, of the four most common symptoms: cough, rhinorrhea, nasal congestion and post nasal drip; the correspondence between symptoms and CT anomalies are greater when the clinical picture is characterized by rhinorrhea, cough and hypo/anosmia [21]. Age represents the most important risk factor associated with CRS, with a drop in prevalence of the disease after 6-8 years of age 9 [22]; in symptomatic patients, detection of CT anomalies has been observed in 73% of patients aged between 2-6 years, in 74% between 6-10 years, while a sharp reduction, equivalent to 38%, was in patients aged over 10 years. The impact of CRS on the quality of life of young patients is extremely negative: from questionnaires on QoL examined by Cunningham et al. [23], a surprising statistic emerges, showing suffering even higher than what is caused by chronic diseases such as asthma, attention deficit hyperactivity disorder, epilepsy and even rheumatoid arthritis. Faced with a chronic or recurrent rhinosinusitis in pediatric age, systemic factors must always be considered which may underlie this disease, such as allergy, immune disorders, cystic fibrosis, primary ciliary dyskinesia, in addition to the role played by adenoidal disease. Allergy has always been considered the main predisposing factor in chronic rhinosinusitis, but no study has yet been able to demonstrate a definite causal relationship between the two diseases. The relationship was also placed under discussion by Piacentini's study of 351 children afflicted with CRS, in which positivity to allergy tests was present in a percentage (30%) not dissimilar to that of the general population (32%) [24]. However, the comorbidity relationship between asthma and CRS is definite: after clinical remission of sinusal disease, achieved with medical treatment or with surgical repair, Rachelefsky [25] described a significant improvement in the asthmatic condition of 80% of patients studied through spirometry testing and detection of inflammation markers in the liquid from nasal irrigation.

A lot of interest is being paid today to defining the role of GERD in CRS. In a study by Phipps [26], 24-hour Ph-tests detected GERD in 63% of patients afflicted with CRS, with distinct symptoms improvement following introduction of a therapy for the treatment of reflux. A study conducted on a wide sample of children (1,980 patients with GERD and 7,920 controls) at the Texas Children Hospital [27], the number of patients afflicted with CRS proved to be significantly greater in subjects with GERD (4.1%) than in the control group (1.35%).

There is an even more definite relationship between a deficiency of immunocompetence and recurrent or chronic rhinosinusitis in pediatric age. Although immaturity of the immune systems is physiological in early infancy and tends to resolve itself after 7-10 years, it should not be overlooked that recurrent or chronic rhinosinusitis represents the most common mode of clinical presentation of a variable common immune deficiency; in a recent systematic review of the literature, Mazza et al. [28] demonstrated that a condition of immune deficiency significantly favors the onset of a CRS, with immune deficiency present in a percentage ranging from 10 to 54% of cases.

The Anglo-Saxons have coined the acronym SPUR (severe, persistent, unusual, recurrent), to indicate the characteristics that a clinical picture has to possess to establish the suspicion that a deficient immunological competence underlies the CRS: in these cases the patient must be directed towards a full immunoallergological study, which can help to resolve the pathological condition. CRS is considered a self-limiting disease, destined to improve with gradual maturity of immune defense systems; antibiotic treatment is therefore indicated in cases of frequent flaring infectious episodes. Antibiotics are substantially the same as those used for the acute form, but the treatment should be extended even for another three/four weeks. In consideration of the efficacy and safety demonstrated in treatment of allergic rhinopathies, local steroid therapy is considered the first line for chronic rhinosinusitis in pediatric age. In prevention of recurrence of acute episodes, some Authors find comfort in the use of bacterial lysates [29], whilst there is a lot of discussion on the possibility to improve clinical conditions in patients afflicted with chronic diseases (bronchiectasis, CRS), especially when sustained by immune deficiency, through antibiotic prophylaxis using azithromycin [30], erythrocin [17] or roxithromycin [31]: along with reduction of episodes of inflammatory re-acutization, an increased resistance to macrolides has actually been recorded, induced by *S*taphylococcus aureus and Streptococcus pneumoniae. There is still the fact reported by Ragab et al. [17] of an analogous improvement, in CRS symptoms and endoscopic findings, in patients treated with prophylaxis based on erythromycin, compared with those subjected to surgery: this fact gives confirmation to the Authors that CRS should be treated initially with an aggressive medical therapy before giving any surgical indication. In the latest position paper, jointly drafted by ARIA and EPOS and published in March of this year in Euforea (European Forum for Research and Education in Allergy and Airway Diseases) [32], aimed at principles of precision medicine in treating allergic rhinitis and CRS, the importance is underlined of correct typification of CRSs, on the basis of physiological, functional and pathological characteristics, in order to be able to predict risks of progression of the disease or its recidivity, so that the best available treatments can be used and new therapeutic strategies identified (Fig. 8.4).

The candidates for endoscopic surgery of paranasal sinuses are those children presenting with signs and symptoms of chronic rhinosinusitis and who do not respond to adequate medical therapies, that is, to the "maximum conservative therapy". Other treatment procedures taking into account age, predisposing factors and comorbidity may be useful before turning to the solution of surgery. As in glue-ear, chronic rhinosinusitis may progress towards spontaneous resolution when the child reaches the age of seven-eight years, with progressive maturing of the immune system. ESS therefore represents the last resort in treatment of rhinosinusitis and should be taken into consideration only after all other treatment options have been tried, including massive medical treatment using antibiotics for 10-14 days taken orally and, if symptoms persist, by intravenous means, checks on comorbidity, treatment of predisposing factors and, if necessary, adenoidectomy, whose appropriateness will be discussed later. Although there are no universally accepted guidelines on surgical treatment of chronic rhinosinusitis, most otorhinolaryngologists dealing with pediatric diseases believe that the complicated forms are definitely indicated for endoscopic surgery. In the same way, children who present with systemic conditions such as CF, immune deficiencies, fungal infections or neoplasias may present with forms of sinusitis which require surgical therapy.

Surgical techniques are substantially the same as those used in adults: PESS, however, requires extreme attention to preservation of anatomical structures and has different characteristics regarding its indication, preoperative measures, type of anesthetic and post-operative treatment. An essential premise to surgical treatment is represented by full understanding of the anatomical and pathological condition of the paranasal sinuses, obtained from CT Imaging and enabling the sinuses to be studied in axial, coronal and sagittal projections. These images, if correlated with a CANS, can also provide high precision and relative safety in reaching zones that are surgically difficult to approach, such as the anterior basicranium.

Study with 3D cone beam, if properly done with appropriate instruments, may also provide detailed images, with significantly less exposure to radiation: relying on this type of investigation is preferable in cases where frequent radiological checks are necessary.

"Minimal" surgery, limited to opening the anterior ethmoid (often represented only by the bulla ethmoidalis) and middle meatotomy, is generally suitable for resolution of most of the recurrent rhinosinusitis. If necessary, surgery may be extended to the posterior ethmoid and sphenoid sinus, which may also be sites, especially in later childhood, of mucoceles, mycetomas or neoplasias. Post-operative therapy includes the use of antibiotics taken orally, nasal decongestants and topical steroids.

In children it is necessary to perform a check under anesthetic after two weeks following the operation, in order to remove fibrin and encrustations that would encourage formation of synechiae and expose the patient to recurrence of inflammation.

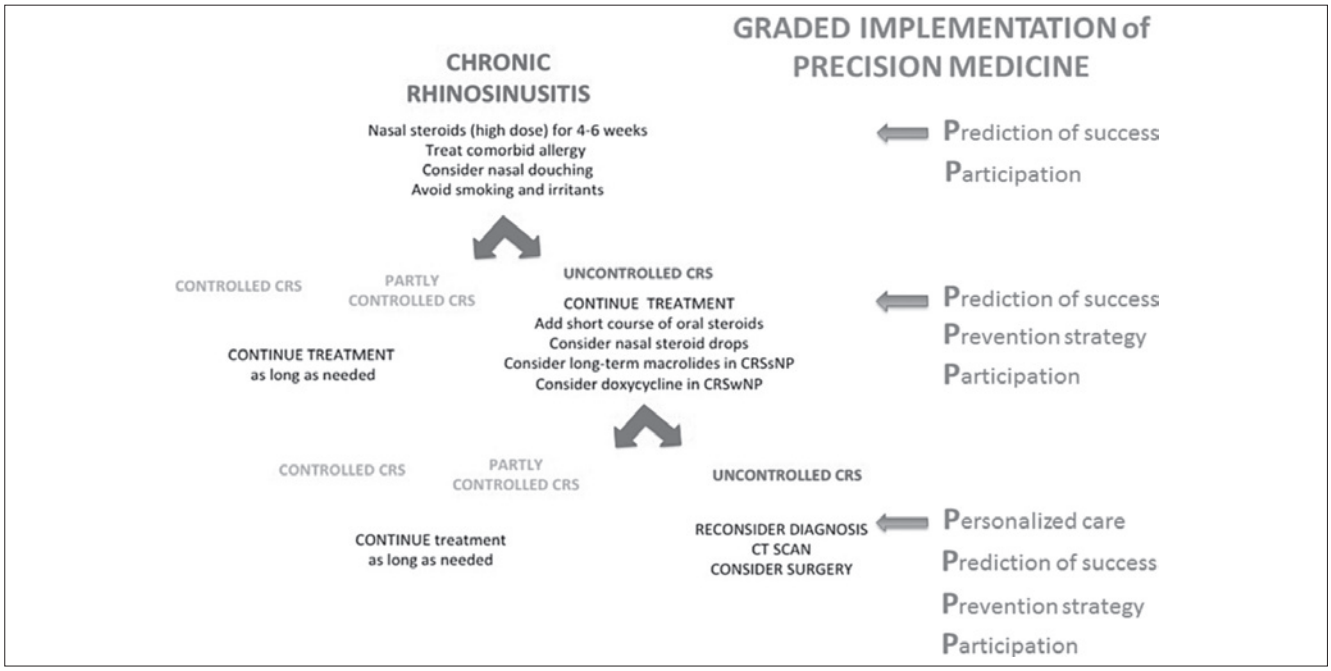

Fig. 8.4. Graded implementation of precision medicine in chronic rhinosinusitis.

Adenoidectomy represents a possibility in treatment of chronic rhinosinusitis, even though it has not yet been fully established what role the adenoids play in the genesis and resolution of chronic rhinosinusitis.

Adenoidectomy removes a source of mechanical obstruction, with consequent stagnation of catarrh and difficult drainage of paranasal sinuses, as well as persistent condition of inflammation caused by bacterial biofilms. One of the first studies on the role of adenoidectomy in treatment of chronic rhinosinusitis was published by Rosenfield [33], who recommended a therapeutic protocol setting out various steps in treatment of this pathological condition: the first step was represented by appropriate medical treatment followed by adenoidectomy and by ESS as the final step to fall back on, with average success rates of 32, 70 and 89% respectively.

Ramadan reports different statistics, demonstrating that 77% of children undergoing ESS progress towards symptomatological resolution, compared to a lower percentage (47%) of patients undergoing adenoidectomy alone. For most of the Authors who have studied the subject, adenoidectomy enables resolution of the CRS in 50% of cases and should be taken into consideration initially in children aged under six years, with modest findings on CT, not affected by asthma and who have not resolved the pathological condition with appropriate medical therapy (so called "*maximum medical therapy*"). In this connection, a lot of interest is paid today to the possibility of preventing chronic inflammatory processes affecting the rhinopharynx and paranasal sinuses through a therapy of bacterial competition (probiotic therapy), by administering benign germs capable of colonizing the human rhino- and oropharynx and competing with pathogen germs through production of cytokine. The first studies appearing in the literature and the results of a recent research conducted by the ENT Unit of our hospital, which are currently in the press, are encouraging in terms of stimulating mucous and systemic immunity, reducing use of antibiotics and giving greater quality of life to young patients.

CF represents a chapter on its own, being the cause of inflammation of paranasal sinuses in an extremely high percentage of cases: every time a bilateral nasal polyp is found in a child, CF should be suspected and the patient directed towards genetic definition of the pathology. CT generally shows massive opacification of the maxillary and ethmoidal sinuses, with hypopneumatization of the frontal and sphenoid; the lateral wall of the nasal fossae shows bulging of the medial wall of the maxillary sinus, with decalcification of the uncinate process.

Two main indications exist for endoscopic sinus surgery [34 35] in cystic fibrosis: the first one is the necessity to remove the polypoid formations, which in the majority of these patients occupy massively the nasal fossae, thus restoring nasal permeability. The second indication arises from the observation that the paranasal sinuses, and particularly the maxillary sinuses, when involved in a chronic

inflammatory process, may represent a sort of reservoir for germs which remain confined in an anaerobic environment because of persistent sinus obstruction. This condition may favour the development of resistances to many antibiotics (multi drug resistant germs) and may cause deterioration of pulmonary condition through a mechanism of chronic inhalation. Confirmation of this theory comes from detection of the same type of germs (mostly Pseudomonas aeruginosa and S. aureus) in nasal secretion and liquid obtained from the bronchi through BAL.
Surgical repair of the maxillary sinuses can enable immediate improvement in the condition affecting the upper and lower airways, with distinct rise in FEV1 at spirometry.
A more limited option, in pediatric age, for treatment of chronic rhinosinusitis, is balloon dilation [36], a method which is widely applied in surgery for choanal atresia and laryngeal tracheal stenosis; as previously mentioned, benefit may be derived from drainage of the frontal sinus, when necessary (Fig. 8.5).

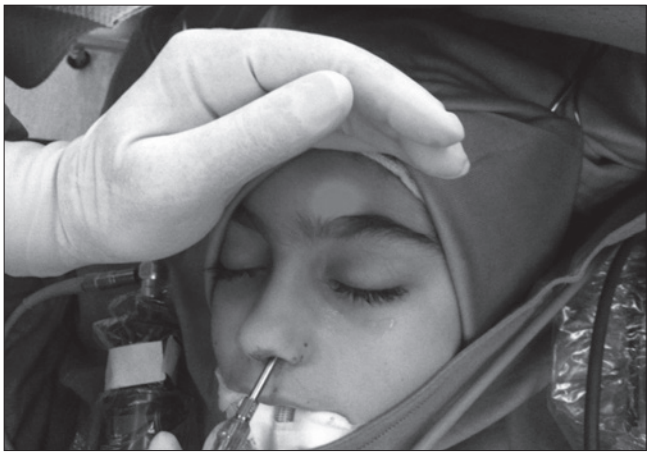

Fig. 8.5. Balloon dilation of access to frontal sinus.

