Sexually Transmitted Infections

Advances in Understanding and Management

Antonio Cristaudo
Massimo Giuliani
Editors

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Preface

The decision today to edit a handbook on Sexually Transmitted Infection (STI) is a difficult one, mainly due to the complexity of this field and to the growing challenges arising from this wide spectrum of infections for clinicians, researchers, and public health authorities after the year 2000.

STIs remain a global health priority because of their dramatic impact on women and infants and their well-documented biological and epidemiological inter-relationships with HIV infection. The health complications which arise from STIs represent relevant causes of morbidity and mortality for a large part of the planet's population and a main source of spending health financial resources in high-income countries. WHO and UNAIDS have estimated that nearly one million people become infected every day with a bacterial STI and that about 750 million people live with a viral STI such as HIV, HSV, or HPV infection.

Clearly, STIs disproportionately affect low-income and middle-income countries, but since 2000 there has been a disturbing resurgence in Western countries with higher incidence rates in most vulnerable groups, coupled with declining susceptibility or outright resistance of some STI pathogens to large-use antimicrobial agents.

Despite their high global impact, STIs remain a neglected field for clinical and public health practice and for research, and many issues are still incomplete, with several questions unanswered, particularly in the area of basic research and of the behavioral key drivers.

For all these reasons, even though other comprehensive texts and e-publications about STI have already been accessed by many specialists in this field, we have accepted the challenge to design a handbook which focuses on some of the high-priority contents that now concern the STI field. This approach has forced us to make specific choices for building a text based on some selected issues concerning the modern STIs, such as; the novel epidemiology, the challenges of prevention and of basic research and the new clinical aspects for many of the infections.

Thus, the book cannot be considered a typical STI handbook because it is not a complete guide regarding STI, but a streamlined text that tries to enhance knowledge about some selected contents which are proving to be the most decisive for modern STI control.

Naturally, we could not have done all this by ourselves and without the close cooperation of a panel of international experts who agreed with us to take on this challenge. We are honored and proud to have received their pre-

cious dedication. We would like to express our gratitude for their efforts, support for our ideas about the framework of the opera, and the sacrifice of their valuable time. Additional thanks go to the contributors who have enriched the texts with graphics, tables, and algorithms and donated color photographs to improve the didactic value of the texts.

We would like also to thank the Editorial Team at Springer Medical Editions for their assistance and support in the finalization of the different chapters and for the autonomy of decision they granted us as Editors concerning the contents, form, and collaborations. In particular, our special thanks go to Dr. Juliette Kleemann for her trust, her assistance, and precious advice and to Beauty Christobel Gunasekaran who provided support in all the phases of collection, revision, and management of the published materials.

We hope, together with all the contributors, that we have produced a useful text for all those who today are engaged or interested in the study of modern "Venereology."

Happy reading to all!

Antonio Cristaudo
Rome, Italy Massimo Giuliani

Contents

About the Authors

Massimo Andreoni is a specialist in Internal Medicine and in Infectious Diseases, has been President of Italian Society of Infectious Diseases (2013–2016), and currently he is Scientific Director of the Italian Society of Infectious Diseases. Chief of Ward of Infectious Diseases, Policlinico Tor Vergata, Rome, Italy, Professor Andreoni is Director of Integrated Care Processes Department at Tor Vergata Hospital and Director of the Department of Medicine of the Systems, University "Tor Vergata," Rome, Italy. Professor Andreoni has been a project leader of several national and international research programs, and he is the author of more than 300 indexed articles and several chapters of books.

Sevgi Okten Aral has been the Associate Director for Science in the Division of STD Prevention, Centers for Disease Control, since 1993. She holds professorial appointments at the University of Washington in Seattle, University of Manitoba in Winnipeg, and Emory University in Atlanta. Dr. Aral has authored more than 250 scientific articles and edited 17 journal issues and 3 books. She has served on many national and international work groups, boards, and committees and has consulted for the World Health Organization, the European Union, and the World Bank. She has also received the ASTDA Achievement Award and the Thomas Parran Award. Dr. Aral's research interests have included social and behavioral aspects of sexually transmitted disease; epidemiology and prevention including gender, age, and race effects; mixing patterns; sexual and social networks; contextual factors; social determinants; and most recently, program science.

Anna Carannante after the degree in Biological Sciences and Biology for Biomedical Research received the Specialization in Microbiology and Virology from the University of Rome *La Sapienza*. She is currently a researcher in the Department of Infectious Diseases of Istituto Superiore di Sanità.

Her professional interests focus on specific aspects of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. In particular, she is involved in the Italian Surveillance System for antimicrobial resistance in *N. gonorrhoeae*. Her routine activity is to perform, on collected strains by Italian outpatients, antimicrobial susceptilility testing and molecular typing, including whole-genoma sequencing (WGS).

Moreover, she is involved in serology and molecular typing of *N. meningitidis*. The so-called MATS (The Meningococcal Antigen Typing System) ELISA and the SBA (Serum Bactericidal Antibody) assay are the main field of activities. Her main area of studies includes the molecular typing of meningococcal virulence determinants. VPD-Reference Labs Unit, Department Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

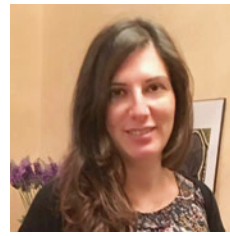
Manola Comar received her degree in Biology from the University of Trieste, Italy, and is currently an Associate Professor of Clinical Microbiology in the Department of Medical Sciences at the University of Trieste. She is Director of the Advanced and Translational Diagnostic Microbiology Department at IRCCS Burlo Garofolo, Trieste, Italy. She specialized in virology and clinical microbiology at the University of Brescia.

Her main professional interests focus on sexually transmitted microorganisms and host interactions She is scientific principal or co-investigator in research projects on co-infections of vaginal microorganisms, tumor viruses, vaginal microbiome and its relationship with host innate immune response. In addition, she serves as an expert in sexually transmitted infection (STI) for the Italian Ministry of Health and the Institute of Superiore di Sanità, is a member of the Italian Society of Microbiology, and was with the Premio Natta Copernico for her contributions to molecular microbiology research. She is the author of more than 150 papers in the field of molecular microbiology.

Antonio Cristaudo is a dermatologist and venereologist with a vast experience in STI prevention, diagnosis, and therapies. In 1987, he started his clinical and research activity at the San Gallicano Dermatological Institute in Rome, a research and health institute, his main duties are the management of patients with STI and HIV infections. In April 2013, he became the head of the STI department at the San Gallicano Dermatological Institute. He is a PI of trials and scientific projects on STI, HIV, and HPV infections. He is author of more than 200

scientific articles in high indexed journals, most of which are in the field of STI. He is the current Past-President of the Italian Association of Hospital Dermatologists (ADOI).

Marco Cusini graduated in Medicine at the University of Milan in 1980. He obtained his Specialization in Dermatology and Venereology in 1983. He worked at the Clinic of Dermatology since then mainly in the field of sexually transmitted diseases. Dr. Cusini became head of the Sexually Transmitted Disease Center of Milan in 1990. He became a member of IUSTI in 1994, organizing the XXIV IUSTI Europe Meeting in Milan in 2008. Dr. Cusini was the Scientific Chair of the XXX IUSTI Europe Conference held in Budapest in September 2016. He is an IUSTI officer in charge of the Educational Branch and Italian representative for IUSTI. He is the co-organizer of the IUSTI Basic Summer Course held every 2 years in Bertinoro and of the Advanced IUSTI Course held every 2 years at the site of IUSTI Europe conference. He is a member of the IUSTI Guideline Committee. Dr. Cusini is the present president of SIMAST (the Italian Society for Sexually Transmitted Infection); he is also a member of EADV and a member of the EADV task force for STI and HPV. He published more than 100 papers in scientific journals and is the coauthor of the book *Atlas of Male Genital Disorders* edited by Springer. He was awarded with the IUSTI Silver Medal. He is still working in the field of STIs and is planning a co-operation with Lacor Hospital (Gulu Uganda) for the prevention of HPV diseases in Ugandan women.



Sarah Dal Zoppo received her degree in Medicine and Surgery from the University of Brescia (Italy) in 2008 and then obtained her postgraduate degree in Infectious Diseases from the Clinic of Infectious and Tropical Diseases—University of Brescia in 2014. Her professional interests focus on the treatment of HIV-infected people, STDs management, and tuberculosis diagnosis and treatment. Recently, she served as a research fellow at the Clinic of Infectious and Tropical Diseases—University of Brescia. She currently works as consultant physician in the Infectious Diseases Division of "C. Poma" General Hospital in Mantova. She is a member of the Italian Society of Infectious and Tropical Diseases (SIMIT).

Serena Del Bue received her MSc degree in Medical Biotechnology in 2000 at the University of Milan. Since then, she decided to focus her research on the virology field and strengthened her methodological skills with a fellowship at the Center for Neurovirology and Cancer Biology, Temple University, in Philadelphia. She completed her PhD in Molecular Medicine at the University of Milano. Currently, she is an Assistant Professor of Clinical Microbiology in the Department of Biomedical, Surgical and Dental Sciences at the University of Milano, and Adjunct Assistant Professor in the Department of Neuroscience at Temple University.

Her scientific interests specifically regard the pathogenesis of the human polyomaviruses, with focus on the infection of the immunosuppressed host (organ transplant recipients, HIV-positive patients, patients affected with tumors). For her studies in these fields, she received the Heine-Medin Award from the European Society of Clinical Virology in 2017. She is the author of more than 80 papers in the field of molecular microbiology.

Davide De Santo was born in Cosenza (Italy) in December 16, 1968. He obtained his Medicine degree summa cum laude in 1999 at the University of Rome "LASAPIENZA" and then specialized in obstetrics and gynecology summa cum laude at the University of Trieste (Italy) in 1999. In March 2003 Dr. De Santo completed his doctorate (PhD) in Perinatal Medicine, Developing Pediatrics and Perinatology at the University of Trieste (Italy) and during this period he spent 1 year, as exchanging scientist, at Thomas Jefferson University, Philadelphia, USA. In 2003 he became a consultant in Obstetrics and Gynecology at the Monfalcone Hospital (San Polo) (GO-ITALY) until 2007. Since September 2007 until present, he was hired at the IRCCS Burlo Garofolo hospital of Trieste as a consultant in Obstetrics and Gynecology. His personal areas of interest vary from infections in obstetrics and gynecology and minimally invasive surgery. He is a coauthor of more than 150 papers of which more than 20 are peer reviewed. He is a member of many scientific societies mainly in infections in obstetrics and gynecology and minimally invasive surgery.

Francesco De Seta (Birth Date April 9th 1968) is an assistant professor of obstetrics and gynecology at the University of Trieste, Italy, where he is involved in clinical practice, teaching, and research in the areas of premature birth and obstetrics/gynecology infectious diseases. After receiving his medical degree from the Medical School of Pavia in 1991, he did his residency in obstetrics and gynecology in Trieste where he now works with an internationally recognized group that was responsible for the first paper on the proteomic analysis of human cervico-vaginal fluids published in the journal *Proteome Research* and later in collaboration with Sergio Crovella studied Mannose-binding lectin in recurrent vaginal infections. In 2007–2008 he was Research Assistant Professor in the gynecologic infectious disease research program at Des Moines University (Des Moines, USA) and has continued in that position until 2010 where he continued to make short-term research visits to pursue genomic and microbiomic studies in women who deliver prematurely. He was also associated with the international prematurity research group organized by the World Health Organization PREBIC (Preterm Birth International Consortium) that connected him with physicians and scientists who study the causes and complication and interventions for prematurity at locations around the world. Now he is continuing clinical studies and research on vaginitis and vaginosis with next-generation sequencing methods. Since 2018 Dr. De Seta is part of an organizing Committee for a "Permanent School on Human Microbiome" (Turin, Italy). He is currently a member of several scientific societies as ISIDOG (International Society for Infectious Diseases in Obstetrics and Gynecology) and vice-president of SIMAST (Italian Society for Sexually Transmitted Infections).

Maria Gabriella Donà received her degree in Biological Science and her PhD in Biology from the University of Roma Tre (Rome, Italy) and is currently a Senior Researcher at the San Gallicano Dermatological Institute IRCCS (Rome, Italy).

Her professional interests focus on the molecular epidemiology of human papillomavirus (HPV) infection and on the role of HPV in the development of anal and oropharyngeal carcinomas, as well as nonmelanoma skin cancer. Her current projects include the study of (1) the natural history of anal and oral HPV infection in high-risk males with and without HIV-1 infection; (2) the role of HPV-related biomarkers in anal cancer screening; (3) the diagnostic and prognostic role of HPV-related biomarkers in oropharyngeal cancer; and (4) the epidemiology of cutaneous HPV infection in (pre)malignant skin lesions. In addition, she is a member of the Scientific Committee of the biannual Workshop "Emerging Issues in Oncogenic Virus Research," organized by the International Agency for Research on Cancer (IARC) and the German Cancer Research Center (DKFZ), and a member of the Scientific Committee of "Sinergia & Sviluppo" training company for healthcare professionals.

Mark C. Fernandez graduated from the University of Washington in 2014 with a BSc in Microbiology. As an undergraduate student, he began studying syphilis in Dr. Sheila Lukehart's laboratory. After graduating, Mark worked at the Washington National Primate Research Center as a research scientist in the reproductive biology group, where he developed innovative genetic tools to establish a nonhuman primate model of fragile X syndrome. In 2017, he joined the laboratories of Dr. Sheila Lukehart and Dr. Lorenzo Giacani to work toward developing a syphilis vaccine. He recently entered into the University of Washington Pathobiology PhD program and is currently working on his dissertation project on the molecular pathogenesis of *Chlamydia trachomatis* infection. Mark was awarded a prestigious scholarship through the University of Washington STD/AIDS Research Training Grant for his innovative proposal to characterize the microenvironment of the *Chlamydia trachomatis* inclusion lumen and identify essential host factors that are recruited into this compartment during infection.

Pasquale Ferrante is an MD, specialized in public health and in microbiology and virology. He is full professor of Microbiology and Clinical Microbiology in the Department of Biomedical, Surgical and Dental Sciences of the University of Milano and Adjunct Professor in the Department of Neuroscience at Temple University, Philadelphia.

He has been working on neurovirology for more than 40 years and is one of the world leader in the study of the pathogenesis of the human polyomaviruses, with particular attention to their interaction with the central nervous system. Prof. Ferrante is the chief of the Laboratory of Translational Research at the University of Milano. The laboratory applies the most advanced molecular techniques for the research and the molecular characterization of the viral strains in the clinical specimens collected from patients affected by demyelinating diseases of the central nervous system. He published more than 250 papers on international peer-reviewed journals, receiving about 4000 citations.

Antonio Carlos Gerbase is a Senior Consultant in Sexually Transmitted Infections (STI), HIV/AIDS, and Global Health.

He worked for WHO from 1994 to 2013 as Medical Officer in the Department of HIV/AIDS in functions such as program manager, prevention, partnerships, country support, key populations, and prevention of mother to child transmission of HIV.

Born in Brazil, he also holds Italian and Swiss nationality. He worked from 1977 to 1991 as Public Health MD at the Health Department, Rio Grande do Sul, Brazil, having worked with people affected by leprosy, HIV/AIDS, and STIs. At the national Brazilian level, from 1979 to 1991, he contributed to the establishment of the Brazilian National HIV/AIDS/STI program as a member of the National Commission for AIDS control. He worked with WHO as a consultant in South and Central America from 1986 to 1993. Dr. Gerbase studied medicine at the Federal University of Rio Grande do Sul, Brazil (1974) and became a Resident in Dermatology (1976) before pursuing a Mastership in Clinical Medicine (1989). In 1991, he was granted a Fellowship in Public Health at the Johns Hopkins University, Baltimore, USA, School of Hygiene and Public Health. He has also been active as a plastic artist from 2010.

Lorenzo Giacani earned a B.S. in Molecular Biology cum laude at the University of Bologna in 1998. His interest in STIs and syphilis began after being drafted into the Italian Army after graduation, where he was tasked with administering aptitude tests to soldiers and obtaining serum samples for assessment of syphilis infection. At the end of his service, Dr. Giacani enrolled in a PhD program in Medical Biotechnology also at the University of Bologna, which he obtained in 2005. As a graduate student, Dr. Giacani focused primarily on the study of the virulence factors of the syphilis agent, developing an ongoing interest for this disease. In 2002, he accepted a position as an exchange scholar and moved to the University of Washington, in Seattle (WA), USA, where he also stayed for his postdoctoral training. Dr. Giacani is currently an Associate Professor at the University of Washington, in the Departments of Medicine and Global Health, where he keeps studying syphilis pathogenesis and evaluating vaccine candidates for syphilis. Throughout his career, he has received the ASTDA Developmental Award and the Vallesina Award for his scientific achievements.

Cristina Giambi is a medical doctor, epidemiologist, and public health specialist, working in the field of vaccination and communicable disease control since 2004. She is working in the Infectious Diseases Department at the Istituto Superiore di Sanità, the national public health institute in Italy since 2008. Her interest in epidemiology and public health has led her to complete a PhD in Methodologies in Preventive Medicine and Therapy (2006–2009) and the European Programme for Intervention Epidemiology Training (EPIET) (2011–2013). She has participated in numerous national and international research projects in the field of vaccination programs, with a focus on vaccine hesitancy and HPV vaccination. Among several activities, she is involved in the Italian surveillance system for congenital rubella and rubella in pregnancy and has been the project manager for the VENICE (Vaccine European New Integrated Collaboration Effort) project that supports vaccine programs in EU/EEA countries. Currently, she is working in the Prevention Department of the Local Public Health Unit in Latina (Italy), dealing with infectious diseases surveillance, vaccination programs, and public health promotion at the local level. She has published over 40 peer-reviewed journal articles related to her work.

Enrico Girardi graduated in Medicine (1981) at Sapienza University in Rome, Italy, where he also specialized in Internal Medicine (1988) and Medical Statistics (1992). He is presently Director of Clinical Epidemiology Unit and Head of Public Health Activities at the National Institute for Infectious Diseases, Lazzaro Spallanzani, Rome, Italy.

His research interests include epidemiology and control of HIV-associated tuberculosis, tuberculosis in migrants, epidemiology and control of HIV infection, cost-effectiveness of control interventions for HIV infection and tuberculosis, and epidemiology of viral hepatitis. His current projects are focused on the HIV continuum of care, on strategies for HCV elimination in high-risk populations, and on intervention to control HIV-associated tuberculosis.

He served as a member of several Italian national and regional committees on tuberculosis, HIV, and hepatitis C. He also served as consultant to WHO on tuberculosis and HIV and to ECDC on tuberculosis control. He is presently a member of the Italian National AIDS Committee.

Massimo Giuliani graduated in Psychology at the University of Rome "La Sapienza" (1982) and became Clinical Psychologist before pursuing a training in Cognitive Behavioral Sciences and Psychotherapy (1985). Since 1987, he earned governamental and international masters in Epidemiology of the Infectious Diseases, particularly focused on sexually transmitted infections and HIV-1. He has been Temporary Advisor for WHO on projects concerning the Social Aspect of AIDS and Counseling for

HIV prevention. He worked for the Italian Institute of Health (Istituto Superiore di Sanità) as a consultant researcher, and he contributed to the establishment of the National STI Surveillance System in 1992, which he coordinated until the year 2009. He is currently a clinical psychologist and professional counselor in the STI/HIV Unit of the San Gallicano Dermatological Institute (IRCCS) in Rome, Italy, where he is scientific coordinator of the largest Counseling & HIV Testing program (COROH Project) targeted to men who have sex with men (MSM) in Italy. His current research and intervention priorities include management of funded STI prevention projects based on urban point-of-care test and STI behavioral counseling, and of longitudinal cohort studies on the natural history of anal HPV infection in at-risk males. Dr. Giuliani is also a reviewer for various leading biomedical journals in the field of STI and HIV-1, and he is a member of the scientific commission of the Italian Multidisciplinary Society for STI (SIMAST).

Secondo Guaschino graduated in Medicine and Surgery at the University of Pavia in 1973. He obtained his postgraduate degree in 1977. He was an Associate Professor of Obstetrics and Gynaecology from 1983 to 1990 and Full Professor from 1990. From 2011 to 2012, he was Director of the Department of Medical Sciences of the University of Trieste, Italy. From 2012 to 2016, he was Director of the Department of Obstetrics and Gynaecology of the University of Florence at Careggi Hospital. He was past president of the Italian Society of Menopause and Elected President of European Society of Infectious Diseases in Obstetrics and Gynecology (ESIDOG). He was a member of the International Infectious Diseases Society in Obstetrics and Gynecology—USA (I-IDSOG-USA).

Prof. Guaschino's primary focus is to apply gynecological sciences expertise to women's health with a special focus on the prevention, management, and care of infectious diseases of the genital tract. His current interests include contraception, menopause, perinatal medicine, and human reproduction. He published more than 200 papers in scientific journals, and he is the author and coauthor of many chapters of books.

Matthew Hogben received a doctorate in social psychology from the State University of New York, followed by postdoctoral training in the Department of Preventive Medicine and Community Health at Downstate Medical Center in Brooklyn, NY. He is currently the Chief of the Social and Behavioral Research and Evaluation Branch of the Division of STD Prevention at the US Centers for Disease Control and Prevention.

Dr. Hogben's primary focus is to apply social and behavioral science expertise to STD prevention and sexual health problems, especially through translational research and evaluation. His current research priorities include analyses of sex and STD-related behaviors data in nationally representative data, management of STD and HIV in networks through enhanced partner services and

disseminating prevention, and implementation of STD prevention behavioral counseling in primary care.

Dr. Hogben also serves on the editorial boards for *Sexually Transmitted Diseases* and *Sexual Health* and was the Program Definition and Prevention Services track chair for the 2018 STD Prevention Conference in Washington, DC.

Bryan Larsen serves as associate dean for biomedical sciences, Marian University College of Osteopathic Medicine since 2011. Previously, he was dean for university research and biomedical graduate studies at Des Moines University and executive director of the Iowa Center for Translational and Clinical Research at Mercy Medical Center in Des Moines, Iowa.

Throughout his faculty career, which started at Marshall University School of Medicine, he has been a teacher of microbiology and clinical research methods and ethics. His research career, spanning over four decades, has focused on host-microbe interaction in the obstetrical and gynecologic patients, consistently melding laboratory science and clinical research. More than 150 articles and book chapters have resulted from his work, including a microbiology text which was commissioned by the Council of Resident Education for Obstetrics and Gynecology.

Dr. Larsen contributes to the discipline by serving on the editorial boards of *Annals of Clinical and Laboratory Sciences*, *Infectious Diseases in Obstetrics and Gynecology*, and reviews for several clinical and basic science journals. He also serves on NIH special emphasis grant review panels and engages in post-publication peer review through Faculty of 1000 (Medicine). The Government and industries supported his research, and he received the prestigious Johnson and Johnson Focused Giving Award as well as the Beehler Mentor of the Year Award (OMED 2009) and headed the development of an electronic Osteopathic SOAP note.

He has presented his research at numerous national and international meetings, has been a visiting professor in China and Germany, and collaborates with researchers around the globe. He served as chairperson of the Des Moines Hospitals Joint IRB and served on the inaugural board of directors of the John Stoddard Cancer Center, Des Moines, Iowa. He currently serves on the board of the Indiana Institute for Medical Research. Numerous graduate students and medical students have been mentored over the course of his career, and he continues to guide research students in the laboratory.

Alessandra Latini received her Degree in Medicine and Surgery at the University of Rome "La Sapienza" (Italy) in 1993. She is a specialist in Dermatology and Venereology (1997) and in Infectious Diseases (2014). From 2002 she moved to the Dermatologic Infective Department of the San Gallicano Hospital at the Sexually Transmitted Infections (STI)/HIV Unit where she started to work in prevention, diagnosis, and STI and HIV infection. Her professional interests focused on the management of bacterial and viral STI and HIV-1 infection. Her daily work is directed to clinical management of HIV patients on combined antiretroviral therapy (cART). In addition her area of interest is focused on the management of co-infected patients, in particular those with HPV, HHV8, and Chlamydia trachomatis infections. She is a member of the International Union against Sexually Transmitted Infections (IUSTI).

Jami Leichliter is the deputy associate director for science in the Division of STD Prevention at the Centers for Disease Control and Prevention. She received her PhD in applied experimental psychology from Southern Illinois University with a specialization in measurement and evaluation in public health. Prior to joining CDC in 1999, she was an associate scientist for Southern Illinois University Student Health Programs. Her current research activities include evaluating STD-related laws and policies, access to and use of sexual and reproductive health services, the infrastructure for STD prevention in the United States, and behavioral surveillance.

Valentina Marchese received her degree in Medicine and Surgery in 2009 at the Second University of Naples, Naples (Italy), and obtained her postgraduate degree in Infectious Diseases in 2015 from the same academic institute. She is currently a PhD candidate at the University of Brescia and is working as a consultant physician in the University Department of Infectious and Tropical Diseases at the ASST Spedali Civili in Brescia (Italy). Previously, she worked as an Infectious Diseases consultant for newly arrived migrants in Sicily (Lampedusa and Trapani) and at the Centre for Tropical Diseases, Sacro Cuore-Don Calabria Hospital, Negrar, Verona (Italy). Her professional interests focus on tuberculosis, STIs, and tropical diseases. Her current projects include screening activities for STIs, parasites, and tuberculosis among migrants.

Alberto Matteelli received his Degree in Medicine and Surgery from the University of Pavia (Italy) in 1985 and in the same Academic Institute he received the Postgraduate qualification in Infectious Diseases in 1989. He is currently Associate Professor in Infectious Diseases at the University of Brescia (Italy). His professional interests focus on TB-HIV infections and sexually transmitted infections (STI). He is the director of the Units of Community Infections and Imported Disease of the Spedali Civili General Hospital in Brescia (Italy). Since 2010 he is the director of the WHO Collaborating Centre for TB/HIV and TB elimination.