A separate chapter is represented by *invasive mycotic infections* [37], which arise in immunosuppressed children who undergo organ transplants or are afflicted with oncohematological diseases. The stage of deep neutropenia following transplant favours the local growth of fungal hyphae, in the absence of the phagocyte function of the neutrophils: inverse isolation of patients with laminar airflow chambers or by positive pressure and air filter systems (HEPA filters) represent the only truly effective means of prevention of aspergillosis, because at the moment there are no effective antifungal prophylaxis schemes for patients with acute leukemia under induction chemotherapy, while fluconazole prophylaxis in doses of 400 mg/die has proved useful in patients undergoing bone marrow transplant.
Early diagnosis is of fundamental importance in avoiding complications linked to orbital and intracranial invasion, but initial symptoms are relatively non-specific: intense facial algia, especially, retro-orbital algia and nasal congestion should lead to perform a nasal endoscopic examination, which enables detection of typical paleness of the mucosa, associated with brown-blackish eschars, result of necrosis by ischemia of the tissues. In *mucormycosis* (Figs. 8.6, 8.7), in particular, marked tropism for blood vessels causes hematogenous dissemination of the fungus, resulting in vascular thrombosis, infarction and necrosis of surrounding tissues. CT may document the extension of the pathological process and the necrosis of tissues; diagnostic certainty is transferred to biopsy, preceded by a pre-operative transfusion. For purposes of prognosis, the widespread tissue demolition recommended in the past is no longer considered necessary, but promptness of surgical intervention is essential, enabling removal of necrotised tissue, reduction of fungal load and reventilation of the sinuses: systemic and local antimycotic treatment with amphotericin B will be needed until the disappearance of neutropenia, until when the possibility of a real recovery is postponed.
In conclusion, acute rhinosinusitis and chronic rhinosinusitis in pediatric age are notably prevalent, correlated with frequency of viral rhinitis and the condition of physiological immaturity of the immune system, to which is added the role played by the adenoids as a cause of dysventilation and as a reservoir of bacterial biofilms.
ARS is a self-limiting disease, but antibiotic treatment is encoded in international guidelines, in order to accelerate healing times and prevent dangerous complications affecting the orbit and the central nervous system, which are favoured by their delicate anatomical structures and immune deficiency conditions.
CRS recognizes basic diseases as favourable conditions, such as allergies, cystic fibrosis, ciliary dyskinesia and deficiencies of immunocompetence, which have to be identified and treated in order to resolve the rhinosinusal inflammatory picture satisfactorily. The majority of pa-

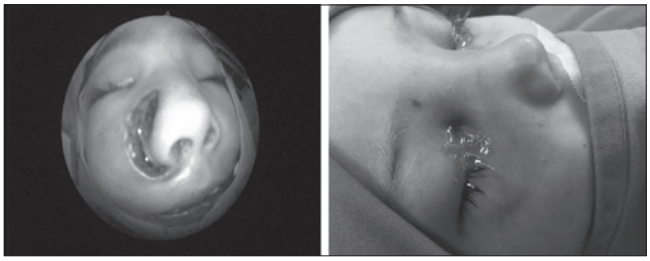

Fig. 8.6. Mucormycosis in patients with ALL.

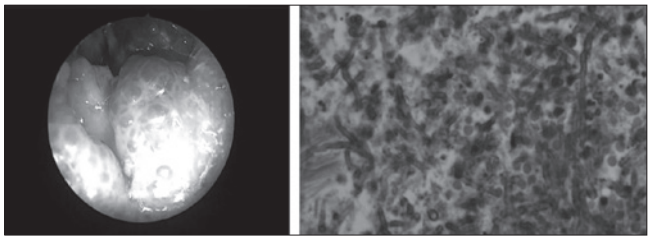

Fig. 8.7. Mucormycosis: endoscopic and histological detection.

tients respond to an adequate medical therapy. Surgery represents the last therapeutic choice, when conservative treatments have proved unsuccessful; in these cases endoscopic surgery represents the first choice treatment and in the literature there is wide agreement on the high percentage of success and low morbidity of this technique.

8.2 Complicated otomastoidites

During the course of an AOM, the mucosa that covers the mastoid cells always plays a part, even though in a variable manner, in the inflammatory process. This phenomenon is particularly significant in early infancy, an age in which the Eustachian tube is shorter than in adults and runs mainly horizontally, there is a relatively large mastoid antrum, amply communicating with the middle ear, and mastoid pneumatisation is mainly ensured by the antral and periantral cellularity. The remaining cellularity forms gradually over the first 2-3 years of life and pneumatisation develops around the compact bone structures of endochondral derivation. This is why, especially in the age range from 1 to 3 years, acute otitis media is inevitably associated with "mastoid tenderness", and acute otomastoiditis constitutes a relatively frequent occurrence. Consequently, complications from otomastoiditis involve around 80% of cases in pediatric age, as reported by Singh et al. [38]. In pre-antibiotic times, acute mastoiditis was associated with mortality rates of around 50% [39]. Despite the introduction of antibiotic treatment which dramatically reduced undesirable outcomes to less than 2%, the potential impact in terms of morbidity and mortality remain important. As a matter of fact, in recent decades the drop in mortality has not been matched by a decrease in complications, due to increasingly frequent immune deficiencies secondary to various diseases, selection of antibiotic-resistant microorganisms and migratory movements. Complicated otomastoiditis means a mastoidal inflammatory/infectious process which features at least one of the following characteristics:

- erosion of the bony intercellular septa and merging of mastoid cells in wide cavities, a condition known as mastoid empyema;
- erosion of the mastoid cortex;
- extension of the infectious process beyond the confines of the mastoid, in an extracranial or intracranial direction.

Identification of these phenomena, which are often coexisting, is highly important, because it will make it possible to set up treatment of the complication promptly and effectively.

Traditionally, complications from otomastoiditis are distinguished between intracranial and extracranial.

Extracranial complications are more frequent than intracranial ones, and seem to be mostly connected with osteolytic activity of the infectious/inflammatory process. When the inflammatory infiltrate blocks the aditus ad antrum, the purulent material stays "captive" in the mastoid cells, causing lacunar osteitis and therefore destruction of the bony septa, with the creation of a single mastoid cavity overfilled with purulent secretions, a picture known as mastoid empyema. When this condition is misinterpreted and not treated adequately, pre-existing bone dehiscence or erosive phenomena affecting the Fallopian canal can lead to the appearance of facial paralysis. A mastoid empyema must be suspected in the case of otalgia and otorrhea persisting for at least two weeks or in the case of recurrence of symptoms after a period of remission of 7-10 days.

Exteriorisation of a mastoid empyema through direct destruction of the cribriform area of the external mastoid cortex produces a retro-auricular subperiosteal abscess, characterised by the flattening and final disappearance of the retro auricular crease and the anteroinferior displacement of the auricle. Touching the area will give the feel of a fluctuant swelling, while the cutis will appear hyperemic, shiny and stretched. Among the clinical signs, the disappearance of the retroauricular crease is considered the pathognomonic finding, allowing differential diagnosis with retroauricular lymphadenitis or complicated external otitis, in which there may be anteriorisation of the auricle and local swelling, but the retroauricular crease remains intact.

Other, rarer, extracranial complications are represented by: Bezold's mastoiditis, with propagation of the infectious process below the sternocleidomastoid muscle, with consequent stiff neck; Bezold's pseudo-mastoiditis, with propagation inside the sheath of the sternocleidomastoid muscle, which may become further complicated in phlegmonous myositis; Mouret's jugulodigastric node mastoiditis, caused by deep exteriorisation at the level of the jugulodigastric triangle, characterised by parapharyngeal or retrostyloid abscesses with pharyngodynia, odynophagia and trismus; temporal zygomatic mastoiditis propagation anteriorly to the cellularity of the zygomatic process; and

posterior mastoiditis, with propagation towards the rear of the lateral sinus and formation of nuchal abscesses.
Treatment of extracranial mastoid complications consists firstly of intravenous administration of broad spectrum antibiotics and corticosteroids in a hospital environment. Absence of improvement in the clinical picture 48-72 hours after starting intravenous therapy represents an indication towards thin layer CT scan of the temporals and urgent surgical treatment. The operation consists of a mastoidectomy or anthrotomy by retroauricular route, with opening and repair of all cellular groups involved in the suppurative process and, lastly, placing of short term transtympanic drainage to allow maintenance of adequate mastoid ventilation in the subsequent months; an external drainage is also normally placed, to allow performance of daily cleansing of the surgical cavity with antibiotic solution in the 3-4 days following the operation.
Complications affecting the pars petrosa of the temporal bone include petrositis, labyrinthitis, labyrinthine fistula and paralysis of the facial nerve. In around 30% of adult individuals a marked pneumatisation may be seen of the apex of the petrous bone. In these cases, the osteitis processes spread to the cellularity of the apex and cause severe pictures which cannot be resolved after a simple surgical repair of the posterior cellularity, such as the Gradenigo syndrome or petrous apicitis, characterised by trigeminal neuralgia with corneal and facial cutaneous hypoesthesia, paralysis of the abducens nerve and oppressive pain in the retroorbital area.
Labyrinthitis is caused by bacterial infections involving the membranous labyrinth, with increasing loss of auditory and vestibular function. With acute or chronic riacutised otitis media in course, the infectious process may propagate through weakening or dehiscence affecting the oval window membrane, in malformations such as Mondini dysplasia or in enlarged vestibular aqueducts. The clinical picture is characterised by accentuated hypoacusia, which becomes neurosensory, with tinnitus and intense objective dizziness; nystagmus beats in the very early stage from the diseased side and subsequently from the healthy side. In purulent labyrinthitis, after a few weeks the central compensation mechanisms allow for resolution of crises of vertigo, while cochlear damage is irreversible once it has started.
Paralysis of the facial nerve in the course of otomastoiditis may be early, due to toxic or infectious neuritis from spreading of the process through dehiscence of the II portion of the Fallopian canal or may be late, that is to say secondary to an osteitis of the Fallopian canal. In these cases, facial paralysis is frequently incomplete and rarely persists longer than 3 weeks.

Among intracranial complications, the most frequent and feared is without doubt meningitis. In the last two decades there has been an epidemiological decline, linked to vaccination for the most severe pathogens, while in the past Streptococcus pneumoniae and Haemophilus influenzae were responsible for most of the infections. Below two years of age and in AOM, the mechanism for spreading the infection is more frequently hematogenic and the picture is associated with a better prognosis. Over the age of 2 years, this complication runs concurrently with predisposing factors, such as tegmen defects with or without herniation of cerebral tissue, fracture of the temporal, congenital malformations such as Mondini dysplasia, congenital stapes fixation and enlarged vestibular aqueduct. Similar risk factors are associated with a worse prognosis. With regard to cerebral abscess, this condition has also seen a significant decrease in incidence and mortality in the last few decades [40]. In a case study involving 122 cases [41], the "otogenic" propagation route was identified as the third etiopathogenic method, preceded by cardiac malformations and neurosurgery trauma/operations. "Otogenic" cerebral abscesses are found in almost all cases on the same side as the primitive inflammatory process, in the cerebellum and in the temporal lobe in equal amounts, the morphology is irregular and multi-located, but encapsulated. Two thirds of patients with otogenic cerebral abscess are affected simultaneously by other intracranial complications [42]. The majority of cases are secondary to cholesteatoma, 50% of cases appear in the second decade of life and two thirds in the male gender [43 44]. In addition to the clinical signs described previously, which are typical of intracranial bacteremia, there are also inexhaustible horizontal nystagmus, dysmetria and tremors. Cases have also been described of homolateral vision loss, controlateral hemiparesis and various focal neurological signs on the basis of localisation.
Epidural or extradural abscess is a coalescent collection of pus encircled by adherences from the dura mater. The most frequent localisation is the middle cranial fossa and the condition is frequently diagnosed in association with thrombosis of the lateral sinus. There are no specific clinical signs for this disease, which only leads to neurological signs in the case of ample extension and compression. Patients usually complain of deep oppressive pain in the mastoid area and diagnosis is only achieved intraoperatively.
Subdural empyema is a fulminant purulent infectious process which develops between the membranes of the dura and pia mater. It represents one of the most severe neurosurgery emergencies: when the infection propagates into the subdural space, the pus spreads rapidly and causes a

rapid thrombophlebitis of the cortical vein, with devastating neurological consequences, characterised by increased endocranial pressure and fulminant decline of the state of consciousness. Lumbar puncture in these cases is not recommended, because of the risk of transtentorial herniation due to high endocranial pressure.

When intracranial complication is suspected, an accurate clinical and instrumental assessment is of crucial importance, both because of the young age and poor collaboration and because of the need to begin an appropriate treatment promptly. Conditions such as meningitis, cerebral abscess and subdural empyema can alter various levels of the state of consciousness: it has been reported that in a population of 268 patients, 15% reached clinical attention in a drowsy condition, 18% were in stupor, while 2% were in coma [38]. Chronological assessment of the evolution of the infection, therapy administered, sequence and entity of symptoms, all together with rapid access to diagnostics for imaging and lumbar puncture are all the basis for a correct diagnostic focus in the emergency regime. For example, differentiating a cerebral abscess from a subdural empyema or from a meningitis, in a limited time period, is crucial, in consideration of the fulminant progression of the latter two morbid conditions.

The critical clinical elements to look for are intense headache, nausea, fever and cervical rigidity, sometimes accompanied by photophobia, hyperesthesia and alteration of the state of consciousness to a variable extent. The decisive diagnostic exams are rachicentesis and cranial CT scan to allow differential diagnosis with cerebral abscess, cerebritis, subdural empyema.

An intracranial complication of mastoiditis that merits a separate explanation, owing to the peculiarity of its clinical picture and the treatment strategy, is represented by thrombosis of the sigmoid sinus, caused by extension of the inflammatory process to the perisinus cells. The etiopathogenetic mechanism may be constituted from diffusion of necrosis and infection surrounding the sinus, with stimulation of platelet aggregation and formation of infected mural thrombi, which obstruct the sinus. Alternatively, it may arise from a primitive thrombophlebitis of the sinus, in which the pre-sinus bony plate remains intact. Retrograde propagation of the thrombus may involve posteriorly the transverse sinus up to the superior sagittal sinus, inferiorly the jugular bulb and internal jugular vein and anteriorly the superior petrous sinuses up to the cavernous sinus. A further formidable complication is hematogenic dissemination of septic embolisms with systemic bacteremia. Clinically, this picture may be suspected in the presence of picket fence fever, resistant to antibiotic treatment and intense high lateral cervical pain, especially in the case of obstruction of the dominant venous axis, which in most cases is the right. It is not rare to find the Griesinger sign, characterised by erythema and edema immediately posteriorly to the mastoid process. Signs of endocranial hypertension due to altered venous drainage are indicative of an advanced picture, associated with high mortality rate, which in the pediatric population is around 5-10%. For diagnostic purposes it is fundamental to carry out an urgent CT by means of contrast and "Angio" sequences: the pathognomonic sign is represented by "empty delta", where the contrast outlines the coagulation as a triangular filling defect. To supplement the CT images, Magnetic Resonance with contrast allows greater sensitivity in viewing contrast enhancement at the level of the sinus walls.

The therapeutic approach to otogenic thrombosis of the lateral sinus is still subject to debate: the low level of evidence, due to the presence in the literature of few retrospective cases, does not currently allow a consensus to be reached (see Wong, 2015, for a systematic review on the subject) [45].

International guidelines on treatment of cerebral venous thrombosis in pediatric ages recommends (level 1B) to start, immediately after reaching a diagnosis and in the absence of associated intracranial hemorrhage, an anticoagulant therapy with low molecular weight heparin (recommendation level 1B) [46].

A retrospective review of clinical data of patients with diagnosis of otogenic thrombosis of the lateral sinus taken from 2006 to 2017 at the central Audiology and Otosurgery Unit of the Pediatric Hospital of Bambino Gesù took as its objective the better characterisation of these patients and the definition of a diagnostic therapeutic protocol. First of all, it was attempted to identify the clinical factors that might determine the wide variability of the outcome, understood as achieved recanalisation of venous flow in the lateral sinus at one month after diagnosis with Angio-CT or Angio-NMR.