Between 2013 and 2015, Prof. Matteelli has been the Medical Officer of the TB/HIV and Community Engagement Unit, Global Tuberculosis Programme at the World Health Organization (WHO). In 2016 he joined the Technical Advisory Group on Tuberculosis Control (TAG-TB) of the European Region of WHO, collaborating in the preparation of the "Management of latent tuberculosis infection for people living with HIV, household contacts and other at-risk populations: consolidated guidelines." He has been WHO Consultant (TB/HIV) of many countries with high TB burden, like Uzbekistan, Belarus, Ukraine, Tanzania, Azerbaijan, Kyrgyzstan, and Russian Federation. Prof. Matteelli has also been coordinator of the Sexually Transmitted Infections (STI) working group of the Italian Society of Infectious Diseases. He is a member of many internationally accredited scientific organizations: committee of the Interdisciplinary Society of Sexually Transmitted Diseases (SIMaST), Italian Society of Medical Parasitology, International Society of Travel Medicine (ISTM), and Italian Society of Tropical Medicine (SIMET).

Aldo Morrone is a dermatologist with documented expertise in tropical pathologies and poverty-related diseases. In the last 30 years, he has developed transcultural medicine and helped focus the attention of public and institutions to the health of migrants and the groups at risk of exclusion. From 2018 he is Scientific Director of San Gallicano Dermatological Institute of Rome, Italy. He is Scientific Director of the International Institute of Medical Anthropological and Social Sciences (IISMAS), a nonprofit association working in medical, scientific, and formative projects for the most disadvantaged groups in developing countries. He is the founder of the *International Journal of Migration and Transcultural Medicine*. He is the coordinator and head of several clinical and scientific missions in Africa, India, Latin America, and South-eastern Asia. Prof. Morrone is the author of over 500 publications in national and international scientific journals, original articles, clinical and scientific research studies, epidemiological reports, and abstracts presented at national and international congresses. He is the Scientific Director of the annual International Workshop "Culture, Health and Migration" held at the National Council for Research in Rome, and of three International Congresses on Dermatology and Infections in Ethiopia, in collaboration with Tigray Ministry of Health.

Massimo Puoti was born in 1959; he graduated in Medicine in 1983 at the "Sapienza" University in Rome. He completed his first Specialization Course in Gastroenterology and Endoscopy in 1987 at the same university, and then took a second Specialization in Infectious Diseases in 1993 at the University of Brescia, where he worked as Head of the Viral Hepatitis Department.

He moved to Milan in 2010, when he became Head of the Infectious Disease Department at the ASST Grande Ospedale Metropolitano Niguarda. He is a member of the multidisciplinary Niguarda Transplant Center for the management of solid organ transplantation. He founded the "Niguarda Hepatitis Center," a multidisciplinary department for the management of viral liver disease including ID specialists, gastroenterologists, and transplant surgeons.

He is a member of several national and international scientific societies, including the European Association for the Study of the Liver (EASL) for which he has been in the steering committee of the European Guidelines for the treatment of HCV. The European Medicine Agency has named him Infectious Diseases European Expert since 2008. He is the author of more than 270 scientific papers with a cumulative Impact Factor above 750. His publications published since 1996 have been cited more than 7958 times with a Hirsch Index of 42.

Virginia Quaresima received her MSc in Medical Biotechnology from the University of Modena and Reggio Emilia (Italy) and is currently a PhD candidate at the University of Brescia—Clinical Department of Tropical and Infectious Diseases (Italy). Her professional interests focus on diagnostics in low- to middle-income countries, and her current projects are specifically regarding malaria and tuberculosis. From 2018 Dr. Quaresima has been working on a study concerning Malaria and Gender in Ghana and is actively collaborating with the Emerging Bacterial Pathogens Unit (EBPU)—WHO Collaborating Centre in Tuberculosis Laboratory Strengthening, San Raffaele Scientific Institute (Milan, Italy). In addition, she serves as Laboratory Manager for the international humanitarian NGO "*Médicins sans frontières* (MSF)," in implementing and strengthening the diagnosis of tuberculosis in South Sudan (2016) and in Papua New Guinea (2017). She has also contributed to the scientific organization of the first edition of the ESCMID postgraduate course in "Migration Health," held in October 2017.

Stefano Ramoni graduated in Medicine in 2003 and obtained his Specialization in Dermatology and Venereology at the University of Milan in 2007 with a thesis about the epidemiology of sexually transmitted infections (STIs). Since then he has been working at the Clinic of Dermatology of the University of Milan in the field of STIs and as a dermatologist in private practice. He published a lot of papers in scientific journals, mainly in the field of sexually transmitted infections, and he collaborated on the drafting of some chapters of books about dermatology and STIs. Dr. Ramoni has been invited speaker in several STI Conference, and in 2016 he was called as member of the International Scientific Committee of the 30th IUSTI European Congress held in Budapest.

He is a member of many scientific societies: SIDEMAST (Italian Society of Dermatology and Sexually Transmitted Infections), IUSTI (International Union against Sexually Transmitted Infections), and SIMAST (Italian Interdisciplinary Society for Sexually Transmitted Infections).

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He has worked for the Italian Ministry of Health, the World Health Organization in Geneva, the Italian Cooperation, and the European Union. He has been a contract professor at the University of Sassari and ran courses at the University of Roma ("La Sapienza" and "Tor Vergata"). He has been Chairman of the Administration Board—and he is now President of the Scientific Board—of the Consortium called "Collezione Nazionale dei Composti Chimici e Centro Screening" (CNCCS), a public/private initiative including ISS, National Research Council, and IRBM. He served as a member of several national and international committees and is currently a member of the Italian NITAG.

He has carried out epidemiological investigations in Italy and abroad, regarding both community and hospital outbreaks. He has been the principal investigator of several research projects and is the author of more than 400 articles indexed in PubMed and 4 books.

Finally, he is the editor of the "infectious disease epidemiology" section of BMC Public Health, is a member of the editorial board of *AIDS* for a 6 years period, editor of the supplement of *AIDS* for 2 years, and is acting as a reviewer for several international scientific journals.

Benedetta Rossi graduated in Medicine at the University of Brescia in 2016. In collaboration with the Department of Infectious Diseases of Brescia, in 2018 she has worked on a research project for the implementation of End TB Strategy in Italy, aiming at reducing the MDR-TB burden in the country. She is now working as a resident in Infectious Diseases at the Policlinico Tor Vergata-Rome.

Roberto Rossotti was born in 1979; he graduated in Medicine in 2004 at the University of Milan and took his Specialization in Infectious Diseases in the same university in 2008. He was Head of the HIV Clinical Trial Unit at the "A. Manzoni" Hospital in Lecco from 2008 to 2010; in 2011 he moved to the Infectious Diseases Department of the ASST Grande Ospedale Metropolitano Niguarda in Milan. Since then, he has worked in the Outpatients HIV clinic and has been involved as sub-investigator in several Phase II-III-IV Clinical Trials, especially for the development of anti-HCV direct-acting agents. He also joined the "Niguarda Hepatitis Center," a multidisciplinary Department for the management of viral liver disease, with the task of treating HIV/HCV co-infected patients.

In 2018 he created the STI Clinic not only for the treatment of sexually transmitted infections but also for the management of HIV pre- and post-exposure prophylaxis with a special focus on the MSM population.

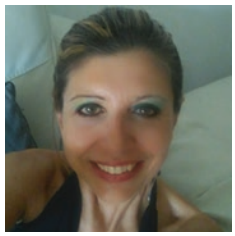
He is a member of the steering committee of ICONA, the largest Italian cohort of HIV patients. He is the author of more than 50 publications on HIV and viral hepatitis.

Vittorio Sambri was born in Modena on May 22, 1960, and graduated summa cum laude in Medicine at the Alma Mater Studiorum, the University of Bologna, in 1986. In 1988 he attended the Laboratory of the ID Division, the University of California at Los Angeles, working under the supervision of Michael A. Lovett on genetic transformation of spirochetes. He received his PhD in Microbiology from the University of Genoa in 1991, discussing a thesis on the microbiology of spirochetal infection. Presently he holds a position as Associate Professor of Clinical Microbiology at the University of Bologna and Director of the Unit of Clinical Microbiology, Greater Romagna Central Laboratory, in Cesena, Italy. Dr. Sambri has been recently involved in the research on emerging vector-borne infections and on the molecular diagnosis of bacterial infections. He also serves as vice-president of APSI (Association for the Study and Prevention of Infections). His work resulted in 200 scientific publications in international journals with an h-index of 42 and more than 5700 citations. Dr. Sambri is a reviewer for many journals, including

Clinical Infectious Diseases, *the Journal of Infectious Diseases*, *the Journal of Medical Microbiology*, *PLoSONE*, *PLoSNTD*, *Clinical Microbiology and Infections*, *Journal of Clinical Microbiology*, and *the Journal of Infection*.

Loredana Sarmati was born in Rome, Italy, on August 13th, 1957, is married, and has three sons. She is a medical doctor specialist in Internal Medicine (1988) and in Infectious Diseases (1992). Since 2012, Loredana Sarmati is an Associate Professor of Infectious Diseases at Tor Vergata University of Rome with teaching and research activities. She has carried out research in the field of epidemiology, clinical diagnostics, and laboratory infectious diseases with regard to viral (HIV, herpesvirus, and HHV8) and fungal (*Pneumocystis jirovecii*) infections in the immunocompromised host. The results of her scientific activity have been the object of more than 150 PubMed indexed articles. From June 2018, she is the president of the Lazio section of the Italian Society of Infectious Diseases.

Lawrence Stanberry is the Associate Dean for International Programs and Director of the Global Health Initiative at Columbia University's Vagelos College of Physicians and Surgeons. During the last decade, he served as the Reuben S. Carpentier Professor and Chairman of the Department of Pediatrics and Pediatrician-in-Chief of the NewYork-Presbyterian/Morgan Stanley Children's Hospital. He has served on numerous advisory boards and review panels including serving as the chair of the Vaccine Study Section and the Pediatrics Review Panel at the National Institutes of Health. He is a pioneer in the areas of therapeutic vaccine development to control chronic viral diseases, topical microbicides/pre-exposure prophylaxis for the prevention of reproductive tract infections, and the development of herpes simplex virus and influenza virus vaccines. Dr. Stanberry has authored over 200 scientific articles and chapters. His current work focuses on the preparedness of children's hospitals globally to prevent, detect, and respond to infections with pandemic potential.



Paola Stefanelli is the Head of Vaccine Preventable Diseases (VPD) Reference Labs Unit at Istituto Superiore di Sanità and the Head of the WHO Collaborative Centre for Polio. She coordinates national and international level surveillance activities together with research programs on important pathogens, such as *N. meningitidis*, *N. gonorrhea*, *B. pertussis*, *Polio* and *Enterovirus* no-polio associated. Her studies have the objective of translating the insights obtained into benefits for human health. Microbiological data are combined with demographic and clinical information of patients. Dynamic modeling and phylogenetic investigations on genomic analysis of RNA

and DNA are in place. Her interest is focused on understanding and evaluating the impact and duration of vaccination of VPD using ELISA, SBA, and Ab titer on cell lines. Head of VPD-Reference Labs Unit, Department Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

Christof Stingone was born in Rome on 26 February 1986, graduated from the University of Rome La Sapienza on 24 September 2014 with a Bachelor of Medicine and Surgery, and obtained his medical license to practice on 5 February 2015.

His medical interest has been focused on infectious diseases since the beginning of his studies, and he now works as Resident Doctor attending the Postgraduate Training in the Infectious Diseases ward and clinic at Tor Vergata University Hospital.

His main activities include working in HIV clinic, Sexually Transmitted Infections clinic, Pre-Exposure Prophylaxis clinic, and Infectious Diseases Ward. In 2018, during a training period in London, he attended 56 Dean Street Clinic (Chelsea and Westminster Hospital) in Soho, where he focused on High Resolution Anoscopy Clinic and STD Clinic, studying the use of PrEP among those practicing Chemsex. He also attended the HIV ward at the Royal Free Hospital Foundation Trust, studying the phenomenon of HCV re-infection among MSM individuals.

Massimo Tommasino is Head of the Infections Section and of the Infections and Cancer Biology Group at the International Agency for Research on Cancer (IARC) in Lyon, France. He obtained in 1981 his Doctorate in Pharmacy at the University of Bari, Italy, and has been working for more than 30 years on oncogenic viruses in different European leading cancer research institutes, i.e., ICRF, London (recently renamed CR-UK), DKFZ, Heidelberg, and IARC. Massimo Tommasino is involved, as coordinator or participant, in several international collaborative programs focused on the elucidation of the role of infectious agents in human carcinogenesis. He is the author of more than 200 papers.

Martí Vall-Mayans received his MD and PhD degrees from the Autonomous University of Barcelona and MPH degree from the University of Texas. He has been working in the field of infectious diseases for 40 years, the last 20 years in STI, and is currently Faculty Specialist at the STI Unit of the Department of Infectious Diseases, Hospital Vall d'Hebron in Barcelona.

His professional interests focus on the clinical and epidemiological aspects of STI, and his current projects include research on syphilis and chlamydia including LGV. He also collaborates abroad in an intervention project about nonvenereal treponematosis.

In addition, he serves as a member of the editorial board of the STI Guidelines of the International Union against Sexually Transmitted Infections (IUSTI), is a member of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), and was honored with the Award Virgilio Palacio for STD research on LGV in 2009 and the IUSTI-Europe Medal of Merit for his contribution to the organization of the European STI congress in 2015.

Antonio Volpi is "Docens Turris Virgatae" at the University of Rome Tor Vergata, Italy, and Professor of Infectious Diseases at the Catholic University "Our Lady of Good Counsel," Tirana, Albania. He earned his Degree in Medicine and Surgery (1969–1975) from the University of Rome and was trained as a Specialist in Infectious Diseases (1975–1978) from the same university.

Previously, he served as President of Hospital Infection Committee and Head of Infectious Diseases Clinic at Policlinico Tor Vergata Rome, Associate Professor of Infectious Diseases, Deputy Director of Scuola Iad at the University of Rome "Tor Vergata" Project officer EPI/Health UNICEF Ethiopia, fellow at the University of Alabama in Birmingham, USA, and Tutor at National Somali University, Mogadishu.

He has served on numerous advisory boards and review panels. He has received research funding from the EU, Italian government, vaccine and pharmaceutical companies. He is vice-president of SIMAST and a member of SIMIT. Professor Volpi has authored over 200 scientific articles, abstracts, and chapters. He has been the editor or co-editor of *Herpes,* the journal of IHMF, and the book *Genital Herpes*. His areas of research include diagnosis and therapy of herpesviruses infection, basic studies of the pathogenesis and immunobiology of HSV and VZV, and the special problems of healthcare-associated infections.

Nunzia Zanotta received her degree in Biology from the University of Trieste, Italy, and is currently a researcher in the Advanced and Translational Diagnostic Microbiology Department at IRCCS Burlo Garofolo, Trieste, Italy.

Her professional interests focus on tumor viruses and their relationship with host innate immune response. Her current projects include sexually transmitted microorganisms, vaginal microbiome, and the study of interactions between the host innate immune response and sexually transmitted pathogens. She is the author of 30 papers in the field of molecular microbiology.

Charifa Zemouri received her Master's in Infectious Diseases and Public Health in 2013 at the Vrije Universiteit in Amsterdam. Zemouri will finalize her PhD (2020) on microbial challenges in dentistry focusing on the ecology of biofilms, aerosols, and the oral cavity at the ACTA. Her work takes place in clinics, laboratory, and desk research. Her professional interest focuses on microbial challenges in public health, reproductive health, poverty-related diseases, politics, and human migration and human right. Zemouri worked as a researcher for several institutes. Zemouri has previously written medical book and published peer-reviewed papers. She contributes to research work relating STIs and tuberculosis and STI point-of-care test with members of the WHO. Zemouri is also an expert panel member at the International Life Sciences Institute in Brussels and an external auditor and consultant to the Kyiv Medical University in Ukraine. Furthermore, Zemouri is a volunteer journalist writing on health and politics in the Middle East and North Africa region for Morocco World News. She loves to continue her career combining science and international public health in the diplomatic field.

Gabriella Zito graduated in Medicine and Surgery at the University of Trieste. She specialized in Gynecology and Obstetrics, and in 2017 she obtained the European Doctorate in Reproductive and Developmental Sciences, carrying out a research project in collaboration with the Instituto Valenciano de Infertilidad (IVI), en Barcelona (Spain). She works at IRCCS Burlo Garofalo Maternal and Child Institute in Trieste as reproductive gynecologist, and she also deals with oncofertility. Her research areas include infertility, gynecological endocrinology, and oncology.

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Part I

Global Epidemiology

Massimo Giuliani

Enhancing our knowledge concerning the global distribution of a disease and their determinants across populations and core groups represents the cornerstone of any effective multi-level control strategy. This is particularly true for STI and HIV infection, which in the beginning of the Third Millennium are becoming among the most common communicable diseases worldwide. According to the latest estimate by WHO, nearly one million people become infected every day by one of the four major curable sexually transmitted infections (STIs), such as trichomoniasis, Chlamydia, gonorrhoea and syphilis. Moreover, almost 400 million people live worldwide with genital herpes, 290 million with HPV infection and over 36 million with HIV infection.

With these reasons in mind, this part focuses on the epidemiology of STI and HIV infection, and particularly on the methodological issues that can better describe the dynamics of diffusion of the different infections according to the criteria of the "where", "when" and "who".

Antonio Gerbase will guide the reader through the modern methods of descriptive epidemiology applied to the global surveillance of STI and will go beyond the simple concept of numbers and explore the extent of burden. It was precisely some epidemiologists who, at the beginning of the 1980s, noticing an excess of opportunistic pneumonia in young immunosuppressed males, revealed the presence of a new severe disease, such as HIV infection. From these facts, Enrico Girardi started to structure an overview on the modern epidemiology of HIV-1 infection, which, for more than three decades, has largely transformed the methods of surveillance and investigation of all the other infectious diseases.

It is well known that it is the synergistic relationship between individual, environmental and epidemiological factors that play a relevant role in the spread of STI and HIV infection in a defined social context. On this matter, Matthew Hogben, Jami Leichliter and Sevgi Aral have drawn up a comprehensive chapter on the socio-behavioural determinants of STIs where many data and interesting insights will be critically brought to our attention.

1 Global Epidemiology of Sexually Transmitted Infections in the Twenty-First Century: Beyond the Numbers

Antonio Carlos Gerbase and Charifa Zemouri

1.1 Introduction

Present since the dawn of humanity sexually transmitted infections (STIs) continue to pose a challenge for individuals, societies, and public health authorities. It is well known that STIs are present in all populations, but their extension and impact vary according to vulnerability, prevention interventions, individual behaviour, and access to health services. In a recent policy brief, the World Health Organization (WHO) states, "Their impact of morbidity and mortality worldwide resulting from sexually, genitally and extragenitally transmitted pathogens compromises quality of life, as well as sexual and reproductive health, and new born and child health. Moreover, it is widely recognized that STIs facilitate sexual transmission of HIV and trigger some cancers that are common across the world. Both oral and anal sexual exposure, involving bacteria, viruses and protozoa, can cause significant morbidity" [1]. The new "WHO Global health sector strategy on Sexual transmitted infections, 2016–2021. Towards Ending STIs—WHO, 2016" [2] aims to "contribute to a radical decline in new sexually transmitted infections and in deaths related to such infections (including still births and cervical cancer), while improving individual health, men's and women's sexual health, and the well-being of all people". This will be soon reflected in the objectives of any local, national, or regional public health intervention. Quantitative targets are appealing and necessary to modern public health interventions, which should be measurable and show the value of the time and funds invested in a specific issue.

The global goal is to end STI infection epidemics as major public health concerns in 2030. The ambitious targets are:

- 90% reduction of *Treponema pallidum* incidence globally (2018 global baseline).
- 90% reduction in *Neisseria gonorrhoea* incidence globally (2018 global baseline).
- ≤50 cases of congenital syphilis per 100,000 live births in 80% of countries
- Sustain 90% national coverage and at least 80% in every district (or equivalent administrative unit) in countries with the human papillomavirus vaccine in their national immunization programme.

The global STI estimates, based on consistent methodology, are issued regularly and have been used for advocacy purposes since the end of the 1990s. The present ones [3] state that "it is

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estimated that annually there are 357 million new cases of fourcurable sexually transmitted infections among people aged 15–49 years (see Fig. 1.2): *Chlamydia trachomatis* (131 million), *N. gonorrhoeae* (78 million), syphilis (6 million), or *Trichomonas vaginalis* (142 million). The prevalence of some viral sexually transmitted infections is similarly high, with an estimated 417 million people infected with herpes simplex type 2 (HSV-2), and approximately 291 million women harbouring the human papillomavirus (HPV). The prevalence of these STIs varies by region and gender".

Estimates, which try to reflect the reality, are based on statistics generated at local and national level. This needs to be well understood. Health authorities will lack insights on the magnitude of the STI epidemics when STI surveillance systems are poor. The data occurring from the surveillance systems inform on incidence, prevalence, STI syndromes' aetiology, and resistance patterns and estimates. In this circumstance, at any level, when quality data is not available, the STI numbers will be inaccurate and low reliable. In general, STI reporting is usually very poor in any health system. The exact magnitude of the STIs burden is frequently unknown. Although passive STI surveillance systems exist in some countries, the data is not always reliable and complete. Due to stigma and discrimination form many health care providers, people seek care from alternative providers or do not seek care at all. All these cases are not reported. Furthermore there is lack of case finding and screening leading to the absence of diagnosis and reporting. STIs are frequently asymptomatic and any reporting systems based on STI syndromes cases heavily underestimate the total number of new infections.

1.2 Objectives and Components of STI Surveillance

Epidemiological surveillance is the ongoing, systematic collection, analysis, interpretation, and dissemination of data regarding a health-related event for use in public health action to reduce morbidity and mortality, and to improve health. Continuous collection of STI incidence and prevalence rates are important for understanding the epidemiology, trends, and monitoring for interventions (success or fail). The retrieved data can be used to follow emerging diseases, trends within a specific population, success or failure of applied interventions such as condom programmes. But also, to know the magnitude of all STIs, which includes the proportion of the population infected (prevalence), the number of new cases per year (incidence), the aetiology of the different STI syndromes and the patterns of antimicrobial resistance. Besides reporting on incidence and prevalence, periodically determining aetiologies of STI syndromes are necessary together with AMR monitoring to inform treatment recommendations, improve treatment and patient care. With prevalence and incidence data STI estimates, for advocacy and planning purposes, can be developed. The core components of the STI surveillance are visualized in Fig. 1.1.

The objectives of the STI surveillance are [4]:

- Measure the magnitude of STI problem in populations
- Assist in programme planning
- Monitor trends over time and identify emerging infections
- Define needed resources
- Provide data to advocate mobilization of resources for intervention activities
- Guide implementation of appropriate intervention measures
- Assist in valuation of effectiveness of the response

1.3 Case Reporting: Incidence

Case reporting is the source to calculate STI incidence (number of new cases of disease occurring in a population during a defined time interval). Case reporting is the most common attempt to measure STI magnitude. Cases are reported from health services or laboratories to public health authorities. The type of information provided depends on how health services diagnose STIs

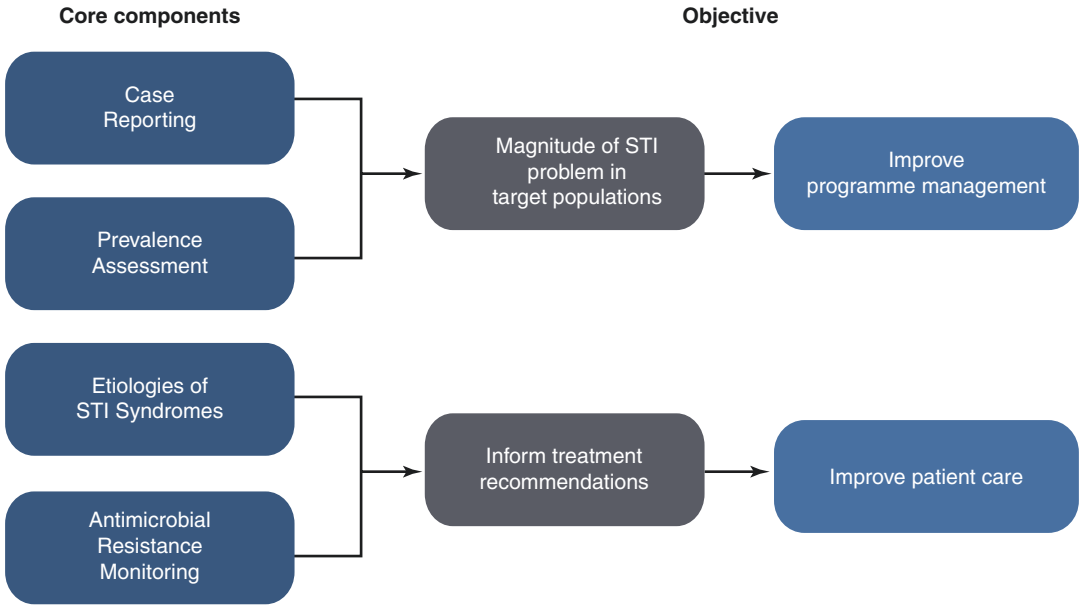

Fig. 1.1 Core components and objectives of STI surveillance. Source: Strategies and laboratory methods for strengthening surveillance of sexually transmitted infection 2012. Geneva: WHO; 2012

and how the national surveillance systems are organized. In the 2014 evaluation of the WHO STI strategy it was recognized that STI case reporting systems are not uniform. Even in the European Union (EU) area data is very difficult to compare due to inconsistent reporting between countries. Stable information systems and sources result in a more reliable tool to assess incidence, trends, and inform planning. Unfortunately, underreporting is occurring consistently. Gender based factors such as stigma, fear for STI testing, masculinity, or little resources for STI testing, and also the perception of low risk to STIs are linked to underreporting.