The criteria for inclusion taken into consideration were pediatric age (0-16 years), otomastoiditis complicated by thrombosis of lateral sinus confirmed by radiological exams and availability of exhaustive clinical data.

The analysis took into consideration the following independent variables: age, sex, presence and type of genetic mutations/variations favouring thrombophilia, alterations at coagulation tests, type of inflammation trigger (acute or chronic otomastoiditis), presence of neurologic clinical signs at start, surgical intervention of mastoidectomy, extension of thrombosis to internal jugular vein, clear erosion of internal mastoid cortex.

On the indications of the specialist hematologist, the hematochemical parameters indicative of thrombophilia

examined as routine in these patients are factor V Leiden, MTHFR, homocysteine, Protein C and S, LAC, Antithrombin-III and mutation of prothrombin factor II. The thrombophilia risk is therefore categorised as "high" (homozygous for factor V Leiden or MTHFR, or presence of multiple mutations) or "low" (for example carriers of mutation in heterozygous form).

For "presence of neurologic clinical signs at start", manifestations were considered of at least one from headaches, vertigo/instability, diplopia, alteration of the state of consciousness, coma. Erosion of the internal mastoid cortex was defined as a continuous solution of at least 3 mm, identified by means of CT of the temporal pyramids with high resolution. Surgical intervention, when carried out, consisted of antromastoidectomy with accurate opening of perisinusal cellular groups and placing of transtympanic drainage. The variable "age" was made binary by subdivision into higher than/equal to or lower than the mean age of the sample (i.e. 6 years).

A total of 24 patients (8 females, 16 males) were included in the study who were afflicted with otomastoiditis complicated by thrombosis of the lateral sinus, making a total of 25 ears, because of one of the patients having a bilateral disease. On clinical debut mean age was 63 ± 33 months, (range = 6-139 months), and 8/24 patients (33%) showed exclusively otologic signs and symptoms, in the absence of neurologic involvement. In 16 patients (66%) the otologic symptoms were accompanied by neurologic ones, most frequently headaches and diplopia. The trigger inflammatory event was acute otomastoiditis in 9 cases (36%) and chronic in 16 (64%). Two patients were affected by cholesteatoma, one of which bilaterally. The thrombosis presented extension to the transverse sinus or to the jugular bulb in 15/25 cases (60%). In 22 patients (92%) thrombophilia was demonstrated: in the majority of cases there was a MHTFR mutation, in 11/22 heterozygous (50%), in 1/22 compound heterozygous (4.5%), in 2/22 homozygous (9%). Two patients presented with homozygous factor V Leiden mutation (9%), while 6 patients presented with association between the two mutations. As a surgical intervention mastoidectomy was performed by three surgeons with proven otologic experience in 15/24 patients (62%).

In nine patients (38%) surgical treatment was not indicated and treatment consisted exclusively in intravenous administration of ceftriaxone (50-70 mg/kg) and desametasone (0.1 mg/kg). Anticoagulant therapy was prescribed in all cases and included enoxaparin in the acute stage and maintenance with aspirin in the subsequent months. The time lapse between onset of symptoms and the start of anticoagulant therapy was a mean of 8 ± 6 days (range = 1-30 days).

At radiological control at one month, recanalisation of the sinus was verified in 44% of cases (11/25). The exact Fisher test did not show a significant association between recanalisation and sex ($p = 0.434$), trigger event (acute or chronic otomastoiditis; $p = 0.434$), high or low thrombophilia risk ($p = 1$), extension of thrombosis to the transverse sinus or internal jugular vein ($p = 0.24$), erosion of the internal mastoid cortex ($p = 0.35$), mastoidectomy ($p = 0.43$). The variables that are significantly associated with increased possibility of recanalisation are age at diagnosis < 6 years ($p = 0.027$) and presence of neurologic signs at start ($p = 0.043$): 6/8 patients (75%) without neurologic symptoms had a favourable prognosis, while only in 5/17 (29%) patients with neurologic symptoms was recanalisation observed at control after one month. Analysis of multivaried logistic regression showed that only the variable "age at start" was significantly associated with favourable outcome (OR = 0,94, 95% CI = 0.89-0.99, $p = 0.03$), while the associations between the other variables and outcome do not prove statistically significant. As confirmation of this data, comparison between the mean age of the subgroup with recanalisation and the subgroup with failed recanalisation shows a significant difference, with the former significantly lower than the latter ($t = 2.46$, $p = 0.002$).

In the light of the results obtained from our retrospective case study, which is one of the more numerous, and of the international guidelines on treatment of infantile cerebral venous thrombosis, we have outlined a protocol for diagnosis and treatment, summarised by the flow chart in Figure 8.8.

In view of the detection of thrombophilia in 92% of cases, all of our patients regularly undergo hematological advice and a thrombophilia screening panel immediately after diagnosis.

On diagnosis, all patients start anticoagulant treatment with low molecular weight heparin (100 U/kg), administered subcutaneously, associated with broad spectrum antibiotic and corticosteroid treatment intravenously [47]. The use of low molecular weight heparin is particularly suited to the cases that present with surgical indications, owing to the short half-life of the drug and the possibility of greater "convenience" of perioperative management. Indications to mastoidectomy, however, are not made in all patients, but with an elective criterion based on specific case, even though mastoid repair is recommended by the majority of the authors [48-50]. In particular, there is a surgical indication in cases of mastoiditis with radiological evidence of mastoid empyema or erosion of critical anatomical structures, such as tegmen and Fallopian canal, or in cases of suspected chronic cholesteatomatous oto-

mastoiditis. On the other hand, simple discovery of fluid flow in the mastoid cellularity does not represent, in our opinion, indications for mastoidectomy.
The observation that age at start < 6 years constitutes a significant factor associated with favourable outcome might be explained by considering that thrombosis started a shorter time ago has a greater chance of recanalisation; on the other hand, it is plausible that in older children this complication started earlier and that long-term mastoid infections has produced a perisinusal inflammatory impairment that is more difficult to treat. As a corollary, this result suggests the importance of early diagnosis and treatment as a factor capable of affecting the result.
The presence of neurologic signs at start is also associated with lower probability of resolution, determining greater severity and extension of the picture; these signs dictate more interventionist conduct associated with close monitoring of vital functions. In the presence of marked neurologic signs there may be the suspicion of wide extension of the thrombus, longer duration of the complication, possible other associated endocranial complications and of progression towards septicemia and septic thromboembolism. In any case, it is curious to note that other markers of severity of clinical picture, such as extension of thrombosis to the transverse sinus or internal jugular vein, or erosion of the mastoid cortex, do not associate with unfavorable outcome.
In conclusion, our data supports the role of a multidisciplinary approach, shared by otorhinolaryngologist, hematologist, pediatrician and specialist in infections in managing this dangerous clinical entity, for which a therapy must necessarily be identified. Clear guidelines do not exist and the majority of authors propose personalised observations based on series of less than 10 patients. Broader case studies, such as that proposed by us, show new prospects regarding treatments considered established, such as mastoidectomy, performed more on a traditionalist basis than on real scientific evidence.

8.3 Laryngeal papillomatoses

Laryngeal papillomatosis is a disease of viral etiology, which can strike all age groups and is characterised by the appearance of exophytic lesions at the level of the upper airways, with tendency to recur and possibility of spreading to the whole respiratory system.
A distinction can be made, based on the age of patients,

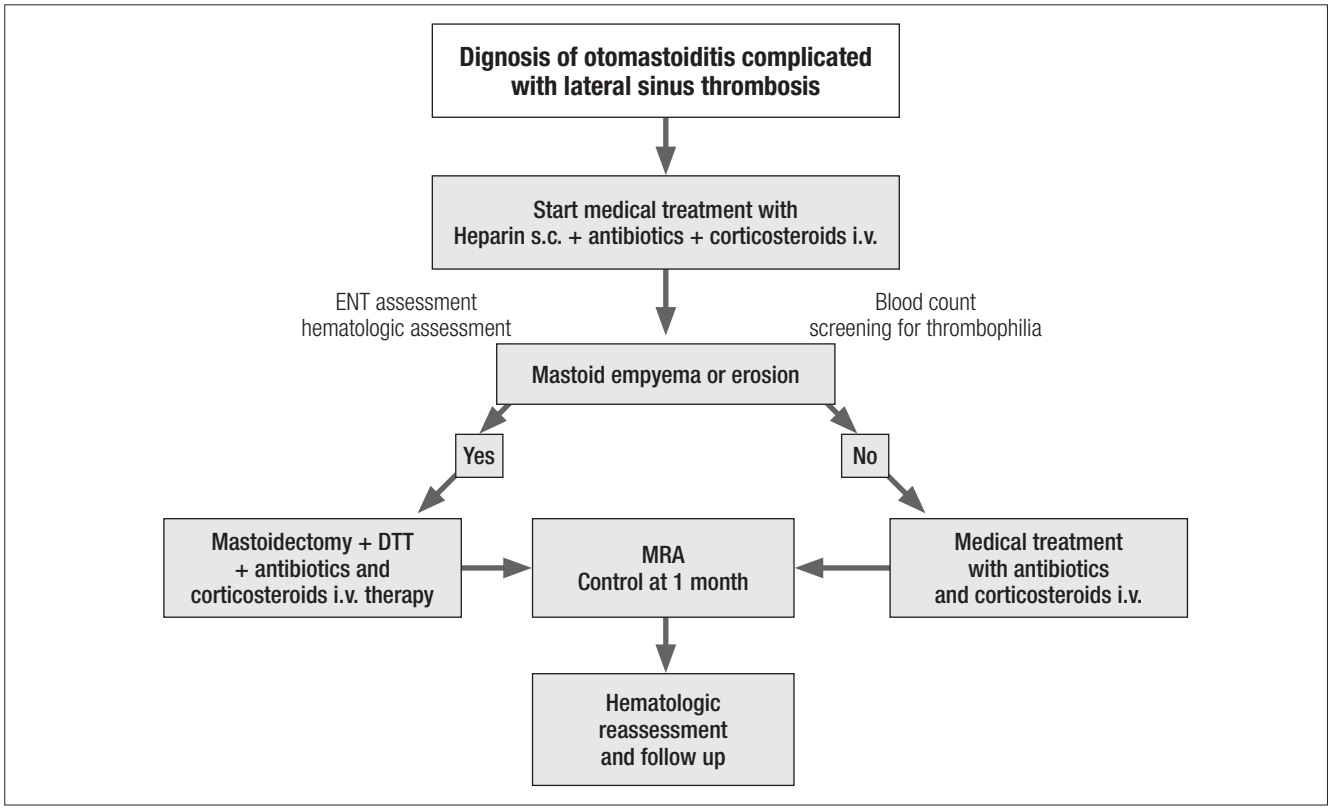

Fig. 8.8. Flow-chart of treatment of otogenic thrombosis of the sigmoid sinus proposed by the Audiology and Otosurgery Unit of the Pediatric Hospital Bambino Gesù.

Juvenile Onset Recurrent Respiratory Papillomatosis, for onset up to 12 years of age, and adult onset RRP, over 12 years of age.
In pediatric patients it represents the most frequent laryngeal neoplasia and the second cause of dysphonia, after the vocal nodes.
Apart from the laryngeal localisation, papillomatous lesions may be found at the level of the nasal vestibule, the soft palate, the trachea and the main bronchi.
The first description of a case of papillomatosis dates back to the second half of the 1800s, by an English physician (Sir Morell Mackenzie), one of the pioneers of pediatric laryngology, but not then with association of neoplastic manifestations with viral etiology, which was discovered in the early 1900s, with the advent of molecular genetic techniques.
In western countries incidence of laryngeal papillomatosis is of 1-4:100000 new cases per year, with an increase in countries with lower socio-economic development, and a mean age at first detection less than 5 years [51]. It is certain that the lower the age at onset, the greater is the risk of progression.
This is a benign disease, with very low mortality, mainly caused by respiratory complications, but with high probability of recurrence [51].
For the pediatric form the peak of incidence for the number of cases is around 7 years, while in adult age there is a double peak, at 35 and at 64 years [52].
The infectious nature of the disease has been definitely confirmed, with the etiological agent being the human papillomavirus (HPV) and especially the subtypes 6, 11, 16, 18, 31, 33.
The most frequently found are the types 6 and 11, the same that can be found in around 90% of genital warts [53].
This latter consideration certainly lends weight to the theory of vertical transmission during passage through the birth canal.
The pathogenesis is still not very clear and is linked to high tropism of HPV with the squamous epithelia, where it strikes the cells of the basal layer and stays quiescent for some time in latent form, before manifesting with anomalous activation of the EGF and consequent exophytic growth of papillomatous formations [53].
An important role appears to be played also by an anomalous function of the T-cells on the part of the host during pregnancy and childbirth, with influence certainly deriving from a prolonged vaginal delivery (first child) and from high viral content of genital warts [53 54]. Local traumas, such as intubations or gastroesophageal reflux, compete in worsening the picture of the disease and encouraging its extralaryngeal spread [54].
In around 50% of patients with laryngeal papillomatosis, maternal history of vaginal warts can be found and up to 75% of cases are first born, which associates with frequent longer duration of delivery of first born and consequently longer stay in the birth canal [55].
Another fact to highlight is that incidence of papillomatosis is less than 1% in patients born by caesarean, which confirms vertical transmission, but might also suggest the possibility of hematic transmission of the infection [55].
The first manifestation of disease is increasing dysphonia, arising from early predominant involvement of the glottal plate. Then, depending on the extension and aggressiveness, the patient may present with respiratory symptoms, such as stridor, chronic cough, dyspnea, distress (Fig. 8.9) or dysphagia, if the diffusion involves the digestive tracts.
It is certain that the presence of dysphonia, stridor and respiratory distress in a child younger than 5 years is strongly suggestive of recurrent respiratory papillomatosis [56].
Evolution of the disease is very variable and unpredictable and it is possible to talk about a form of low aggressiveness (around 25% of cases), when less than five interventions are needed to achieve resolution of the picture, moderate aggressiveness, when it is not resolved with a limited number of procedures, but is easily controllable, with the possibility of spontaneous regression (it sometimes remains quiescent, to manifest itself again several years later). The form with high aggressiveness, on the other hand, has early onset (before 2-3 years of age), with the need for more than 40 interventions, increase in mortality rate and possible need for tracheotomy [57].
Early onset, extensive involvement of airways and frequent recurrence after treatment are indicating factors of aggressiveness. In any case, if possible tracheotomy and intubation are to be avoided as they have, as a consequence, greater risk of tracheobronchial diffusion.
Malignant transformation is infrequent and has been described in the forms of high aggressiveness at a minimum of 15 years from first onset.
Diagnosis of papillomatosis is, above all, endoscopic, with the first finding that is often made even in outpatient regime awake by means of fibrolaryngoscopy, which is followed up by endoscopic assessment under sedation through direct laryngoscopy in suspension with rigid lens at 0° (4 mm/2.7 mm depending on the age of the patient). It is also indispensable to make histological assessment of a bioptic fragment, to enable confirmation of the diagnosis and by means of molecular biological analysis to find the HPV subtype involved. In the last decade a further auxiliary to endoscopy has been introduced, assessment with narrow band imaging (NBI), which offers in some

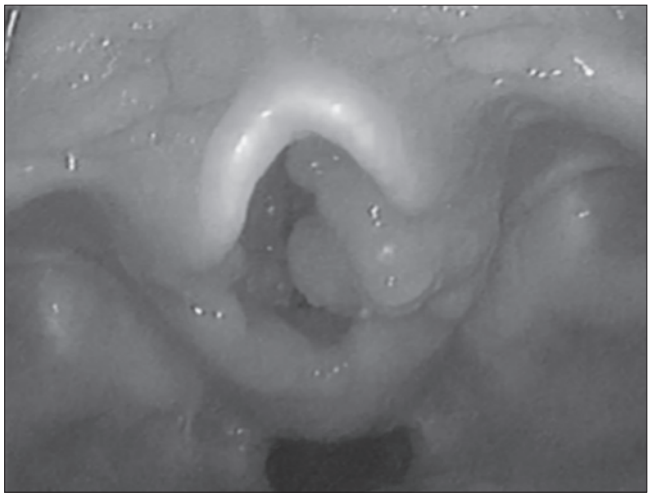

Fig. 8.9. Marked obstruction of the respiratory space in a case of massive laryngeal papillomatosis.

cases more accurate identification and better viewing of the papillomatous lesions than with white light [58].