Most case reporting data is not published in peer review literature and must be obtained in a country-by-country basis. The EU and Central Asian countries report to European Centre for Disease prevention and Control (ECDC), which regularly produces reports, For the reasons above are difficult to make sense based on these reports. Recently the WHO started to build a Global database on reported STIs [5] to follow up trends, inform estimates, and stimulate countries to improve STI case reporting and STI surveillance in general. Core data elements should include diagnosis, reporting site, date of visit, gender, age group, and age or date of birth. Additional data elements, when possible, are residence, education or socio-economic status, syndrome (for etiologic reporting), anatomic site of infection, date of symptom onset, behaviour elements, pregnancy status, history of STI, and previous or ongoing treatment.

Studies have also described men's structural or sociocultural barriers to sexual healthcare services, such as their fear of STI testing procedures, for example, a genital examination or a urethral swab.

According to the UNAIDS/WHO Guidelines for Sexually Transmitted Infections Surveillance [6], case reporting has several purposes and uses:

- Assess disease burden by providing an indicator of minimum incidence of recently acquired infections
- Monitor trends in incidence of recently acquired infections.
- Provide information required for management of patients and their sex partners
- Provide information on which providers in the health care system are diagnosing and report-

ing the major STIs, to assist in planning and managing programme efforts
- Provide other data necessary for managing health services (e.g., pharmaceutical
- distribution)

STIs may be reported either syndromic (based on de most common STI syndromes, mainly urethral and vaginal discharge and genital ulcer) or etiologic (based on the aetiological agent, e.g., *N. gonorrhoea, T. pallidum*), depending on the availability of laboratory tests in clinical care settings. In most developing countries, syndromic case reporting is the only option. Syndromic case reports require no laboratory diagnostic tests. When using syndromic reports, no assessment of asymptomatic STIs is available. The amount of information collected is variable. As the trend is to have integrated national surveillance systems minimal data is usually available. However, these data sets are the most frequently available at country levels compared with the other STI surveillance elements. Etiologic case reporting requires diagnosis based on laboratory testing. An advantage of etiologic case reporting is that specificity for STI agents is high, providing a highly credible assessment of the minimum disease burden and facilitating efforts at counselling and treating.

Figure 1.2 describes the trends of urethral discharge and genital ulcer syndromes in the Midlands province Zimbabwe, 2001 to 2016. The decrease on reporting in 2005 is linked to the fact that at this year charges for consultation were introduced and attendance felt accordingly. Many are the factors influencing reporting and potential bias influencing trends must be taken into account.

1.4 Prevalence Assessment

Measuring STI prevalence (proportion of persons in determined population who have a STI at a specified point in time) is together with case reporting fundamental to construct a reliable vision of the STI situation in a specific country or

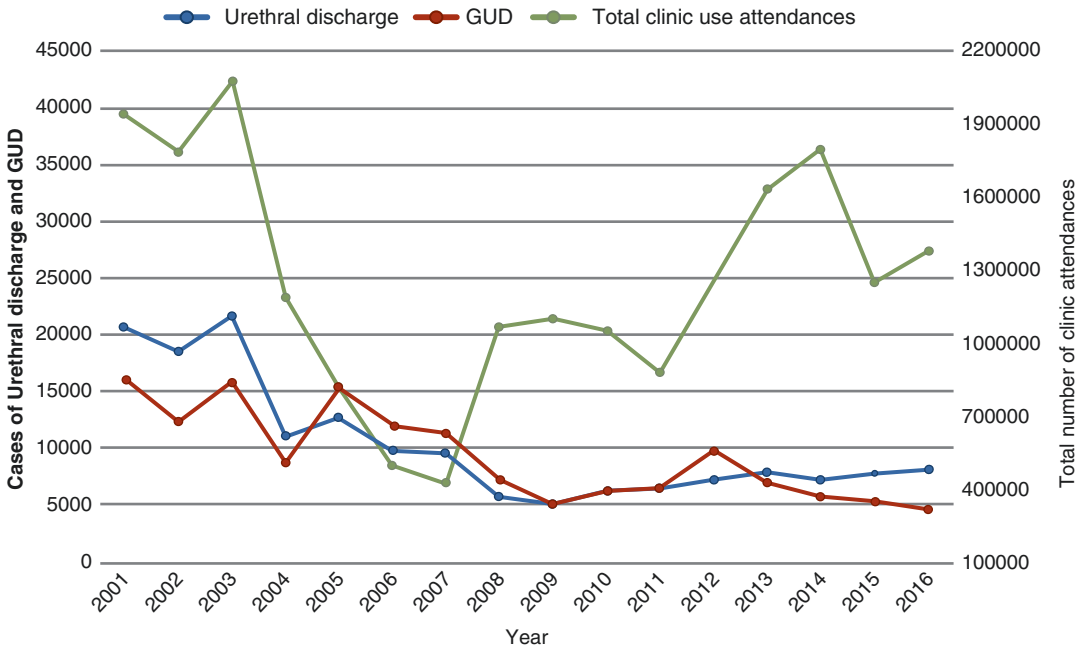

Fig. 1.2 Urethral discharge and GUD syndromes, Midlands Province, Zimbabwe, 2001–2016 (Courtesy Francis Ndowa)

area. Prevalence data are the main input for the development of global and national estimates of STIs. The determination of prevalence among persons screened in is called prevalence assessment. Prevalence monitoring is the monitoring of trends in prevalence over time. The objectives of STI prevalence assessment and monitoring are to identify population subgroups with high prevalence of STIs and monitoring its trends. This permits to guide funding and resource allocation and to monitor effectiveness of STI and HIV prevention programmes. Populations to be used to assess STI prevalence include pregnant women and selected populations such as men having sex with men, sex workers, and young people. Frequency is continuous for pregnant women, and every 2 or 4 years for other populations.

Asymptomatic patients usually seek services for reasons that are unrelated to STI (for example, family planning clinic clients and women seeking antenatal care). In contrast, prevalence of symptomatic disease (i.e., STI syndromes) in clinical care settings will be heavily biased compared with community disease rates, because these patients are presenting for care. Recent data, using NAAT, suggest that substantially more cases of gonococcal and chlamydia infections in men are asymptomatic than previously thought. Tests that do not require gynaecological or genital examinations can facilitate screening (and prevalence assessment). Urine tests for gonorrhoea and chlamydia based on nucleic acid amplification methods can be used for this purpose, although their cost may limit their use. Because assessment of prevalence necessarily focuses in most settings on diseases that are asymptomatic and persistent, reporting of prevalence based on laboratory diagnosis is necessary. Sample sizes vary according to expected prevalence and usually are situated between 200 and 400 subjects.

Prevalence of STIs that are often asymptomatic (e.g., chlamydia and gonorrhoea in women; syphilis, determined through serologic testing) may provide insight into the disease burden in the population from which those attending the clinic is drawn. Trends in prevalence may be altered substantially by changes in the population being screened because of changes in the characteristics of the clinic, in the population's patterns of health care seeking, or criteria used to select persons for screening. Any such changes should be recorded and taken into account in the interpretation of trend data. Changes in diagnostic tests, which often vary in sensitivity and specificity, in the use of confirmatory tests, and in the type of specimen collected (e.g., endocervical swab versus urine) should also be recorded and considered when interpreting these data.

Figure 1.3 is an example on how syphilis prevalence data was used to produce syphilis estimates in Morocco [7].

Screening a population is done in a cross-sectional set-up providing a point prevalence estimate. Based on previous cross-sectional or longitudinal screening of populations, diseases can be monitored. In the case of syphilis, screening of pregnant women combines prevention of mother to child transmission of *T. pallidum*, treatment of pregnant women and their partners and continuous generation of prevalence data. This is done strictly in ANC settings. Table 1.1 presents the number of reporting countries per WHO region and the median seroprevalence. Screening in this case works as a preventive method for transmission. Another potentially important source of syphilis seroprevalence data in many countries is blood donors. These screening data are often available, but STI programmes often do not receive, organize, or report these data. Despite the limitations of these data, they can be especially useful in those countries where it is adequate, those data can be routinely reported to the STI programme. Screening high-risk populations such as female sex workers (FSW) and their clients generate disease prevalence data in specific populations. For example, in China a study found a syphilis prevalence rate of 2.91% versus 0.52% in the general population. Indicating a sixfold higher rate in FSW improves targeted screening programmes and expanding screening in clients

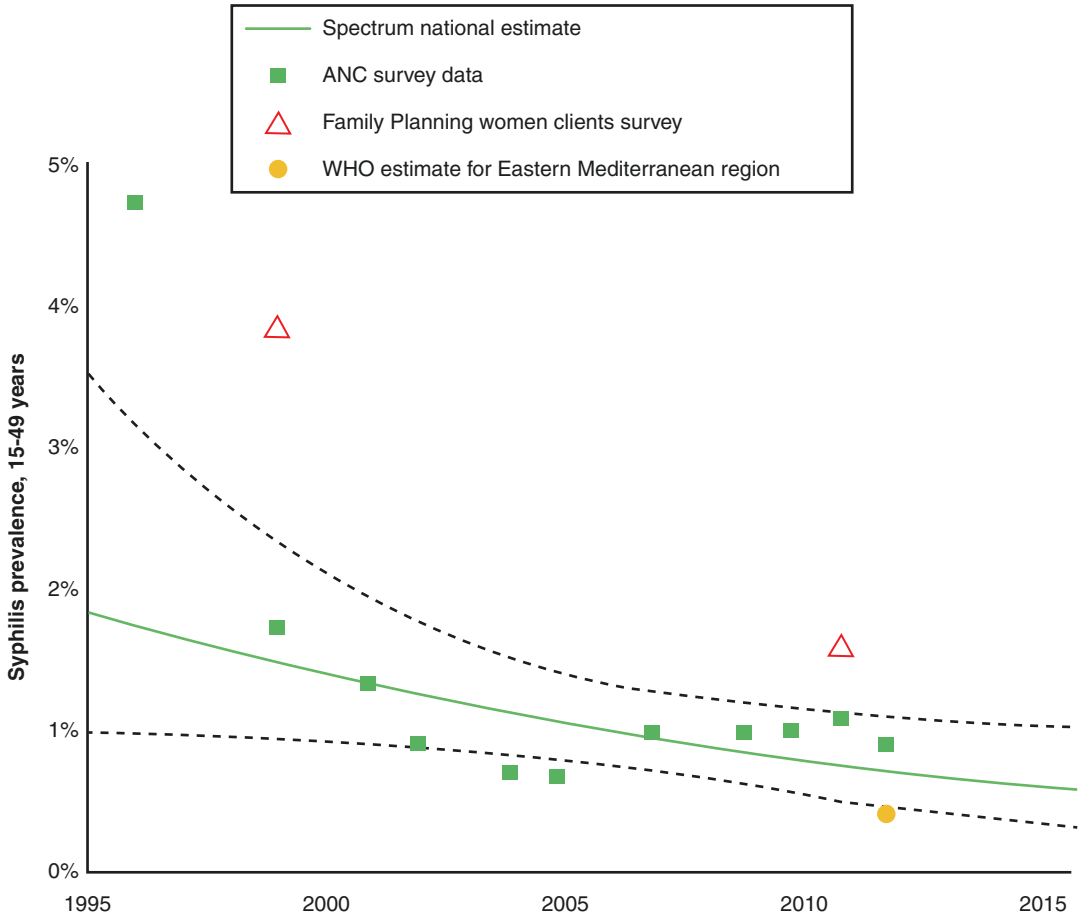

Fig. 1.3 Spectrum-estimated national syphilis prevalence, women 15–49 years, Morocco

Table 1.1 ANC syphilis screening and seroprevalence per WHO region

WHO region	No. countries reporting	Median ANC syphilis seroprevalence (range)
African region	31	1.6% (0–11.3)
Region of the Americans	21	0.4% (0–3.1)
Eastern mediterranean region	4	0.0% (0–1.5)
European region	9	0.1% (0–1%)
South-east asia region	7	0.5% (0.1–1.7)
Western pacific region	13	1.8% (0–13.5)
Overall	85	0.7% (0–13.5)

of FSW and possible transmission to general population. Screening for HIV-facilitators also contribute to the description of the magnitude of disease.

1.5 Aetiologies of STI Syndromes

The assessment of syndrome aetiologies provides data for guiding STI syndromic management, assists in the interpretation of syndromic case reports, and assesses the disease burden due to specific pathogens. [6] Based on research performed in the early 1980s [8] consolidated in

WHO and UNAIDS 1999 recommendations [9], the syndromic approach is applied in settings with low resources or availability of adequate laboratories. As stated in the report "Syndromic case management is based on classifying the main causative agents giving rise to a particular clinical condition (syndrome), such as the syndrome of urethral discharge in men. It then uses flowcharts, which help the health service provider reach a diagnosis and decide on treatment. The treatment covers all the important causes of the syndrome". Presently the assessment of STI syndromes aetiologies is made with multiplex polymerase chain reaction (M-PCR) assay [10].