Various systems of staging have been proposed, the best known and most used of which is that presented by Derkay in 1998 and revised in 2004 [59 60], which classifies the disease in relation to its severity, extension and extra-laryngeal diffusion, but is also a tool for assessment of progression, response to treatment and post-operative follow-up, as well as trying to predict the time lapse between surgical interventions.

Use of systems of discussion is still subject to debate and in many centres follow-up is performed only through assessment of clinical evolution and comparison of recorded endoscopic images.

None of the possible treatments currently available are effective in eradicating recurrent respiratory papillomatosis. The aim of the therapy and, in particular, surgery in these patients is to check on evolution of the disease, while ensuring sufficient respiratory space and improvement in the quality of the voice [53 61]. Treatment carried out should always be conservative with regard to the anatomical structures, especially where there are commissure and subglottic lesions present, because of high risk of formation of synechiae and stenosis (Fig. 8.10).

In treatment of this disease, surgery may be backed up by adjuvant medical therapy, which may improve the severity and the course and reduce the need for interventions. Times and methods for use of adjuvant therapy are not clearly defined. In 2004, Schraff and Derkay outlined some of the fundamental points for introduction of medical therapy in cases where more than 4 surgical procedures are needed per year, where there is rapid recurrence with compromise of respiratory space, where onset is early (before 2 years of age), where extension of lesions widely involves the anterior or posterior commissures, or in cases of multi-level diffusion [62].

The aim of the surgical approach has remained unchanged over the years, but the techniques and methods have changed, with the advent of new technologies.

In the past, debulking of the papillomatous formations was performed with a cold blade, which was then replaced by vaporisation by means of CO2 Laser, which, again in direct micro-laryngoscopy in suspension, ensures greater efficacy and precision in treatment of papillomas, with less bleeding. Use of Laser, though, is not free of potential complications, both in terms of heat damage to tissue and because of risk of diffusion of fumes containing particles of viral DNA.

Some years ago, the Microdebrider was brought into use also in pediatric laryngeal surgery, with excellent results in terms of speed and accuracy in debulking lesions (Fig. 8.11), owing, amongst other things, to better endoscopic viewing during the procedure and absence of the risk of heat damage following laser treatment [63].

In some centres, ours included, the approach with the Microbrewer is coupled with completion of treatment of lesions using Radiofrequency Cold Ablation (Fig. 8.12), which, through an easy to use handle, makes it possible to operate in an even better aimed and more conservative

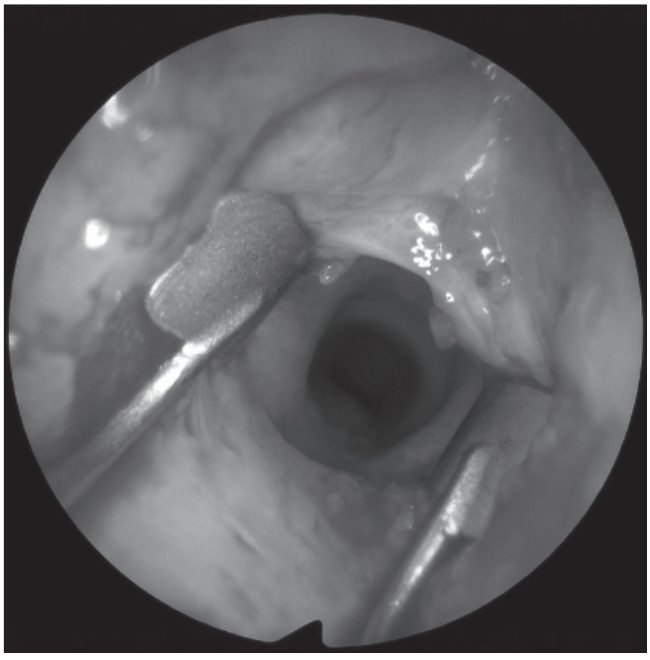

Fig. 8.10. Anterior commissure synechia resulting from surgical treatment of laryngeal papillomatosis.

manner, with minimum risk of heat damage, seeing that the tool's working temperature does not exceed 70° C [64].
Over the last 30 years numerous adjuvant therapies have been proposed to back up surgery in the cases described above. Those most used have been and definitely still are antiviral therapies. During the 1980s, the first to be used was treatment with alpha-interferon, which acts by blocking viral replication, in dosage, for RRP, of 5 million units/m2 s.c. for 28 days followed by 3 times a week for 6 months, then reducing the dosage to 3 million units/m2 in the event of good response and remission of the disease. Treatment with interferon, though, presents important collateral effects, both acute (headache, nausea, myalgia etc.) and chronic (slowing of growth, hypertransaminasemia, leukopenia, fever etc.) [65].
The antiviral that is currently most used in treatment of laryngeal papillomatosis is certainly cidofovir. This drug is used by means of topic injection at the level of papillomatous lesions, both before and after the debulking procedure, with a protocol that sets out repetition of the procedure every 2-6 weeks in a cycle of 4-5 applications with dosage, for pediatric patients, of 2-2.5 ml to 2.5-7.5 mg/ml [66]. In general, the results described are positive, with reduction in the number and frequency of recurrences and in aggressiveness, up to cases of full remission from disease, with a response rate reported in the literature of up to 61% [62].

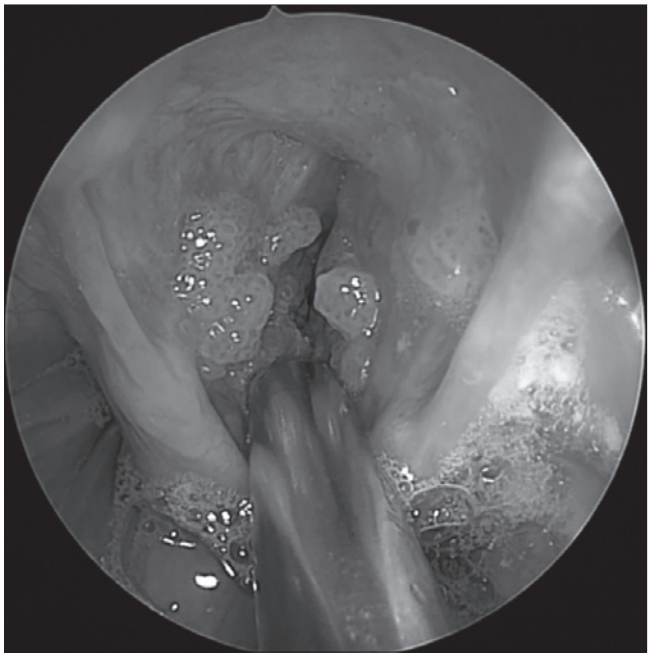

Fig. 8.11. Surgical treatment of laryngeal papillomatosis with Microbrewer.

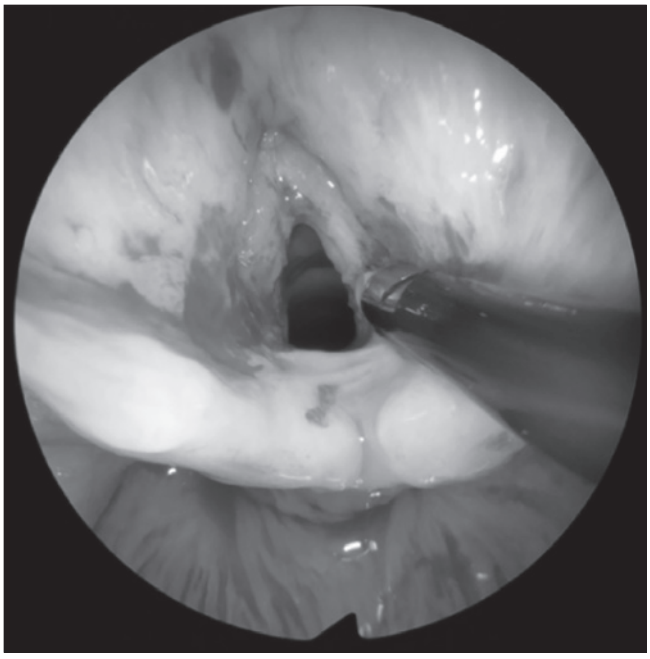

Fig. 8.12. Use of Coblator in treatment of laryngeal papillomatosis.

Good results have also been described with ribavirin (first dose by means of intravenous administration, then by oral administration with dosage of 23 mg/kg/die), in terms of reduction of surgical intervals [67], and with acyclovir, mainly in cases of coinfection (HSV, CMV, EBV), which present with greater aggressiveness [68].
A 2004 study by Rosen et al. shows the reduction of extension of papillomatous lesions after administration of indole-3-carbinol, which acts as inhibitor of the metabolism of estrogens [69].
Bell et. al. in 1988 presented results of treatment with retinoids, in particular 13-cis-retinoic acid, which reduced recurrence times of papillomatous formations, with its capacity to suppress squamous differentiation, but it has collateral effects which may also be serious (teratogenic, psychiatric disorders) [70].
The breakthrough in the battle against RRP came in 2006, with the approval by the Food and Drug Administration (FDA) of the first quadrivalent vaccine against HPV (6, 11, 16 and 18) [71], with administration proposed for all females between the ages of 11-12 years and for women between 13 and 26 years not yet vaccinated. Indication may also be set for those below the age of 11 years in selected cases [72].
The vaccine enables prevention of cervical and anogenital cancer, but also genital warts from HPV infection, with consequent prevention also of maternal transmission during childbirth and therefore of RRP [72 73]. The nonavalent

vaccine was also presented recently and the future of eradication of RRP lies in the global vaccination plan [72].
Use of vaccine has also been proposed in patients afflicted with papillomatosis, with the benefit of greater control over the disease, increased interval between interventions and reduction of aggressiveness, brought about by development of immunity towards other HPV subtypes and increased sero-reactivity with better host response to the disease [74 75].
In conclusion, papillomatosis in pediatric age is a disease with an unpredictable course, potentially fatal and difficult to treat. As of today, there is no unequivocal and 100% effective therapy. The approach most commonly used is surgical, with the aim of ensuring respiratory space, improving the quality of the voice and controlling the evolution of the disease, with the Microbrewer, which is currently the most common tool and guarantees a targeted, mini-invasive approach.
There are various proposals of adjuvant pharmacological therapies and the most effective and most used appears to be topical injection of Cidofovir, but the therapeutic future of RRP is definitely linked to the use and diffusion of vaccine therapy, both in the prevention stage and in that of cure.

9. New bacterial resistance and multiresistant infections

Infections and sepsis are dysfunctions of organs that put the life of the patient in danger as a result of an unregulated response by the host to an infectious aggression [1] and they represent, worldwide, the main problem of public health, with mortality rates that are fairly high, even exceeding in various studies, in cases of septic shock, a rate of 50% [2]. Around two thirds of these infections are caused by MDROs and this is independently associated with 1) a higher rate of mortality than that determined by multisensitive strains, 2) more time spent in hospital and 3) higher global expenditure on health.
The development of AMR is an inevitable natural phenomenon, caused by mutations in the genetic material of bacteria or by acquisition of exogenous genes of resistance transported by mobile elements (plasmids) which can spread horizontally among the bacteria, including different species.
Inappropriate use of antibiotics inside and outside the hospital environments, lack of attention to prevention of infections and to practices of control, have, in a few years, led to selection and spread of strains of pathogens that are resistant to practically everything. Unlike with other drugs, potential spreading of resistant organisms can have a negative impact on the health of subjects not directly exposed [3].
It has been estimated that the annual number of deaths attributed to infections caused by multiresistant pathogens, amounting at the moment to 700,000, will exceed 10 million by 2050. Annually in Europe alone there are 50,000 deaths from infections caused by multiresistant pathogens (MDROs).

9.1 Types of resistance to antibiotics

Two types of resistance to antibiotics are described: natural and acquired.
Natural, here, means that the bacterium is intrinsically resistant to certain antibiotics by natural selection on a genetic basis [4].
Acquired resistance occurs by genetic mutation of bacterial DNA by horizontal gene transfer by means of conjugation, transformation or transduction.
The possible biochemical mechanisms of bacterial resistance to antibiotics are:

- bacterial wall becoming impermeable to the antibiotic and/or rapid elimination from thetarget site;
- alteration of the target site;
- production of enzymes that deactivate antibiotics.

On a global level there is now a major problem with infections, both in hospitals and in the communities, from Enterobacteriaceae, producers of extended spectrum beta-lactamase, enzymes which confer resistance against most of the beta-lactamase antibiotics, with the exception of carbapenems [5].
Moreover, to the antibiotic resistant pathogens that were already attracting attention and causing concern on a global level, have added in recent years the emergence of strains of enterobacteria that are also resistant to carbapenems [6 7], the antibiotics that represent one of the last therapeutic resources for infections caused by multidrug-resistant Gram-negative bacteria. The lost effectiveness of this class of antibiotics leaves very little margin for therapeutic intervention and requires reliance on combinations of antibiotics which include molecules that have not been used for many years, and which may also be relatively toxic, or the use of the latest generation of antibiotics [8].
In February 2017, the WHO published a list of emerging resistant pathogens, for development of new antibiotics with different degrees of priority (Table 9.I) [9].

9.2 Infections from multidrug-resistant gram-negative germs (MDROs)

The current emerging problem in treatment of complex infections from MDROs, as stated above, essentially concerns the gram-negatives, which have different effective molecules from gram-positives, both of the older and the latest generation.

Table 9.I. List of emerging resistant pathogens, for development of new antibiotics, with different degrees of priority (WHO February 2017).

Priority 1: CRITICAL
Acinetobacter baumannii carbapenem-resistant
Pseudomonas aeruginosa, carbapenem-resistant
Enterobacteriaceae, carbapenem-resistant, ESBL-producing
Priority 2: HIGH
Enterococcus faecium, vancomycin-resistant
Staphylococcus aureus, methicillin-resistant, vancomycin-intermediate and resistant
Helicobacter pylori, clarithromycin-resistant
Campylobacter spp., fluoroquinolone-resistant
Salmonellae, fluoroquinolone-resistant
Neisseria gonorrhoeae, cephalosporin-resistant, fluoroquinolone-resistant
Priority 3: MEDIUM
Streptococcus pneumoniae, penicillin-non-susceptible
Haemophilus influenzae, ampicillin-resistant
Shigella spp., fluoroquinolone-resistant

Enterobacteria

The preferred drugs for treatment of infections from ESBL producer enterobacteria are considered to be the carbapenems, though this has not been confirmed by prospective studies, but only by numerous retrospective studies, even though these are very consistent [10]. Unfortunately, wide use of carbapenems has led to an initial domino effect, contributing to the spread of enterobacterial strains that are producers of carbapenemase [11], which has, in turn, led to a drive to find alternative "carbapenem sparing" treatments for infections from ESBL + producer enterobacteria. The drugs most used as alternatives to carbapenems are the combinations of BLBLIs. Strains of ESBL + can often prove sensitive to BLBLIs. Some studies have assessed the importance of the MIC of piperacillin/tazobactam on the outcome of this treatment. According to Delgado-Valverde et al. MIC values lower or close to the breakpoint of 16 mg/L does not influence outcome, while, if the MIC clearly exceeds this level then the percentage of success drastically falls [12]. Use of piperacillin/tazobactam, in fact, is currently guided by the MIC (MIC driven strategy). Within the "carbapenem sparing" strategy of ESBL + , together with old combinations of beta-lactams plus BLBLIs, we have new molecular associations today that are represented both by new beta-lactams plus old BLBLIs (ceftolozane/tazobactam) and by old beta-lactams plus new BLBLIs (ceftazidime/avibactam), but this is all dependent on molecular biology tests capable of identifying the specific pattern of resistance, known as the companion test strategy. For example, in the case of an infection from ESBL + with MIC to piperacillin/tazobactam > 16 mg/L ceftolozane/tazobactam can be used instead of a carbapenem, as long as molecular biology has excluded specific resistance patterns, such as KPC and metallo-betalactamases (VIM, IMP, NDM), which would make the drug totally ineffective. Another interesting carbapenem sparing option in treatment of infections from ESBL + pathogens, is temocillin, a beta-lactam that is active not only against ESBL + strains, but also against the AmpC strains. AmpCs beta lactamase are present in Enterobacteria and their production is mediated by chromosome genes present, especially, in some species such as Enterobacter spp., Citrobacter freundii and Serratia marcescens. In these cases, chromosome resistance can be induced and may not be expressed. For example, in Enterobacter spp. there is sensitivity to all cephalosporins except cefoxitin, which acts as an inductor. More recently, AmpC type enzymes encoded by transferable plasmids have also emerged in Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae and Salmonella. AmpC resistance mediated by plasmids is generally expressed phenotypically and interpretation of the pattern of sensitivity is often easy. In fact, in these cases the MIC of cefepime remains lower and is often within the range of sensitivity (≤ 1 mg/L) compared to the other cephalosporins. Ceftolozane/tazobactam is also active against AmpC strains of Enterobacteria and of Pseudomonas aeruginosa. In a meta-analysis, cefepime did not prove inferior to carbapenems. Ceftazidime-avibactam, shortly to become available in Europe, is also active against AmpC producer enterobacteria, since avibactam is a class A and C beta-lactamase inhibitor. At present, studies carried out have not enlisted many patients with AmpC infections and there is no experience of bacteremia from AmpC producer enterobacteria [13]. Temocillin, a 6-alpha methoxy derivate of ticarcillin, is stable to the hydrolytic action of many serine beta-lactamases of class A (ESBL, KPC) and of class C (AmpC) as stated above. Although the experimental studies are promising, there is a lack of prospective clinical studies. One retrospective study compared piperacillin/tazobactam with amoxicillin + temocillin in hospitalised cases of serious pneumonia: the outcome was the same but piperacillin/tazobactam selected more Clostridium difficile than the other combination [14]. Temocillin, though, must be used at adequate dosage, which is a minimum of 4-6 gr in 2-3 refracted doses for adults. In an experimental study on mice, temocillin also proved active against KPC producer strains with MIC ≤ 16 mg/L [15]. The increasingly frequent spreading of CRE has in turn generated a second domino effect, represented by increasing use of colistin and by constantly growing selection of strains resistant to colistin itself, which is held by many to be molecular rescue. Classically, resistance to colistin was

determined by chromosome mutations, which made this pattern of resistance, however, not easily transmissible to other strains; this was all completely overturned when, in the early months of 2016 *Lancet* published the work of Liu et al., which was the first to show a new method of transmission of R to colistin between one bacterium and another, using a plasmid vehicle called mcr-1 [14]. This method of resistance spread rapidly worldwide and arrived first in Europe [16] then also in Italy.

Klebsiella pneumoniae

According to the data in the 2016 Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net), coordinated by the European CDC, Italy, together with Greece, is a country with a percentage of carbapenem resistant Klebsiella pneumoniae above the average for Europe. Other countries with important percentages are Cyprus and Romania, but percentages of resistance, although still low, are increasing in numerous countries, especially in the Mediterranean area and eastern Europe [17]. The European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) study, financed by the European CDC, was aimed at improving surveillance of CRE in Europe: Italy has been considered an endemic country for CPE since as long ago as 2013 [6].

In Italy, data from the antibiotic resistance surveillance sentinel, coordinated by the Superior Institute of Health, which supplies data on Italy to the European surveillance EARS-Net, indicate a considerable increase in invasive infections from carbapenem resistant Klebsiella pneumoniae since 2010, with a proportion of antibiotic-resistant strains that remains much higher than the European average. In 2009 in Italy only 1.3% of bacteremias from Klebsiella pneumoniae were caused by carbapenem resistant strains, while in 2011 there were 27% and in 2015 33%. Amongst enterobacteria, the species most frequently reported as CPE are Klebsiella pneumoniae and Escherichia coli [18].

The first strain of Klebsiella pneumoniae resistant to colistin, after acquisition of a plasmid denominated mcr-1-2 by a strain of E. coli, was isolated in Italy [20]. Resistance to colistin appears to be associated, at least in infections caused by KPC producing Klebsiella pneumoniae, with an increase in mortality and in some studies it is itself an independent risk factor of mortality [21 22]. At present, the therapeutic strategy for treating infection from KPC producing Klebsiella pneumoniae, which is endemic in Italy, is based on an algorithm which divides the treatment into two groups, depending on whether the MIC to meropenem is < or > than 16 mg/L. In the case of MIC < 16 mg/mL the treatment combination which has proved most effective is that represented by meropenem at high dosage in extended infusion (2 gr every 8 hours in 3 h or better in 5 h) + colistin (9 million UI in loading dose in 3 h followed by 4.5 million UI every 12 hours still in 3 h) + tigecycline at high dosage (100 mg every 12 h) and/or gentamicin (5 mg/kg/die) [23 24]. In the case of MIC > 16 mg/mL, on the other hand, carbapenem is excluded, in favour of using, in colistin sensitive strains, the combination colistin + tigecycline + gentamicin at the dosages reported above and, in colistin resistant strains, tigecycline + gentamicin + fosfomycin at high dosages (6 gr every 6 h) [23]. Gentamicin is an aminoglycoside active against KPC. The Vitek2® automatic system often overestimates resistance to gentamycin and to tigecycline, and MIC of 4 or 8 mg/L to gentamycin or > 1 mg/L to tigecycline on Vitek2® are often not confirmed in broth dilution (gold standard) [25]. The clinician, therefore, in this particular patient setting, has to insist on accurate MIC, in order not to exclude a priori drugs such as gentamycin and tigecycline, which are often the only therapeutic solution in this setting. A recent Spanish study showed that gentamycin is the drug to combine with colistin and tigecycline in KPC, because it is the only way to achieve reduction in mortality, compared to the use in combination of carbapenem [26]. In reality, by optimising the pharmacokinetic/pharmacodynamic parameters of meropenem it is possible to reach, in extended infusion at high dosage, the therapeutic target also for MIC > 16 mg/mL but anyway lower than 64 mg/mL [27]. In various Italian situations, however the usual MIC to meropenem in Klebsiella pneumoniae KPC + ST512 strains (the most frequent variant among those isolated) are extremely high above 512 or 1024 mg/mL, making use of the drug totally ineffective, on the one hand, and selector itself of resistance, on the other hand. The current alternative is represented by ceftazidime/avibactam at dosage of 2.5 gr every 8 h. It is a common opinion among the experts, however, that even that drug should preferably be used in association with gentamycin and/or fosfomycin. At the same time as using a carbapenem, when indicated, in combination regime for infections from carbapenemase producer Klebsiella pneumoniae, a proposal has also been put forward to use double carbapenem, on the theory that one of the two may play a competitive inhibitor role on the carbapenemase, on the basis of greater affinity, while the second would be free to act [28]. However, evidence of the efficacy of this approach is solely anecdotal and the presumption underlying the use of double carbapenem has no substantiation from biochemical data, which indicate a catalytic efficacy of the various carbapenemases produced by Klebsiella spp similar towards the different carbapenem molecules [28 29]. Most

probably, raising the dosage of carbapenem is the winning element in selected cases, especially within determined values of MIC (< 16-32 mg/L). In this sense, personalising the antibiotic treatment with TDM allows best use of high dosages of meropenem, by adjusting the posology from time to time and in a rapid manner [31].

Pseudomonas aeruginosa

In the case of Pseudomonas aeruginosa, a true warhorse in its ability to acquire resistance, however, the resistance to carbapenem comes essentially from two different mechanisms, represented by an "over-expression of efflux pumps" and by "reduction of the membrane pores", which, in practice, represent the entrance for numerous hydrophilic molecules inside the bacterial cell [32]. This acquisition of resistance, (chromosome mutation), has more difficult transmission from one patient to another than that connected to carbapenemase, (plasmid transmission) and this aspect too, in an attentive clinician who has to make isolation choices, has important practical repercussions for assistance. Other mechanisms responsible for resistance in Pseudomonas include: mutations of topoisomerase II and IV, responsible for resistance to fluoroquinolones; mutations leading to derepression of AmpC type endogenous beta-lactamase, partial or total, responsible for resistance to anti-Pseudomonas penicillin and to third generation cephalosporins; mutations leading to up-regulation efflux pumps, responsible for resistance to fluoroquinolones, aminoglycosides and beta-lactams. To treat severe infections from Pseudomonas aeruginosa which is resistant to carbapenem and sensitive only to colistin, there may be various strategies, but starting from the fact that often the only winning therapeutic option is associative, using new pharmacological combinations of old molecules to exploit their synergies [33]. The colistin rifampicin association has proved to be synergic in Pseudomonas aeruginosa resistant to carbapenems. Colistin acts as a Trojan horse, by disorganising the cell wall of the bacterium and allowing the rifampicin to enter it and inhibit the RNA polymerase at the level of the B-ribosomal subunit. Colistin on its own maintains its bactericidal action only for a few hours. The synergic combination with rifampicin extends the bactericidal action of colistin to more than 12 hours [34]. Another combination strategy is that of colistin + carbapenem synergy. This time the colistin disorganises the cell wall to facilitate exit of the carbapenemase, placed below the external membrane, with consequent reduction of carbapenemase concentration and less lytic effect on its molecules, thus allowing the drug to exert its, which is represented by inhibition of peptidoglycan synthesis. In Pseudomonas aeruginosa, addition of an aminoglycoside, such as amikacin, considerably increases therapeutic appropriacy of the antipseudomonal beta-lactam used [35]. In Pseudomonas aeruginosa again, colistin is synergic with amikacin, unlike what happens in Acinetobacter baumannii, where this combination, on the other hand, is often antagonistic. It is important, therefore, to personalise the treatment, taking into account, among other things, the identified association pathogen-antibiotic and/or antibiotics. In the field of Pseudomonas aeruginosa treatment, there is a new possibility, represented by ceftolozane-tazobactam, which is also active against a lot of multiresistant strains. The potent anti-Pseudomonas action of this new cephalosporin is due to the fact that it is capable of eluding the main mechanisms of resistance which operate against the other beta-lactams (AmpC beta-lactamase, efflux pumps, reduction of permeability from loss of pores). Ceftolozane is, in any case, hydrolysed by metalloenzymes, which are not inhibited by tazobactam. For this reason, the metalloenzyme producer strains are resistant to the new drug. A quick search for this resistance mechanism through molecular biology would help in appropriate application of this drug [36].

Acinetobacter baumanii and Staphylococcus aureus

Acinetobacter baumannii also relies on a wide range of resistance mechanisms, through development of which it is transferred horizontally. Resistance to carbapenems in this case is again linked to production of class D carbapenemase (Oxacillinase and, in particular OXA-48 and OXA-23). Treatment of infections from MDR Acinetobacter baumannii may prove inappropriate. This is very likely in the case of resistance to carbapenems, an occurrence found in around 80% of infections from Acinetobacter baumannii in Italy. In these cases, a colistin plus rifampicin plus tigecycline combination is used, or ampicillin/sulbactam for the direct action of its sulbactam. It should not be forgotten, either, that there is no breakpoint for tigecycline and A. baumannii and that the use of this drug should be based on the theoretical levels of drugs which can reach the infection district at the known doses. Adequate monotherapy is therefore difficult to set up; especially in septicaemia, where the maximum concentration of the drug at standard doses is around 0.6 mg/L. With regard to sulbactam, it was demonstrated recently that its antibacterial action is linked to inhibition of PBP of Acinetobacter (PBP1 and PBP3, but not PBP2) [37]. This action mechanism might also explain the synergic effect observed between sulbactam and meropenem, apparently due to the action of meropenem on the PBP2. Sulbactam at the dose of 1.5-3 g every 6-8 hours has proved effective, in small case studies, against bacteremia and pneu-