The aetiologies of each syndrome are not stable in time and studies are needed to periodically assess the etiological agents of each syndrome. With the change of aetiology, treatments change and therefore guidelines must be revised. The sites selected for assessing syndrome aetiologies must have sufficient number of cases available. Sample size depends on the specific aetiology and the expected prevalence of pathogens. Most studies are conducted with a minimum sample size of 50 or 100 specimens from consecutive patients with the specified syndrome. This will provide adequate information for useful analyses. The frequency of the studies does not need to be more than every four years. This gap is technically adequate but frequently leads to lack of sustainability due to concurrent priorities in the STI programmes. For this reason, to keep the momentum, which is not easy to produce, shorter time between studies should be explored.

For example, a recent study conducted among genital ulcer disease (GUD) patients in diverse clinics in Zimbabwe found that most patients were positive for HSV, syphilis and a small proportion (1%) were positive for LGV-associated strains of chlamydia. No chancroid was detected, while in the 1980s GUD was mainly caused by chancroid [10]. In a 2016 meta-analysis regarding vaginal discharge syndrome, the most common agents identified were *Candida albicans*, *T. vaginalis* for vaginal infections, and *C. trachomatis* and *N. gonorrhoeae* for cervical infections [11]. A national etiological study conducted in Poland, using the highly sensitive and specific assay, reported that none of the study sample reporting abnormal vaginal discharge was positive for *T. vaginalis* [12]. The findings are "similar to results from other EU settings" and may indicate that there is no need to treat for *T. vaginalis* as an etiologic agent for abnormal vaginal discharge. It is also interesting the authors' conclusion that "the need for general screening using NAAT for this pathogen while diagnosing vulvovaginal symptoms in females in Poland appears to be low". Therefore, in this study, the assessment of specific syndrome aetiology provided not only elements for a possible syndromic approach to treat vaginal discharge but also to establish screening criteria. The results of these studies also provide insight on the STI syndromic case reporting data, and in particular, for estimating the burden of disease by pathogen.

1.6 Antimicrobial Resistance Monitoring

Management and control of STIs is challenged by the spread and emerging antimicrobial resistance (AMR). Resistance occurs naturally over time through genetic changes. The overuse and misuse of antimicrobials accelerate drug resistance. Resistance seems also to occur as a result from overtreatment by syndromic management. Among the main STIs, resistance occurs mainly in *N. gonorrhoeae* infections with increasing rates worldwide. *N. gonorrhoeae* and *Mycoplasma genitalium* are evolving into superbugs that can become highly resistant to all available antimicrobials. Gonococcal AMR is a hazard to treatment, which can result in disabilities and sequelae. The management and control of gonorrhoea is compromised by widespread AMR in variable strains of *N. gonorrhoeae*. In most countries extended-spectrum cephalosporins ceftriaxone alone, or in combination with azithromycin, is the only effective treatment for gonorrhoea [12].

The objective of monitoring antimicrobial resistance in *N. gonorrhoeae* is to obtain data necessary for developing guidelines for treatment. A second objective is to detect newly emerging resistance. Demographic and risk information obtained through a sentinel system for monitoring antimicrobial resistance in *N. gonorrhoeae* may also be used to further characterize risk factors for resistance and the local epidemiology of this disease. The procedure to monitoring antimicrobial resistance to *N. gonorrhoeae* is to conduct periodic studies collecting samples from men with urethral discharge. A convenient final sample size of 100 untreated men is usually sufficient. The findings can be extrapolated for all population. The site must be capable to perform culture which is further analysed in a laboratory performing susceptibility testing *for N. gonorrhoeae* using inhibitory concentration (MIC) agar dilution testing of antimicrobial agents. Demographic data should be kept at a minimum. When assessing published data (in peer reviewed journal or in country level documents) on *N. gonorrhoeae* AMR particular attention should be given to the following elements in order to assess their quality and, thus, usefulness to change current treatment practices: study site(s), sample size, populations tested, possible selection bias, and laboratory procedures.

Even though the technical procedure is relatively simple, data on *N. gonorrhoeae* AMR is chronically lacking. As the patterns are particular to each country or groups of countries multiple and regular studies are needed. WHO coordinates the global efforts to foster these studies [13] and developed a global plan in which *N. gonorrhoeae* AMR is one of the cornerstones. In 2012 [14] a total of 62 countries participated in the WHO network, only 50 countries had available data for 2009–2010 on ceftriaxone (or cefixime), azithromycin, and quinolones. Data on quinolones were the most widely available data, whereas data on ceftriaxone (or cefixime) were available for 32 countries and on azithromycin for 29 countries.

A meta-analysis of *N. gonorrhoeae* AMR in China [15] provides sound data and concludes on "*N. gonorrhoeae* resistance rates to penicillin, tetracycline and ciprofloxacin were high in China. Ceftriaxone and spectinomycin remained effective therapy for the treatment of gonorrhoea. It is essential to strengthen *N. gonorrhoeae* resistance surveillance and update treatment guidelines timely". The analysis of 127 studies demonstrated prevalence resistance of gonorrhoea to penicillin and tetracycline of 84.2% (79.7; 88.8) and 82.4% (79.9; 84.7) in 2012, respectively. The resistance to ciprofloxacin has increased from 12.7% (8.6; 16.7) in 1995 to 93.8% (91.9; 95.7) in 2003. With this kind of epidemiological surveillance data national treatment guidelines will be adapted to country needs.

In a recent review, Unemo and Safer [16] raise attention to the "suboptimal control and monitoring of antimicrobial resistance" and how AMR compromise treatment effectiveness, concluding that AMR testing must be performed more frequently to inform treatment decisions. Clinicians need to be aware of the current guidelines on diagnostic procedures, treatment regimes, and therapeutic options for multidrug-resistant bacteria. AMR testing must be performed more frequently, inform treatment decisions and how AMRs compromise treatment effectiveness. Increased awareness of gonococcal AMR among professionals, patients, clinicians, and policymakers is therefore crucial

Resistance can be reduced by prevention of transmission of gonorrhoea, partner notification, and correct antibiotic administration. Furthermore, increased detection and treatment of asymptomatic and pharyngeal gonorrhoea are critical since they pose a potential reservoir in which AMR can emerge. The development of novel antimicrobials for gonorrhoea treatment has the highest priority. The new antimicrobials should be made accessible and strategies to conserve these should be implemented. Novel treatment options should be able to reduce the gonorrhoea incidence to 90% by 2030. However, prevention of transmission remains the major global public health priority.

1.7 STI Estimates

The WHO has produced global and regional prevalence and incidence estimates approximately every 5 years since 1995 (1995, 1999, 2005, 2008, 2012) [3, 17–20] for Syphilis, Gonorrhoea (GC), Chlamydia (CT), and Trichomoniasis. The total global estimates of new cases of curable STIs is 357.4 million (2012), with trichomonas and chlamydia on top accounting for 130 and 142 million cases per year [3]. Estimating prevalence includes collecting and standardizing STI data and generating estimates (world divided into ten regions). Incidence is estimated dividing prevalence by average duration of infection. Efforts are being made to increase quality of data, through standard prevalence protocols. Quality and quantity of syphilis data has been improved once efforts were increased to eliminate vertical transmission of HIV and *T. pallidum* [21]. Recently (2016–2018) a new tool, Spectrum-STI national estimation model is being utilized. [22] In a recent WHO meeting [23] challenges were identified. Presently data, especially for men in relation to gonorrhoeae and chlamydia infection are very limited. Many countries do not have data that met estimates entry criteria. STI estimates are not ideal for evaluating trends over time and too few countries/settings have repeat/comparable prevalence measurements. All these elements keep estimation for incidence even more problematic than for prevalence.

1.8 Conclusions

STI surveillance and monitoring has largely improved over the last decades thanks to improved surveillance systems, systematic monitoring, aetiological studies, and targeted prevention and treatment programmes to reduce the burden of disease. Linking STI surveillance with sound intervention may contribute to better surveillance as has been observed with syphilis. However, it still needs improvement since many positive and asymptomatic cases never reach the data charts. Surveillance needs political adherence, funding, and reduced barriers for key populations to seek screening and health care. The integration with national surveillance systems is increasing making universal reporting of the norm, but this is not linked with better quality of data. Only with the implementation of the four components a better idea of STI magnitude can be achieved.

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The New Epidemiology of Human Immunodeficiency Virus Infection

2

Enrico Girardi

2.1 Introduction

The clinical entity, which later became known as Acquired Immunodeficiency Syndrome or AIDS, was first described in 1981 in five young, previously healthy, homosexual men who presented with *Pneumocystis jiroveci* pneumonia in Los Angeles. In the following months, several additional cases of this rare form of pneumonia were reported in the USA in homosexual men, also associated to other opportunistic infections, and Kaposi's sarcoma. The number of cases increased rapidly in the USA and by 1983, 1000 cases were reported. Moreover cases were also reported among female partner of men with AIDS, in infants born to mothers with AIDS, in recipients of blood and blood products, and in injection drug users (IDUs). The etiologic agent was unknown at that time, but a sexually transmitted infection was strongly suspected [1].

In 1984 virologist from France and the USA identified a retrovirus, the Human Immunodeficiency Virus (HIV), as the cause of AIDS. Availability of a serological test to detect HIV infection allowed understanding that the cases of AIDS recorded were just the tip of the iceberg of a much more widespread phenomenon. It also became clear that the AIDS epidemic was a global phenomenon and, in particular, analysis of cases from Haiti and Africa showed that most of them lacked the "classical" risk factors and demonstrated the impact of heterosexual transmission of HIV.

2.2 Evolution of Epidemic

In the following decade, the epidemic evolved with different characteristics in different areas of the world. Men who have sex with men accounted for the majority of cases in the USA, in western Europe, in Australia and New Zealand, in Latin America. In southern Europe most cases were recorded in IDUs, while in Africa heterosexual transmission was identified as the largely predominant mode of spread of the infection, and mother-to-child transmission emerged as a major issue. By the mid-1990s, more than 20 million persons were estimated to be living with HIV, the vast majority in sub-Saharan Africa. A second transmission through blood and blood products was virtually eliminated in high-income countries thank to screening procedures and new methods of processing blood products, while was reduced but still present in low-income countries. A second wave of the epidemic occurred during the 1990s in eastern Europe and south-east Asia and it was mainly due to injecting drug use.

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2.3 Quantifying the Risk of HIV Sexual Transmissions

HIV can be transmitted through sharing unsterile equipment for injecting drugs, through administration of contaminated blood or blood products, and from an infected women to her child during pregnancy, labor, and breast-feeding. However, globally the overwhelming majority of HIV infections are due to sexual transmission.

Persons living with HIV may have replication competent virus in a variety of body fluids including semen, pre-ejaculatory fluid, and vaginal fluid. Transmission may occur when these fluids come in contact with mucous membranes of an uninfected persons in the foreskin and urethra on the penis, cervix and vagina, rectum and anus. Infection is facilitated by breaches of mucosal integrity; however, it may take place also when the mucous membrane is apparently intact. The epithelium of vagina, ectocervix, foreskin, and rectum contains activated, CCR5 expressing CD4 cells and immature Langerhans cells, and dendritic cells or macrophages are present in the subepithelial lining; all these cell types may serve as primary target for HIV infection. Virus containing cells may then migrate to the submucosa and/or regional lymph nodes, where productive infections occurs [2].

The risk of HIV transmission after a sexual intercourse with a person living with HIV is influenced by a series of factors including the type of sex, the presence of genital lesions, and the level of viremia of the infected person.

Based on a meta-analysis of studies analyzing the risk of HIV transmission through unprotected (i.e., without using a condom) sex [3], it has been estimated that the risk associated with receptive anal sex is 138 per 10,000 exposures (or one transmission per 71 exposures), with a similar risk for male and female receptive partners. A lower risk has been reported for insertive anal sex which has been estimated to be 11 per 10,000 exposures (or 1 transmission per 1667 exposures) (Table 2.1).

A similar difference has been recorded in studies on vaginal sex. Risk of receptive vaginal sex has been estimated to be 8 per 10,000 acts (equivalent to 1 transmission per 1250 exposures), while that of insertive vaginal sex appears to be 50% lower (4 per 10,000 or 1 transmission per 2500 exposures).

Table 2.1 Estimates of per-act HIV sexual transmission risk according to different types of sexual intercourse without condom

Type of sexual intercourse	Risk per 10,000 exposures to an infected source	95% confidence intervals (%)
Receptive anal	138	(102–186)
Insertive anal	11	(4–28)
Receptive vaginal	8	(6–11)
Insertive vaginal	4	(1–14)

No reliable estimate is available for oral sex, although there is consensus that the risk is lower than that of anal or vaginal sex but not zero.

Thus the risk of acquiring HIV infection appears to be generally lower than that estimated for other sexually transmitted infections. For example, the risk of acquiring hepatitis B virus infection or syphilis following receptive anal intercourses have been estimated to be 50 and 14 per 100 exposures, respectively.

Moreover the risk of sexual transmission of HIV may be influenced by other factors [3]. For example, inflammatory process, and in particular infections causing the presence of genital ulcers, may increase the risk of transmission by 2–5 times. In fact, these conditions may both increase the presence of virus containing cells at genital level in the infected partner and of potential target cells in the genital mucosa of the uninfected partner. Male circumcision reduces the risk of acquiring HIV infection both for heterosexual males and for men who have sex with men, probably by reducing the penis area potentially exposed to the virus, and consistent condom use reduces the risk by 90%.