monia from Acinetobacter baumannii. In an Israeli study, ampicillin/sulbactam was the only factor correlated with a reduction of mortality [38]. Yang et al. recently demonstrated the efficacy of a therapy with the association of minocycline and colistin in treatment of serious infections from strains of Acinetobacter baumannii resistant to minocycline itself. This association demonstrated that it was synergic by achieving fractional inhibitory concentration indexes, fully comparable to the better known meropenem/colistin association [39]. However, it should not be forgotten that over 80% of Acinetobacter baumannii strains have relatively low MIC for minocycline, (sensitive according to CLSI, EUCAST at the moment does not provide a breakpoint), and that synergy between minocycline and colistin may partly be explained by the fact that colistin, by increasing permeability of the membrane, allows greater entry of minocycline at the intracellular level and that the latter, by inhibiting protein synthesis, might prevent expression of resistance to colistin. Therapy for infections from MDR gram-positives, relies on old molecules and new ones recently put on the market. Old glycopeptides are drugs that may be bactericidal, but in the event of increase of MIC, this characteristic is lost and the clinician must take this into account when treating infections in fragile patients. There is bactericidal effect only with MIC < 1 mg/L. Anyway, it is well known that, in the case of MIC > 1 mg/L for vancomycin the probability of reaching the therapeutic target of AUC/MIC > 400 will only be reached in a limited number of cases, but at the cost of a distinct imbalance towards the drug-correlated toxic effects. The value of the MIC is associated in inverse proportion to mortality [40]. To be active, vancomycin must reach seric concentrations downstream of 20 mg/L or of AUC/MIC > 400 [39]. Obviously, it is much easier to measure the C_{min}, which is the parameter followed in treatment with vancomycin. The best way to administer vancomycin is continuous infusion, since it is a time dependent antibiotic whose effectiveness is reached when plasma concentration, during the whole period between doses, exceeds the MIC values. Continuous infusion of vancomycin also reduces the risk of nephrotoxicity compared with the group treated with intermittent boli (OR = 1.645; p = 0.007) [42]. Teicoplanin, a glycopeptide of synthesis, is burdened by lesser nephrotoxicity than vancomycin. This drug reaches elevated concentrations in the bone (60% of the hematic concentration), in the cutis and in the lung [43]. The bond with plasma protein is very high and, therefore a loading dose is required, followed by an adequate maintenance dose. In the past, teicoplanin was administered at 200/400 mg die, which for a person of 70 kg corresponds to 3-4 mg/kg/die, when the minimum dose should be at least 6 mg/kg/die. Lee et al., in a retrospective study, demonstrated that teicoplanin at dosage of 12 mg/kg/die had a better outcome in terms of mortality, percentage of septic shock and early disappearance of fever than dosage of 6 mg/kg/die [44]. Optimal loading dose for a subject of 70 kg is 800 mg every 12 h for three times, then followed by a single administration/die, in consideration of the drug's long half-life. Developments of glycopeptides are telavancin, dalbavancin and oritavancin. Telavancin has a double acting mechanism, (that of the vancomycin plus that of the daptomycin), is highly bactericide, has indications in pneumonia associated with the ventilator and is not tied by surfactant, but it is burdened by collateral effects, such as kidney failure, if the dose is not well adjusted on creatinine. Dalbavancin and oritavancin are long-acting drugs with indication only in infections of the cutis and soft tissues, with considerable bactericidal strength. Oritavancin does not concentrate in the bone, so in the case of osteomyelitis it is to be avoided. Linezolid is a drug that is by now well known, while tedizolid has fewer collateral effects and pharmacological interactions and also acts against linezolid-resistant strains, but it is bacteriostatic, like its predecessor. Adembri et al. have demonstrated better pharmacokinetic function for linezolid if administered in continuous infusion rather than in refracted doses [45]. Daptomycin has its place in cellulitis and endocarditis, association with beta-lactams is perhaps the best way to administer it, also for microorganisms with reduced sensitivity to beta-lactams. Daptomycin is a semisynthetic lipopeptide antibiotic, which has a calcium-dependent anti-bacterial action. Daptomycin plus calcium forms complexes capable of depolarising and forming pores on the bacterial membrane, using the lateral chain of daptomycin molecules. Daptomycin may be less active against VISA or hVISA strains of Streptococcus aureus, which have a thicker wall [46]. Bacteria face cellular death without lysis and, therefore, do not release factors capable of stimulating inflammatory reactions which may lead to shock [47]. Daptomycin benefits from synergy with beta-lactams with a mechanism called seesaw [48]. The main action mechanism of this synergy is due to the fact that exposure to beta-lactams increases the negative load on the bacterial wall of gram-positive bacteria, which in turn increases the bond with Ca2 + complexed daptomycin (positively loaded molecule), giving rise to a potent bactericidal synergic effect. This association also avoids the onset of resistance to daptomycin [49]. A typical example is the association between oxacillin and daptomycin, in treatment of severe infections from MRSA, also against strains resistant to oxacillin [50]. Ceftobiprole and ceftaroline are beta-lactams active against MRSA but with prob-

lems as far as their registration is concerned: ceftaroline has been approved for pneumonia in the community but not for MRSA, ceftobiprole for those in the community and nosocomial but not VAP and the study for infections of the cutis and soft tissues has not been approved by the FDA because of the impossibility of verifying the data collected. Therefore, these new, fifth generation cephalosporins, may find ample room in hospitals, either alone or in associations, but probably with off-label indications. Faced by this worrying epidemiological reality, a strong consensus has recently emerged, by which the scientific community must act with the aim of limiting this emerging crisis. To respond to this need, the health institutions are building and promoting Programmes of AMS, in order to optimise application of antimicrobials, with the aim of improving outcomes for the patient, minimising collateral effects and reducing incidence of infections from MDROs. AMS programmes are understood as an interdisciplinary effort in a continuum of care, which aims at achieving the best possible result in terms of treatment and prevention of infections, of survival and of reduction of mortality, quality of assistance and costs, as well as reduction of toxicity and adverse ecological effects, through responsible use of antibiotics, targeted and personalised for the patient [51 52]. Lynchpin of the strategy is rapid determination of the pathogen, which enables an adequate choice of antibiotic as fast as possible, thus reducing and/or preventing transmission and colonisation of MDR strains, through elimination of the reservoir. Microbiological diagnostics are essential for identification of the pathogen responsible for the episode of infection and to understand sensitivity to antibiotics, both of which are important information in choosing anti-infection therapy. Traditionally, diagnosis of bacterial infections is based on cultural examination of materials obtained from the site of infection, with relatively lengthy response times (48-72 hours, or sometimes even longer), depending on times for growth and identification of the main pathogenic microorganisms. Such response times obviously do not allow for awaiting the result of the laboratory exams before starting antibiotic treatment in the case of a serious infection, which should therefore start on an empirical basis and be revised and targeted as soon as possible, in accordance with the good practices of antimicrobial stewardship, gradually as the microbiological results become available. In a time-dependent pathology, such as septic syndrome and, in a particular manner, septic shock, once persistent hypertension has set in, the speed with which the infectious load is reduced to a sub-critical threshold appears to be of crucial importance for survival. This view suggests that elimination of the infectious trigger may be the first, and possibly the most important, step in arresting the development of the septic process, before damage to the organ becomes irreversible [53]. In the literature, although available data indicates that delay in administration of an appropriate antimicrobial treatment is inconsistently associated with the outcome of the septic picture in the absence of shock, this delay appears to play a substantial role in influencing mortality in cases of septic shock. The most common cause of inappropriate therapy is represented by failure of the clinician to estimate the risk of infection from antibiotic-resistant organisms. Moreover, it is vitally important for a clinician, apart from estimating the risk of having an infection from MDR using specific scores [54 55], to stratify the gravity of the septic syndrome very well, since this has greater impact on mortality than simple acquisition of multi resistance: in practice, people die more from septic shock than from sepsis from MDR pathogens. In this scenario, where the time factor takes on such determining importance, availability of innovative diagnostic technologies allowing reduction of response times to microbiological examinations is certainly of great interest to the clinician. The introduction of the latest techniques of rapid microbiological diagnostics means, in fact, that in extremely short times, just a few hours, all the necessary information is available regarding both identification of the pathogen (e.g. mass spectrometry MALDI: TOF) and profiles of sensitivity/resistance to antimicrobials to be used (e.g. molecular tests and rapid phenotype tests) [56 57]. These innovative technologies, however, are extremely expensive and, for the time being at least, reserved for the few, on the basis of precise selection of patients [58]. Their introduction also requires individual specialist technical competence on the part of the operators, which is not always available in all laboratories, and special training courses. Another aspect to consider when introducing these new diagnostic technologies is the kind of information that is supplied, which is totally different from that of conventional diagnostics and requires specific interpretation, for which the clinician is often not prepared. What is fundamental is a continual close relationship between microbiologist and clinician.

Focusing attention on the otorhinolaryngology patient from deep analysis of the literature, it can be understood that the considerations stated above are completely valid also in the specific setting of patients who do not differ in any way from the rest of the population admitted to hospital. We wish to highlight, however, the importance of a correct prophylactic antibiotic strategy, often disregarded by many, and the impact this has on the epidemiology of the ward and consequently on rates of infection from multi-resistant strains correlated with it. A large

part of interventions in ENT surgery are considered clean and do not require antibiotic prophylaxis, except in cases of patients with elevated co-morbidity falling under the ASA ≥ 3 class of risk (first generation cephalosporin cefazolin 2 gr). In clean-contaminated surgery, however, administration of a second-generation cephalosporin (cefuroxime 2 gr) is indicated, alone or in association with clindamycin or metronidazole or, as second choice, a ureidopenicillin, (piperacillin), or a protected penicillin such as amoxicillin/clavulanate.

A wide review of the literature enables us to confirm that adoption of prophylactic strategies, prompt application of mono- or polytherapeutic antibiotic treatment, accurate search for infectious agents and monitoring of indexes of inflammation prevent or drastically reduce infectious and septic complications. It would also be desirable that, given the emergency of multi-resistance, there should be, in every Otorhinolaryngology Department, 1 or 2 operators with sensitivity and knowledge on that issue, to deal with, either alone or in team with an expert in intensive care or specialist in infection, the clinical cases that may present.

10. What is the future for antibiotic treatment? Antibiotic treatment and nanomedicine

At the beginning of the twentieth century, infectious diseases represented the main cause of death in the world. The reduction in morbidity and mortality due to infections during the last century is, in large part, attributable to the introduction of antibiotics to therapy.

Today, unfortunately, important changes in society, technological progress and evolution of microorganisms themselves are contributing to an increase in emerging and re-emerging infectious diseases and to the appearance of resistance to antibiotics [1].

According to the European CDC, in Europe, since 2009, more than 400,000 people have developed bacterial infections resistant to antibiotics. Resistance to antibiotics is considered as the capability that some microorganisms have to survive even in the presence of concentrations of antimicrobials normally capable of inhibiting or killing microorganisms of the same species [2].

The advent and growth of phenomena of resistance to antibiotics has reached critical levels, continuing to invalidate the action of the majority of antibiotic drugs currently used in the clinical field (Fig. 10.1) [3]. The onset and spread of extremely resistant bacteria, particularly those resistant to antibiotics "of last resort", such as carbapenems and colistin, represent a serious problem for public health and a threat to the safety of patients and economies

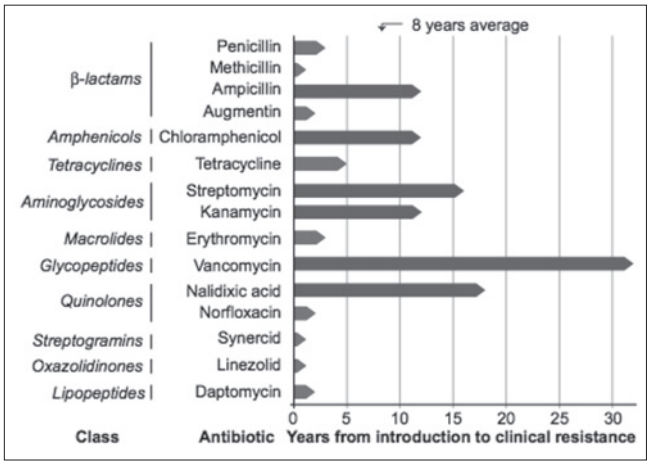

Fig. 10.1. Evolution of antibiotic resistance. The bars show the time lapse from introduction of a clinical antibiotic up to the first described clinical case of resistance, highlighting (red bars) the rapid development of the latter for various classes of antibiotics (from Schmieder et al., 2012 [3], mod.).

at European and world level. The use of colistin, a fallback antibiotic when carbapenems are no longer effective, doubled in Europe from 2010 to 2014 and at nowadays the first cases of resistance have already been registered. A combination of excessive increase in the use of antibiotics and a decrease in discovery of new molecules has led to the situation that many pathological conditions that were previously treatable have today become difficult to eradicate [4].

The problem of resistance to antibiotics is complex, because it is based on multiple factors: increased and continuing use of these drugs (including inappropriate use), spread of hospital infections caused by antibiotic-resistant microorganisms (and limited control of these infections), increase in international travel and therefore greater spread of bacterial strains. Apart from resistance developed by the microorganism towards antibiotics, another factor should also be taken into account, which is capable of influencing the clinical efficacy of the drug, and that is its own capability for spreading itself on a systemic level and therefore in the sites of pharmacological interest (bioavailability). For this reason, when choosing and developing new forms of drugs with antibiotic activity, the pharmacokinetic and pharmacodynamic characteristics of the molecule itself should be taken into account, so that the response can be directed in a bactericidal or bacteriostatic form [5].

The European Commission has also issued a plan of action to combat the growing risks of AMR, in which 12 key points stress how resistance to antibiotics represents one of the main threats to human health which has to be faced

up by a global initiative, in line with the "One Health Initiative" movement (www.onehealthinitiative.com). In particular, point 11 promotes the study and implementation of scientific research to identify new diagnostic and treatment tools capable of combating the ever-increasing growth of resistance caused by antimicrobial agents [6].
The recent scientific research focusing on drug delivery and, in particular, nanotechnologies applied to the pharmaceutical field, can provide and is actually providing innovative, advantageous solutions intended to overcome the problem of resistance to antibiotics.

10.1 Nanomedicine in controlling infections

The use of innovative techniques, such as nanotechnology, can be applied to the immunological field to create new antimicrobial medicines which make it possible to modify the method of drug release and they have proved very promising for overcoming the phenomenon of antibiotic resistance. Nanomaterials showing both marked intrinsic antimicrobial activity and high efficacy and safety in administering antibiotics are called "nanoantibiotics" and their capacity to control infection during *in vitro* and *in vivo* studies has been explored and demonstrated experimentally. Unlike many antimicrobial agents currently used in medicine, nanoantibiotics do not appear to present acute direct collateral effects, even though potential toxicity, due to long term exposure, should always be taken into consideration [1].
Administration of antibiotics, including the use of nanomaterials as carriers, can offer a number of advantages, such as: controlled, uniform distribution in the target tissue, improved apparent solubility of the active principle, possibility of controlling drug release, better compliance for patient, possible reduction of collateral effects and better internalisation.
Here we show some potential systems (Fig. 10.2) which, by exploiting new nanomaterials and nanotechnology, may find a clinical use for administering antibiotics and antimicrobials.

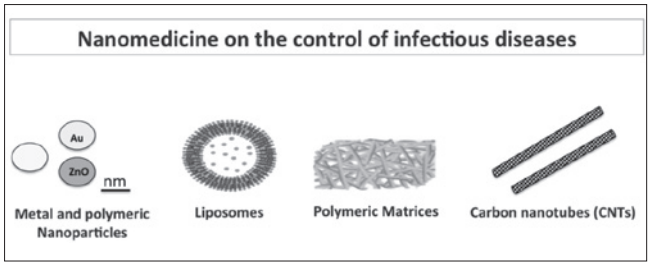

Fig. 10.2. New nanomaterials and nanostructures studied as vehicles of antibiotics and antimicrobial substances.

10.2 Nanoparticles with antimicrobial activity

The term nanoparticle normally identifies particles formed by atomic or molecular aggregates with a diameter indicatively included between 2 and 200 nm.
NPs with intrinsic antibacterial activity consist of metals and metal oxides, such as Gold (Au), Silver (Ag), Zinc oxide (ZnO) and Titanium (TiO_2). These metallic NPs can produce species reactive to oxygen (ROS) when stimulated by UV radiation and then extricate antimicrobial activity towards various microorganisms. In addition to their ample surface area in relation to volume and the characteristic chemical and physical properties of various nanomaterials, they can contribute to effective antimicrobial activity [7].
Recent studies have shown that bacteria are less likely to develop phenomena of resistance towards metallic NPs.
The action mechanisms with which NPs act against microorganisms are:

- photocatalytic production of reactive oxygen species which proceed to damage the components of the bacterial cell;
- damage of the bacterial membrane;
- interruption of energy transduction;
- inhibition of enzymatic activity and synthesis of DNA

In the figure (Fig. 10.3) these action mechanisms are laid out in a scheme [8].
As previously mentioned, there are various metallic materials used for synthesize NPs with elevated antimicrobial activity; in the following table (Table 10.I) the main components used are summarised, with related action mechanisms and experimental uses.
Apart from metallic NPs, which act on microorganisms through the effect of intrinsic antibacterial properties, polymer NPs should also be mentioned for their efficacy.