Finally, the risk of transmitting infection is clearly correlated with the amount of circulating virus. It has been estimated that the probability of passing the virus doubles for a log10 increase of virus concentration in plasma, and thus it is highest during the phases of infection characterized by

highest viremia, such as late stage and acute HIV infection. This latter condition is very relevant since many persons with HIV may transmit the virus when they are not yet aware of being infected. Conversely, persons with consistent suppression of viral replication by contemporary combination antiretroviral treatment, with viremia below the detection limits of currently used tests (200 to 50 copies per mL) do not transmit HIV to their sexual partners. This principle, now referred to as U = U (undetectable = untransmittable), is based on the result of several studies involving large populations of persons with HIV receiving effective antiretroviral treatment and their HIV negative sexual partners (both same and opposite gender partners) and it was clearly stated for the first time by a group of Swiss physicians in 2008. In a paper published in the Bulletin des médecins suisses they stated that a person with HIV receiving effective antiretroviral treatment (i.e., with undetectable viremia) with no other sexually transmitted infections and under regular medical care should be considered as non-infectious through sexual route [4]. This statement was considered quite unwise and not based on solid evidence at that time. Subsequent studies however have confirmed this principle, and presently studies involving data on serodiscordant couples having unprotected sexual intercourse over more than 10,000 person years of observation, failed to record a single case of HIV transmission [5].

2.4 The Global Picture Today

The first two decades of the twenty-first century have witnessed an unprecedented international effort to control the HIV/AIDS global epidemics with more than 100 billion of US dollars of development assistance provided by the international community [6]. This effort resulted in important achievements such as a significant reduction of mortality due to this condition, or the rapid scale up of interventions for preventing mother-to-child transmission of HIV [7, 8]. In spite of that, HIV/AIDS continues to be a leading cause of death and disease burden, especially in some areas of the world such as sub-Saharan Africa. In 2016, the Global Burden of Disease Study (GBD), an international research project of disease burden that analyzes mortality and disability from major diseases, published a comprehensive report of levels and trends of HIV/AIDS incidence, prevalence, coverage of antiretroviral therapy (ART), and mortality for the first 35 years of the epidemic (1980–2015) [9]. According to GDB, global HIV incidence increased rapidly during the last two decades of the twentieth century and peaked in 1997, at 3.3 million new infections per year. Thereafter, annual incidence decreased rapidly by approximately 5% per year until 2005 and then it remained constant at about 2.6 million per year (Fig. 2.1a). Of the new cases estimated in 2015, 75% were in sub-Saharan Africa, with large proportions in western, and 8.5% in south Asia (Fig. 2.1b).

The Joint United Nations Programme on HIV/AIDS (UNAIDS) produced a slightly different estimate of incidence trends [10]. In fact, UNAIDS estimates that the global number of new infection decreased continuously from a peak of 3.4 million in 1996 to 1.8 million in 2017. However, this decline appears to be due mainly to change in new HIV infections in sub-Saharan Africa, and globally it is much slower than what is required to reach the milestone of less than 500,000 new infections set for 2020. Moreover, epidemic trends in some regions are still alarming. In the Middle East and North Africa and Eastern Europe and central Asia, the annual number of new HIV infections has doubled in the last 20 years and it is still on the rise.

GBD estimates show that people living with HIV (PLWHIV) increased rapidly, from 2.4 million in 1985 to 28.0 million in 2000. Thereafter, the number of PLWHIV increased approximately by 1% per year, up to 38.8 million in 2015. Similar figures are provided by the UNAIDS report, which estimates for 2017 a global number of PLWHIV of almost 37 million, of whom 18.2 million are women (> 15 years), 1.8 million children (<15 years), and 1.8 million the new infections (Table 2.2).

The most dramatic change in the global epidemic is the decrease in mortality. According to GBD, global mortality reached its maximum in 2005, at 1.8 million, and subsequently decreased

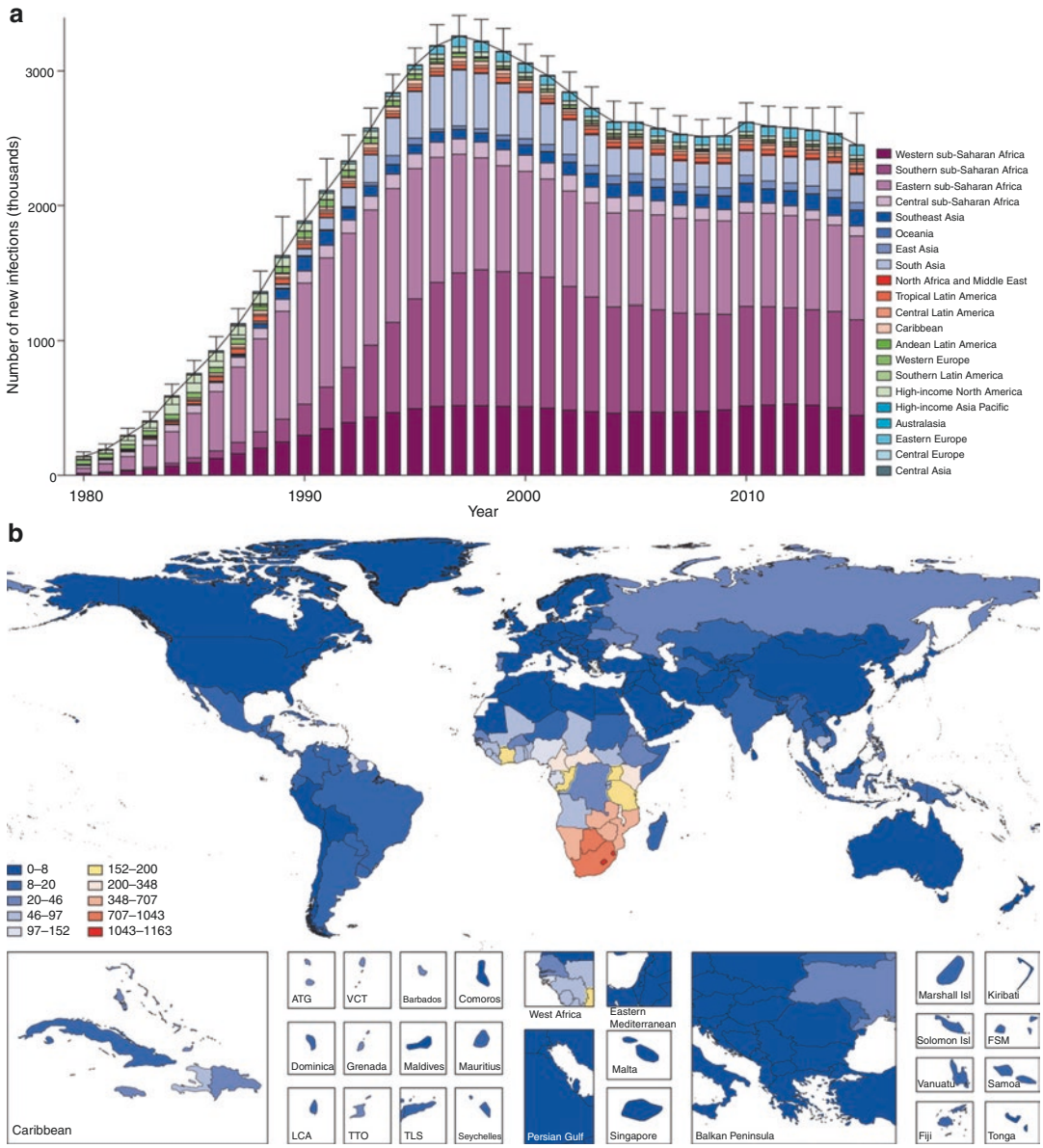

Fig. 2.1 Incidence of new HIV infections from 1980 to 2015, and HIV incidence in 2015. Global number of new HIV infections by region (**a**). Bars show the mean number of estimated new infections within a given year. Error bars represent 95% uncertainty intervals. Each Global Burden of Disease region is represented by a separate color. HIV incidence by country (**b**). We calculated incidence as cumulative new cases of HIV throughout the year divided by the total population at the mid-year. Rates are per 100,000 people. Color bins correspond to the 0–50th, 50–70th, 70–80th, 80–90th, 90th–92nd, 92nd–94th, 96–98th, 98–99th, and 99–100th percentiles to highlight variation within sub-Saharan Africa. *ATG* Antigua and Barbuda, *VCT* Saint Vincent and the Grenadines, *LCA* Saint Lucia, *TTO* Trinidad and Tobago, *TLS* Timor-Leste, *FSM* Federated States of Micronesia. From [8] reproduced with permission

Table 2.2 UNAIDS regional data for 2017

Region	New HIV infections	People living with HIV	AIDS related deaths	People accessing treatment
Eastern and southern Africa	800,000	19.6 million	380,000	12.9 millions
Western and central Africa	370,000	6.1 million	280,000	2.4 millions
Middle east and north Africa	18,000	220,000	9800	63,000
Asia and the Pacific	280,000	5.2 million	170,000	2.7 millions
Latin America	100,000	1.8 million	37,000	1.1 millions
Caribbean	15,000	310,000	10,000	181,000
Eastern Europe and central Asia	130,000	1.4 million	34,000	520,000
Western and central Europe and North America	70,000	2.2 million	13,000	1.7 millions
Total	1.8 million	36.9 million	940,000	21.7 millions

by 5.5% per year to 1.2 million in 2015. Deaths vary substantially by age-group. Among HIV/AIDS deaths in 2015, more females than males died in people aged 15–29 years; after age 35 years, there were more deaths in males (Fig. 2.2a). The Fig. 2.2b shown the global decreased trend and by regions of the mean estimates of HIV/AIDS deaths per prevalent case from 2005 to 2015. This decrease has been paralleled by a sharp increase in the proportion of PLWHIV who receive antiretroviral therapy (ART) (Fig. 2.3). The proportion of PLWHIV receiving ART increased between 2005 and 2015, from 6.4% to 38.6% for men, and from 3.3% to 42.4% for women. UNAIDS estimates for mortality and access to ART, in 2017, are consistent with the same order of magnitude (Table 2.2).

2.5 Progress Toward Epidemic Control

At the beginning of the fourth decade of the global epidemic, UNAIDS launched an ambitious initiative to end the AIDS epidemic as a global health threat. To achieve this by 2030, the number of new HIV infections and AIDS-related deaths will need to decline by 90% compared to 2010, which corresponds to 200,000 new HIV infections and 300,000 AIDS-related deaths per year. Reaching these goals would result in 28 million HIV infections and 21 million AIDS-related deaths averted between 2015 and 2030. As an intermediate milestone, the number of new infections should be reduced to 500,000 in 2020 [11].

We have seen that although progress has been made in reducing new infections and deaths, the pace of decrease should be accelerated to meet the targets. At the same time, we need other metrics to monitor how different countries move toward the control of the epidemic. Two metrics have been recently proposed [12].

The first is the incidence/mortality ratio. This ratio may be used to measure the annual change in the number of people living with HIV within a given population. In fact, when the incidence/mortality ratio is greater than 1, there are more new infections than deaths in a given year and the number of people living with HIV will increase; conversely, when the incidence-mortality ratio is less than 1, there will be a decrease in the number of people living with HIV. However, this metric can be misleading since a high value may also be due to and high mortality which in turn may be linked to a limited uptake of antiretroviral therapy. Thus, it has been recommended that this metric is only used in countries in which coverage of antiretroviral therapy exceeds 80%. According to UNADS data for 2017, only one country, Cambodia, had achieved 80% treatment coverage and had an incidence: mortality ratio under 1 [10].