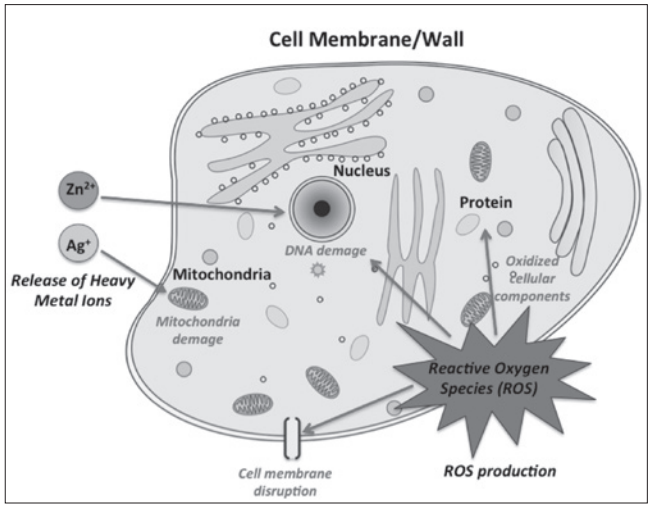

Fig. 10.3. Different action mechanisms of metallic Nps.

Table 10.I. Metallic nanoparticles (NPs) with antimicrobial action: activity, applications and examples.

Metal NPs	Antimicrobial activity	Target	Applications	Examples	Ref.
Ag NPs	Attach respiratory chain and cell division to cell death	Gram + (S. aureus) Gram – (E. coli)	Wound treatment surgical devices	AgNPs Functionalized with ampicillin	(1) (9)
	Release of silver ions with bactericidal activity		Coating for medical and		
	Synergistic action used with antibiotics (Penicillin G, amoxicillin)		Antibacterial agents		
ZnONPs	Destroys lipids and proteins of bacterial membranes	E. coli S. aureus	Non-toxic and biocompatible	PVA NPs coated with ZnO	(1) (10)
	Production of hydrogen peroxide and Zn2 + ions with antibacterial activity		Carrier for drugs and filler material		
TiO_2NPs	Photocatalytic antimicrobial activity with ROS production	E. coli P. aeruginosa S. aureus E. faecium C. albicans L. acidophilus	Foods sterilizing agent	NPs di Ag/(CS)-TiO_2	(11) (12)
	Destruction of bacterial membrane		Air and water purifier		
AuNPs	Interaction with the bacterial membrane and subsequent destruction	Gram + (S. aureus) Gram -	Adjuvant for antibiotic therapy with dose reduction	Au NPs coated with antibiotics Chitosano + Au NPs + ampicillin	(1) (13)
			Photothermic therapy with infrared light		

These NPs are constituted from polymers (composite macromolecules constituted from chains of equal or different monomers joined together through repetition of the same type of bond) and allow transmission and release of antibiotic or substances with antimicrobial action in a site of action. In polymer nanoparticles the polymer membrane, which controls the drug release amount, is used to cover the individual molecule of drug dissolved in water or in an oily solvent, or alternatively the drug is encapsulated inside the polymer matrix as solid end dispersion. In any case the drug will end up distributed evenly within the polymer matrix, with porosity, thickness and degradation times variable according to the polymer used and the desired release mechanism.

The most used polymers for the preparation of these nanoparticles are biodegradable and biocompatible polymers, such as: polylactic acid, polyglycolic acid, polylactid-coglycolic acid, polycaprolactone and polycyanoacrylate; these are used as hydrophobic portion for the formation of the shell, which will contain and encapsulate the drug inside the core, for its controlled release. Externally, these NPs can be made functional with hydrophilic polymers, such as PEG, which avoids the phenomenon of opsonization and slows the degradation of the nanoparticle system, or coated with chitosan, a natural polymer which has been demonstrated to possessing broad spectrum antibacterial activity, or other specific ligands for selective directing of NPs and target release of active.

Systems offer numerous advantages, such as:

- improvement of drug's structural stability in the biological fluid and during the stage of preparation and storage;
- favours internalisation of the NPs through endocytosis on the part of the bacterial cell membrane and therefore release of the drug in situ;
- fine control of the profile of drug release through accurate modulation of dimensions and zeta potential, using adequate solvents, surfactants and polymers;
- ease and versatility of superficial funtionalising for conjugation of drugs or specific ligands.

Polymer nanoparticles are considered a valid means of delivery of antimicrobial agents, showing elevated therapeutic efficacy in treatment of many types of infections [14]. Table 10.II shows some examples of polymer NPs currently being studied for delivery some of the antibiotics used in medicine.

Apart from the polymers shown, which have the advantage of being approved by the main regulatory agencies (FDA, EMA) for use in therapy in humans, studies are ongoing into new polymers which have been demonstrated as advantageous in production of nanoparticle systems. One example is the recent study conducted on Eumelanin, a polymer of natural origin which is biocompatible, biodegradable and has antioxidant properties (radical scavenger). This polymer forms nanoparticles easily, with a self-assembly mechanism. Nanoparticles loaded by ad-

sorption with gentamicin display excellent antimicrobial activity. This polymer's radical scavenger activity can also reduce the toxicity of the gentamicin, due to formation of free radicals [22].

Both metallic and polymer nanoparticles can be formulated in various pharmaceutical forms, such as gels, films, in situ forming, injectable gels, three dimensional scaffolds, in order to enable administration by the desired mode [23-25].

10.3 Liposomes for release of antibiotics

Liposomes are vesicles of varying dimensions, between nanometres and micrometres, consisting of a bilayer of phospholipids with an aqueous core.

The first drug based on a liposome vehicle, doxorubicin (Doxil) was approved by the FDA in 1995, since when much attention has been paid to the use of these carriers for delivery of drugs, as well as enzymes and proteins, for treatment of various pathological conditions. Liposomes prove to be very promising as carriers for delivery of antibiotic drugs because their lipid structure makes them able to mimic the cell membrane, so they can easily interact with bacterial cells; also, both hydrophilic and hydrophobic drugs can be encapsulated in the aqueous core or bonded to the phospholipid layer without undergoing chemical modifications [26 27].

The parameters to consider for use of liposomes as vehicles of antibiotic drugs are:

- chemical and physical property of the lipids constituting the membrane;
- nature of the drug to deliver;
- dimension and polydispersity of the liposome;
- superficial charge (zeta potential);
- conjugation with ligands for a specific target;
- reproducibility of manufacturing technique and product stability.

Use of liposomes for antibiotics administration may present a series of advantages, as in the case of polymyxin B (colistin). This drug, which is active against P. aeruginosa infections, unfortunately presents a series of nephrotoxic and ototoxic collateral effects and causes blockage on a muscular level; delivering polymyxin B inside liposomes displayed how the collateral effects were noticeable reduced, while at the same time antimicrobial activity was increased [28].

Another example involves encapsulation of gentamicin and ceftazidime in liposomes and shows the advantage of prolonging the circulation time and increasing drug half-life, as well as being able to favour a specific localisation at the infection site [29].

Studies carried out by Jones et al. investigated the interaction between liposomes and the bacterial biofilm; for example, by using both cationic and anionic liposomes it was noted that each bacterium in the biofilm absorbs independently from the others and that absorption is lower for anionic liposomes [30]. This has suggested that bacterial variability inside the biofilm may influence the result of the antibiotic treatment, also with the risk that it may be ineffective, and for this reason it may be useful to use a combination of liposomes with different superficial charge (zeta potential).

10.4 Implantable matrices for delivery of antibiotics

Bacterial infection at the level of a surgical site, such as fitting of artificial prostheses, represents one of the most serious problems in the biomedical field [31].

At the tissue level, infection associated with surgical implant is the result of bacterial adhesion to the surface of the material. Immediately after the operation, a sort of competition takes place between integration of the material into the surrounding tissue and adhesion of bacteria to the surface of the implant. In the majority of cases, bacterial adhesion leads to formation of a biofilm, which proves to be much more resistant to traditional antibiotic treatment, with consequent risk of a serious infection associated with the implant. The 6 hours subsequent to the operation are the decisive hours, during which the implant

Table 10. II. Polymer NPs delivering antibiotics and the related action target.

Polymer	Drug	Target	Ref.
PLGA	Sparfloxacin Levofloxacin	P. aeruginosa, S. aureus	(15)
PLGA	Gentamicin	Brucellosis Osteomyelitis	(16) (17)
PA NPs	Gentamicin	Prevents Gram + and Gram- biofilms forming.	(18)
PEG-PECA	Amoxicillin	Helicobacter pylori	(19)
PCL and PLGA	Levofloxacin	E. coli	(20)
CS-PVA/ PLGA	Colistin	P. aeruginosa	(21)

PA NPs = phosphatidylcholine-decorated Au nanoparticles
PEG-PECA = Polyethyleneglycol (PEG)-coated polyethylcyanoacrylate (PECA)
CS-PVA = Chitosan (CS)-Poly(vinil-alcool) (PVA)

is especially susceptible to a superficial colonisation and, therefore, it is fundamental to prevent bacterial adhesion in order to ensure the long term success of the implant.
An alternative approach was recently assessed, to reduce bacterial adhesion by using "active coatings" capable of preventing, or at least inhibiting, bacterial adhesion to the operation site.
Fibrous polymer matrices delivering antibiotics (Fig. 10.4) have been discovered recently as a solution for preventing post-operative adhesion and providing site-specific release of an antibiotic drug. These fibrous matrices, made, for example, with the electrospinning technique, are capable of operating as carriers for hydrophilic or hydrophobic molecules, but also actually they act as support for tissue regeneration at the operation site [32].
Electrospinning is a new production process, which is simple and versatile and capable of producing fibrous matrices consisting of fibres of nanometric dimensions.
These structures are highly promising also as systems for drug release and, additionally, by using various techniques of electrospinning, different loadings of drugs can be obtained (coating, embedded, encapsulated), so as to adjust the release kinetics with accuracy [33].
Some examples are given in Table 10.III for electrospun matrices currently being studied for antibiotic delivery in the treatment of infections.

10.5 Carbon nanotubes and antimicrobial activity

Carbon nanotubes are cylindrical nanostructures consisting of pure atoms of carbon bonded covalently in hexagonal structures and because of their specific optical, electrical, mechanical and thermal properties (high electrical and thermal conductivity, high electron mobility) they have attracted a lot of attention in recent years [44].
A Carbon nanotube may be considered as a sheet of graphene (a hexagonal net of carbon) rolled into cylindrical form.
There are single-wall nanotubes with a diameter of around 1-5 nm and multi-wall nanotubes, consisting of multiple tubes piled one on the other, with a length varying from 100 nm to a few micrometres.
Recent studies have shown antimicrobial activity associated with single-wall nanotubes after implantation in damaged areas that are at risk of infection, since they are capable of interacting with bacterial membranes and putting them under oxidative stress; it has also been verified that these structures can prevent the formation of bacterial biofilm on the surfaces. Experimental tests have demonstrated important cytotoxic action against bacteria (Escherichia coli and Bacillus subtilis) in dispersion, with noticeable reduction of the bacterial count even after just 1-2 hours.

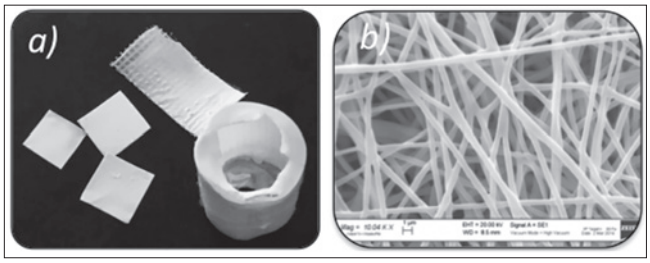

Fig. 10.4. Nanofibrous matrices seen with the naked eye (a) and internal structure of the matrix (b). Made of fibres of nanometric dimensions seen through a scanning electron microscope (SEM).

Their chemical stability and easy functionalisation has made nanotubes potential biomaterials for the prevention and treatment of bacterial infections [45 46].
A lot of attention has been paid recently to the use of graphene and its multiple properties (superior mechanical resistance, excellent load mobility, high thermal conductivity), also in the biomedical field. Researchers at the Faculty of Medicine and Surgery at the Catholic University of the Sacred Heart, jointly with the Complex Systems Institute of the National Research Council (Isc-Cnr) in Rome have discovered that graphene is potentially employable, both in gel and liquid form, for the prevention and treatment of hospital infections.
Graphene oxide (GO) in sheets (sheets of oxygenated graphene with molecules such as epoxide and carboxyl and hydroxyl groups on its surface in a greater number than just graphene) of around 200 nanometres, in aqueous solution, is capable of eliminating, in less than two hours, around 90% of Staphylococcus aureus and Enterococcus faecalis and around 50% of Escherichia coli; this action of oxide of graphene against bacteria has also been demonstrated at low concentrations (10 μg/ml).
Sheets of GO act against bacterial cells by three different action mechanisms:
- cutting the bacterial membrane;
- isolation and suffocation of the bacterial cell;
- cell oxidation and production of oxidative stress.

The antibacterial mechanism of graphene oxide acting with both mechanical damage and with chemical oxidation would make it more difficult for bacterial resistance to occur.
Coating medical instruments with this carbon-based material might contribute to reducing infections, especially after surgery, as well as reducing the use of antibiotics and the onset of resistance [47].
Apart from use on its own, various studies have shown that integration of graphene in matrices and nanostructures gives them increased antibacterial action.

Table 10.III. Electrospun polymer matrices delivering antibiotics.

Electrospun matrices	Drug	Activity	Ref.
PLGA/PEG-b-PLA diblock copolymer/PLA (80:15:5 wt%)	Cefoxitinsodium (Mefoxin®)	Prevent infections after surgery	(32)
PLGA	Tetracyclinehydrochloride	Protein synthesis inhibitors, active against gram + and gram -	(34)
PEUU/PLGA	Tetracyclinehydrochloride	Protein synthesis inhibitors, active against gram + and gram -	(35)
PLA/PCL	Tetracyclinehydrochloride	Protein synthesis inhibitors, active against gram + and gram -	(36)
PLA-collagene	Gentamicin	Post-operative infections due to *S. epidermis*, P. aeruginosa and E. coli	(37)
Halloysite clay nanotubes/PCL/gelatin	Metronidazole	Action on anaerobic and protozoal bacteria	(38)
Chitosan: PEO (90:10)	Ciprofloxacin hydrochloride (CipHCl) or moxifloxacin hydrochloride (Moxi)	Activity against E. coli and S. aureus	(39)
PVA/ SF	Ciprofloxacinhydrochloride	Activity against E. coli and S. aureus	(40)
PEG/PLA	Doxorubicinhydrocloride	Antineoplastic antibiotic	(41)
PDS	Vancomycin (VANC) and/or Rifampicin (RIF)	Activities on biofilms formed by S. aureus and S. epidermidis	(42)
PCL	Ampicillin sodium	Used to treat pharyngitis, tonsillitis and influenza caused by Haemophilus	(43)

PEUU = poly(esterurethane) urea
SF = regenerated silk fibroin
PDS = polydioxanone

Table 10.IV lists some examples of association of graphene with other materials to increase bactericidal activity.

In conclusion, the spread of ineffectiveness of traditional antibiotic treatment is a problem the scientific community is interfacing with over the last few years, with worrying implications for public health. Nanomedicine has received growing interest because of its potential applications in this field and it is demonstrating interesting properties on a clinical level.

Nanoparticles are versatile therapeutic systems; depending on their constituent material, that can fulfil intrinsic antimicrobial activities or be carriers for antibacterial active principles, improving their activity and reducing their collateral effects.

While liposomes were developed mainly between the end of the 1990s and early 2000s, metallic and polymer nanoparticles currently seem to be the focus of greater interest within the scientific community, especially because of their better stability compared to liposome vesicles and the possibility of fine modulation of release of the active carried inside them.

Use of new natural and synthetic biomaterials has shown excellent potential in the clinical field, especially on account of their non toxicity, biodegradability and multifunctional capacity to ensure antibiotic better release. In addition, materials are being developed that are increasingly sophisticated, with appropriate modifications on a chemical and structural level, which can increase their stability over time and slow down release of a drug, to ensure suitable treatment of a disease [55].

Another important advantage of these formulations is their capacity to provide release of active on a local level, which would thus make it possible to reduce waste of active, reduce its enzymatic degradation and also minimise any collateral effects on a systemic level [1].

Over recent years the problem of antibiotic resistance has been involving governments, research organisations and the pharmaceutical industry and unfortunately the problem cannot be simply resolved just with the discovery and creation of more aggressive drugs or ones with greater antimicrobial activity, because the microorganisms will continue to adapt so as to survive the drugs used.

The war against pathogen bacteria calls for the effort of implementation of various strategies, starting from better and more controlled application of the already existing preventive measures to avoid primary infections, better practices of control of hospital infections, better availability of diagnostic instruments enabling clearer and faster recognition of those patients who effectively need curing with antibiotics.

It should also be laid down that antibiotics on sale should be used more rationally, underlining the need to give prec-

Table 10.IV. Graphene-based formulations with bactericidal action.

Polymers	Formulation	Activity	Ref.
PVA/ Chitosan/ G	Nanofibers	Wound healing	(48) (49)
PVP/TiO_2/ZnO/GO	Nanofibers	Antibacterial wound dressings tested on E. coli and S. aureus	(50)
PVA/TiO_2/GO	Nanofibers with interface sol-gel reaction	Photocatalytic and electrochemistry applications.	(51)
PLGA/Chitosan/ GO-Ag-Nps	Nanofibers decorated with NPs	Prevent bacteria surface colonization	(52)
Ag-GO	Nanoparticles decorated on the GO sheets	Antimicrobial activity against the Gram negative bacteria E. coli and P. aeruginosa	(53)
BKB/PDA/rGOG	Hydrogel	Antibacterial activities against both Gram-negative and Gram-positive bacteria.	(54)

G = Graphene
GO = Graphene oxide
Ag NPs = Silver nanoparticles
BKB = benzalkoniumbromide
PDA = polydopamine

edence to the use of low spectrum agents, while reserving use of high spectrum ones for special, high risk circumstances. In this respect, development of innovative, highly engineered pharmaceutical forms (such as nanoparticle drug delivery systems) can enable more targeted and controlled use of antibiotics at the infection site, beginning to combat the excessive and sometimes far from effective use of antimicrobial agents that have been developed over the last few years.

In brief, investment in new anti-infection platforms is essential and urgent and calls for close collaboration between industry, academic world and governments, in order to move ahead with investigating into an effective solution for understanding bacterial resistance and new approaches for controlling it. It would appear, however, that the era in which bacterial infection, whether acute or chronic, was treated with "just" the use of antibiotics is over and that the prospects are of more effective diagnostics, more targeted and combined therapies [56].

11. Considerations on the economic problems and critical issues

11.1 Considerations on health economy policies

Every year 57 million deaths are recorded worldwide and of these around 15 million (25%) are caused by infectious diseases: humanity has to take account of old diseases as well as of new ones, the so-called emerging infectious diseases.

According to the WHO, infectious diseases, emerging and re-emerging ones, pose a global threat which calls for a coordinated, global response: the threat is planetary, since an infection can set in anywhere in the world and pass rapidly to other regions, through means of communication, where environmental and climatic conditions are not incompatible (WHO Doc A48/15; 1995).

The WHO has, in the last five years, detected the onset of more than 1100 epidemic events: and among them there is no lack of new, communicable mortal diseases, such as Severe acute respiratory syndrome (SARS) or avian influenza. The spread of HIV/AIDS is undermining stability and even every attempt at economic and social development, especially in the poorest countries, and this just to give few examples of the social impact of these deseases.

Politico-economic liberalisation is often translated into loosening of interventions and controls on health and sometimes into cuts in investment in health programmes, such that many governments considered it pointless investing resources in controlling infectious diseases. Moreover, development of the global market has led to increased economic competition and greater pressure on governments, which weakens institutional defences against emerging infections, this being considered a problem already solved or else an issue that is only important for certain entities: despite the favourable influence that globalisation has had on general economic development, the same cannot be said for public health policies, especially with regard to infectious diseases.

Only 0.8% of state expenditure in the poor nations is destined for health, while worldwide only 2% of investments in research is used to combat AIDS, malaria, TB and acute respiratory infections.

Underdevelopment and poverty do not only contribute to the spread of infectious diseases, but they are also responsible for decrease in life expectancy, reduced to no more than 40 years in many African nations.

The permanent situation of political instability on a world

scale might also further weaken the watch over infection emergencies.
The fight against emerging and re-emerging infectious diseases is not only achieved through health programmes for adequate treatment of patients, but also with programmes for surveillance, prevention, implementation of monitoring systems for drug resistance and the adoption of policies for use of antimicrobial drugs as well as for effective vaccine cover.
It therefore becomes imperative to carry out cost studies on diseases, to lend support to health policies and provide indications for decision making processes, through the following initiatives:

- identify various components of costs and how much falls on society for diagnosis and treatment;
- estimate costs between various emerging and re-emerging infectious diseases and make comparisons between them, in order to define a classification of health problems according to volumes and costs and project future costs of the diseases under consideration;
- identify the main cost drivers, according to seriousness and stage of advancement of the disease;
- identify the indispensable elements of economic assessment.

Measuring the costs of infections is difficult and the financial impact varies in the various health systems, inasmuch as these diseases are becoming an important cause of morbidity and mortality; unfortunately, there is no data in the literature on the clinical/economic impact for assessment, especially regarding Italy.

11.2 The cost of emerging and re-emerging infectious diseases in ENT

A patient with infectious disease generally reaches our operative units either for diagnostic needs, such as lymphadenectomy for suspect TB or other atypical lymphadenitis or lymphoproliferative diseases in HIV subjects, or for correlated neoplastic diseases (HPV/EBV) or for abscesses or phlegmons of the cervical cephalic area.
For correct economic assessment and description of costs, it is fundamental to define a prospective analysis: points of observation and methodologies may be very different from each other, with consequently differing results. Costs can be calculated from the point of view of the patient, the family, a local health authority, the National Health System or of society and therefore they are set out in a different manner, depending on the point of view of observation.
The direct cost to the NHS is correlated with the costs related to employment of hospital resources, either for primary care for inpatients or for home care, together with costs of drugs. Indirect costs include those related with lost productivity or early retirement; then aspects have to be considered that are connected with possible invalidity and lost years of life.
Co-existence of these diseases generally leads to a lengthy stay in hospital, with increased inpatient costs, increased patient costs in terms of loss of work and costs of relatives/caregivers connected with time and movements for visits during hospital stay.
These diseases also mean an increase in costs of therapy, diagnostic investigation and laboratory, as well as costs of prevention and control of infections, including epidemiological, medical and nursing investigation and handling times (Tables 11.I, 11.II).
The costs connected with isolation of the patient also have to be, including related blockage of a bed and reduced performance of the operative unit.
To this we should also add social costs, that are actually difficult to quantify (Table 11.III).
Lastly, it is useful to consider the general costs as well, which, in accountancy, are all the resources used by many services, departments and programmes (health and administration offices, ancillary services, records, cleaning services, electrical energy, etc.).

11.3 DRG and critical aspects

The fundamental characteristics of the system of payments and tariffs for hospital activities, based on specific predefined tariffs for each diagnosis-related group (DRG), are as follows:

Table 11.I.

Indirect costs
Lost days of work or study
Contagion between subjects
Chronicisation
Deterioration of quality of life of the patient
Temporary/permanent invalidity

Table 11.II.

Direct costs
Hospitalisation
Laboratory and imaging diagnostics
Surgical and anaesthetic procedures
Pharmaceutical expenditure and that of health authorities
Specialist visits
Primary assistance and home assistance, health prevention

Table 11.III. Economic impact.

Hospital inpatient costs increased inpatient stay
Use of antibiotics
Possible stay in intensive care
Costs of assistance potential need for isolation
Use of barriers
Diagnostic and laboratory tests
Working time of health staff
Outpatient/homecare costs additional medical check-ups
Antibiotic treatment
Home visits by GP
Rehabilitation therapy, where necessary
Outcomes/costs for the patient mortality
Morbidity
Lost earnings
Transfer expenses

- the expected payment of the hospital, i.e. with tariffs set before the services are provided by the hospital structure;
- the all-inclusive tariff, i.e. paying for everything needed during the hospital stay to provide the service to the patient.

To function properly, the DRG must inevitably be:

- flexible;
- prompt;
- upgradeable;
- transparent;
- accurate;
- fair;
- homogeneous.

These characteristics would mean that the services provided should be classified adequately, but such a system is absolutely not dynamic, innovative or prompt in updating the ICD 9 CM health ministry codes, so we find ourselves, especially with regard to our specialisation, in a condition in which the level of complexity, the severity and the technological and human resources are not considered. This does not allow for adequate assessment of the clinical and assistance processes provided, especially where there are situations of co-pathologies, which cause increased absorption of resources, as in the case of infectious diseases. To obtain groups of patients that are reasonably homogeneous on a level of absorption of resources, the DRG system should be less simple and it should be supplemented with indicators of severity of disease and complexity of complications.

Homogeneity in each DRG is generally assessed on the basis of variation of the length of hospital stay: the greater the coefficient of variation, the higher is the inhomogeneity of the DRG in question. Unfortunately, the inadequacy of the coding system for otorhinolaryngology, which does not recognise innovative technology that has led to contraction of length of hospital stay, heavily penalises our specialisation and does not allow for identification of exhaustive specific DRGs.

DRGs of MDC 3, which are involved in the case of procedures in patients with infections, are limited in number and with the already known issues of weighting and tariffs:

- DRG 049: major interventions on head and neck;
- DRG 053-054: Interventions on sinuses and mastoid;
- DRG 063: other interventions on ear, nose and throat;
- DRG 168-169: interventions on the mouth, with and without CC;
- DRG 482: tracheotomy (face, mouth, neck).

Therefore, in order to reach the objective of a correct assessment of the provided services, correct compilation of the SDO (inpatient discharge form) becomes the source of adequate correlation between clinical, processing and administrative data and is the best source with regard to the hospital inpatient process.

The SDO should be completed with all the diseases and services, trying to find the best ICD-9CM codes among those available, instead of those that appear more opportunist.

The SDO and its contents are always mostly used by the decision makers in very delicate areas of governance of the systems, for areas related to outcomes, appropriacy and efficacy.

The SDO is, therefore, at present even with its limitations, the best instrument available to allow assessment of performance and economic aspects of the services we provide daily: it is a source of "assessment, monitoring and enhancement" of the assistance for the so-called "complex" patient, as the one who is treated for diseases correlated with an emerging and re-emerging infection (e.g. HPV/HBV or HIV correlated neoplasias of the cervical-cephalic regions in a patient not in perfect general condition and with multiple co-pathologies).

Aspects of remuneration often also penalise us and frustrate the operators and the development of technological innovation: however, it is of fundamental importance to try to enhance our activities by correctly compiling the fields of the SDO, and not to consider this task as a mere bureaucratic exercise.

11.4 The outpatient

Maintaining efficient prevention structures, which control the spread of infections, represents the fundamental element for preventing the emergence or re-emergence of

infectious diseases in economically developed and in developing countries.
The most significant example in the world comes from TB, which has gone through periods of decline and moments of re-acutisation, in parallel with containment by control and aid programmes.
A recent alarm for public health concerned the spread of mycobacterial strains that are multi-resistant to pharmacological treatments (MDR-TB), a fact which really represents a major issue.
This phenomenon has a really considerable social and economic cost, with costs of treatment that multiply from 3 to 100 times, depending on the country and the type of resistance to the bacterial strain.
Increasing costs is even more significant, together with elevated mortality, in patients affected both by TB and by HIV: the correlation between these two diseases has been known for a long time, especially on the African continent, and it is known that decline of the immune system in HIV infection increases vulnerability to mycobacteria and leads to greater difficulty in care, with the need to perform tests for resistance to antitubercular drugs, which take time to complete and are notably expensive, also with associated close, accurate surveillance of antibiotic resistance.
Movement of populations, now more frequent, rapid and numerically substantial, can lead to a change in the advancement and spread of infectious diseases, so surveillance systems have to envisage groupings according to different criteria:

- emerging/re-emerging diseases;
- diseases requiring immediate intervention;
- pathogens resistant to antibiotics;
- diseases requiring strategies and campaigns for vaccination.

From this the importance may be deduced of an adequate, correct assessment of the clinical and economic impact. It is imperative that the decision makers arm themselves with monitoring instruments that evaluate new, adequate methods of treatment and the results of assistance, giving priority to growth of a cultural approach combining various dimensions: programming, introduction of diagnostic and assistance processes, cost of illness, cost-effectiveness analysis, incremental cost-effectiveness ratio, efficiency, integration and usability of services, following a logic of continuity from community to hospitals, fairness of access ensuring adequate standards of services nationwide in Italy.
It is only through development of the idea of an Infection Network, for research, clinical assistance and training, that the goals can be reached of rationalisation of the system of supply, of improvement of assistance practices and of the creation of economy of management.
In conclusion, dealing successfully with emerging and re-emerging infectious diseases requires a clear idea of the decision making processes, of the policy aims of decision makingand of the time required for making decisions. Availability of resources and incentives must also be defined for implementation of programmes and treatments which can lead to more cost-effective use of resources.
Consideration must also be given to the importance of expenditure on research, which leads to a return in terms of public health and savings on the real costs of hospitalisation and assistance.
Having assessed the financial impact that these diseases are having on health systems as well as on society, preventing these diseases means reducing that impact and therefore also means, in a context of growing pressure on health budgets, protecting the population by reducing or avoiding hospital stays, medical intervention, reliance on antibiotic treatment and other drugs: all of which will surely contribute to greater sustainability of health systems.

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11. Considerations on the economic problems and critical issues

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