Another epidemiologic measure that has been proposed is the incidence/prevalence ratio (IPR). Prevalence and incidence of a condition are linked by the average duration of that condition

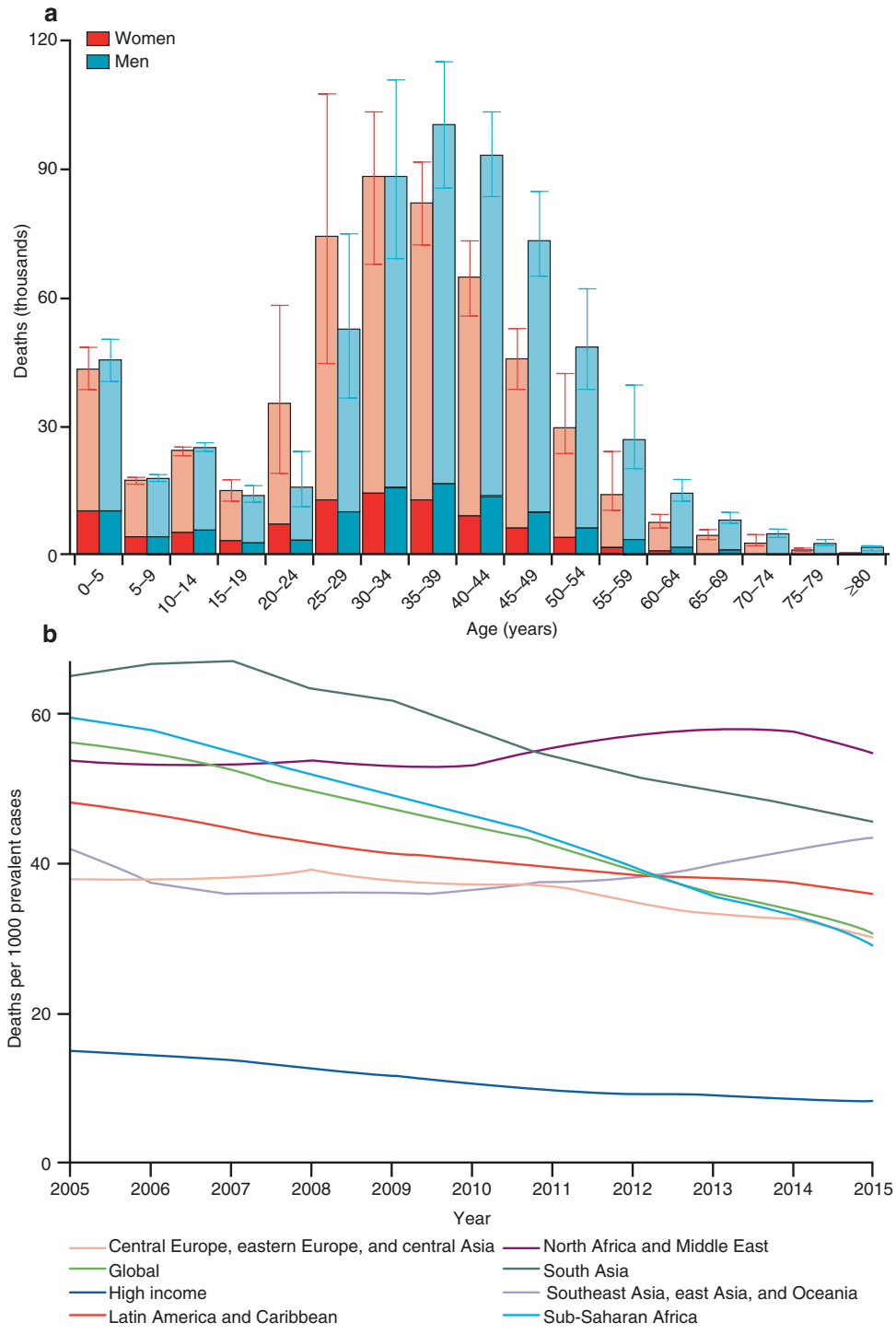

Fig. 2.2 Global HIV/AIDS deaths, 2005–2015. Global deaths caused by HIV/AIDS resulting in either mycobacterial infection (tuberculosis) or other diseases, by age and sex in 2015 (**a**); dark shading indicates deaths caused by tuberculosis associated with HIV; light shading indicates deaths caused by other diseases resulting from HIV; error bars show 95% uncertainty intervals. Mean estimates of global and super-regional HIV/AIDS deaths per prevalent case from 2005 to 2015 (**b**). From [8] reproduced with permission

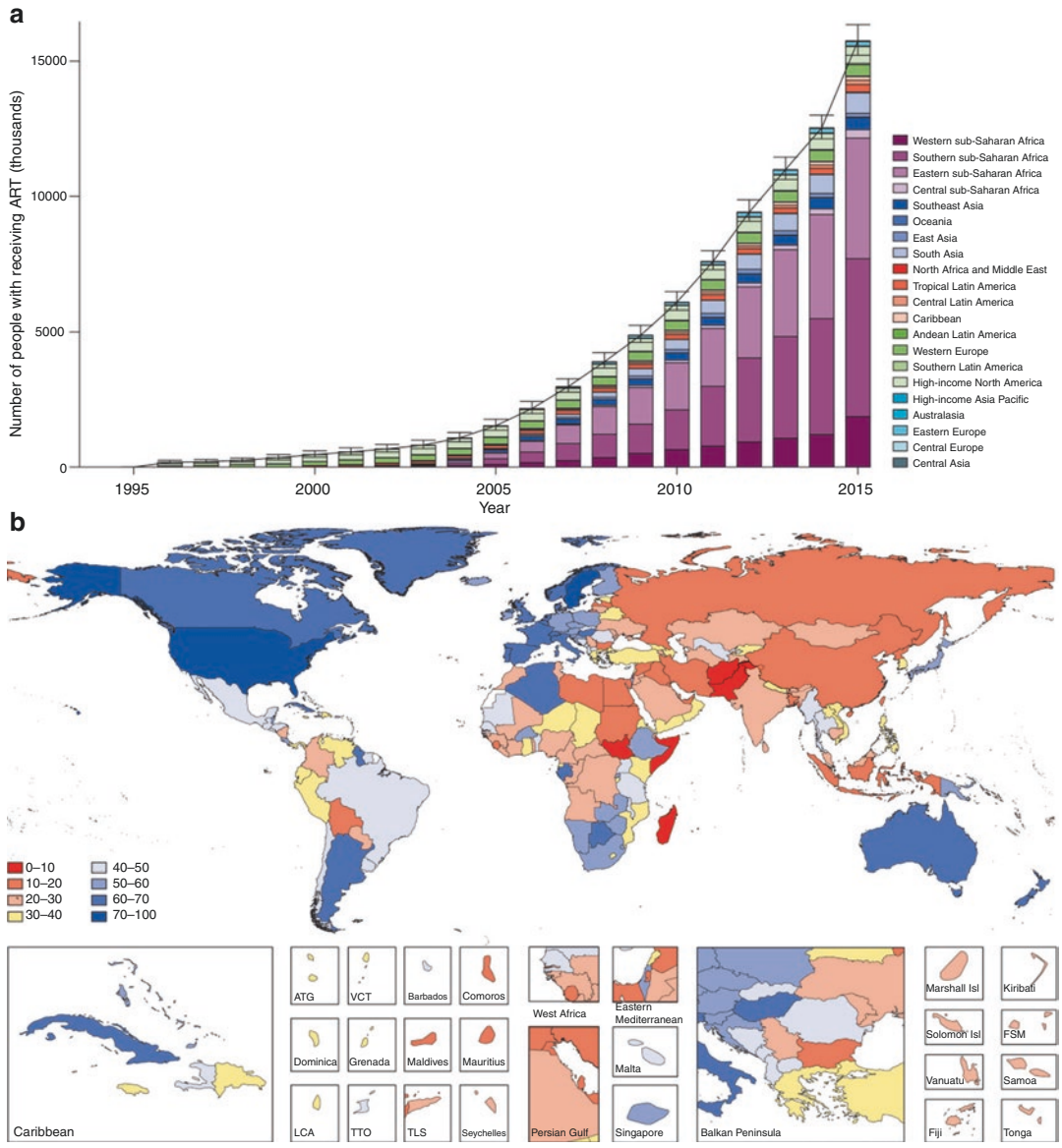

Fig. 2.3 Number of people living with HIV receiving antiretroviral therapy from 1995 to 2015, and the proportion living with HIV receiving antiretroviral therapy in 2015. Number of people living with HIV receiving antiretroviral therapy (ART) by region (**a**). Bars represent the mean number of people living with HIV who received ART within a given year. Error bars represent 95% uncertainty intervals. Each Global Burden of Disease (GBD) region is represented by a separate color. Proportion of people living with HIV receiving ART by country (**b**). The number of people living with HIV receiving ART and the total number of people living with HIV are year-end point prevalences. *ART* antiretroviral therapy, *ATG* Antigua and Barbuda, *VCT* Saint Vincent and the Grenadines, *LCA* Saint Lucia, *TTO* Trinidad and Tobago, *TLS* Timor-Leste, *FSM* Federated States of Micronesia. From [8], reproduced with permission

(Incidence × Duration = Prevalence) thus the ratio of HIV incidence to HIV prevalence is an indicator of the survival of persons with HIV (Prevalence/Incidence = Duration). If we set the survival of persons with HIV acquisition at 33 years, the epidemic will be under control (i.e., the number of prevalent infection will decrease) if in a given year less than 3 new infection will

occur per 100 people living with HIV, which gives an incidence/prevalence ratio = 0.03. A higher number of new infection will result in higher ratios and which are indicative of a growing prevalence of the infection. This metric will not be applicable in the context of rapidly increasing or decreasing incidence nor in areas where there is significant migration.

At a global level, according to UNAIDS data, incidence/prevalence ratio has been declining from over 0.10 in 1990 to 0.05 in 2017. This decline has been particularly marked in eastern and southern Africa where the ratio decreased from 0.11 in 2000 to 0.04 in 2017, most likely because of strong reductions in new HIV infections and AIDS-related mortality. More limited progress has been recorded also in other regions, and the ratio remains high in eastern Europe and central Asia and in Middle East and North Africa (0.09 and 0.08 in 2017, respectively). In western and central Europe and North America, a continuous decline has been observed over the last two decades with a ratio of 0.03 in 2017, which is regarded as indicative of epidemic control. At country level, ratios below 0.03 have been recorded in Austria, Bahamas, Cambodia, Denmark, Ethiopia, France, Italy, Nepal, Netherlands, Portugal, and Spain.

2.6 The 90-90-90 Target

Since combination antiretroviral therapy became available in clinical practice in the mid-1990s, it became clear that it was possible to alter substantially the natural history of HIV infection, with a dramatic reduction of mortality of persons living with HIV. With the availability of increasingly more potent and less toxic drugs, a consensus emerged on widening the indication of antiretroviral therapy in all persons with HIV, including those with minor level of damage to the immune system. The new indication were also based on accumulating evidence, form observational studies and clinical trials, about the lack of transmission of the infection from persons receiving effective treatment and about the potential of the global scale up of antiretroviral treatment to contribute to the control of the epidemic.

Based on this evidence, at the 20th International AIDS Conference in Melbourne, Australia, in 2014, UNAIDS director launched the 90-90-90 target, whereby, by the year 2020, 90% of people living with HIV globally know their HIV status, 90% of those who know their HIV status had access to antiretroviral therapy, and 90% of people on treatment had suppressed viral loads [13].

Important progress has been recorded towards these targets. By the end of 2016 an estimated 70% of people living with HIV knew their HIV status in 2016. Among them, 77% were on antiretroviral therapy, and 82% of those on treatment had undetectable viral load. Significant regional differences however have been observed. For example, the proportion of persons with HIV that is aware of their status ranges from more than 80% in Western Europe and North America to less than 50% in Western and Central Africa. And important gaps between different stage are recorded, for example, in Eastern Europe where less than 50% of those with known HIV infection are on antiretroviral treatment. Nonetheless, it has been estimated that in 2017 at least 7 countries, including Botswana, Cambodia, and the UK, have achieved the overall goal of viral suppression in 73% of all persons with HIV, and more than 10 countries are close to this threshold. Taken together, these observations show that it is possible to achieve the 90-90-90 targets, if gaps across the HIV care cascade are properly addressed [10].

2.7 Epidemic Patterns and the Key Populations

A notable feature of HIV epidemic since its start has been its regional and local heterogeneity in terms of burden and of population groups affected. According to traditional view of epidemic patterns, different countries were classified as having generalized or concentrated HIV epidemic. Generalized HIV epidemic has been defined as characterized by a firmly established spread in the general population that is mainly due to sexual transmission and is documented by HIV prevalence constantly exceeding 1% among pregnant women. In contrast, in concentrated

epidemic HIV has spread in one or more subpopulations, where HIV prevalence is above 5%, but its spread is limited in the general population and HIV prevalence is less than 1% among pregnant women. HIV epidemic has been classified as generalized in most of southern and of eastern African countries, while it has been considered concentrated in Latin and North America, the Middle East, Europe, and Asia [14].

More recently, however, it became clear that even in the context of concentrated epidemics, some population groups are disproportionately affected by HIV infection (Table 2.3). These populations at higher risk of HIV have been termed as key or vulnerable population [15].

The term key population refers to population groups who, almost in all settings, are disproportionately affected by HIV infection. It also refers to populations that are frequently underserved or affected by stigma. Intervention aimed at responding to the need of these populations is key component of an effective response to the epidemic.

According to WHO key populations are:

- Men who have sex with men.
- People who inject drugs.
- Sex workers.
- Transgender people.
- People in prisons and other closed settings.

Epidemics of HIV in men who have sex with men is still on the rise in various regions. High efficiency of transmission through unprotected anal intercourse, high number of sexual partners and, and concomitant injecting drug use may contribute to the high prevalence of HIV infection in this population. Based on published evidence and on country progress reports submitted to the UN General Assembly Special Session on HIV/AIDS (UNGASS), it has been estimated that HIV prevalence ranges from 3.0% in the Middle East and North Africa to 25.4% in the Caribbean. Prevalence among men who have sex with men is above 10% in North, South, and Central America, South and South-east Asia, sub-Saharan Africa and the Caribbean, and in all regions it is at least three times as high as compared to that in general populations [16].

Injecting drug use is increasingly recognized as a global health issue. A review of available data published in 2017 found evidence of injecting drug use for 179 countries, where 99% of adult population lives, 31 countries more than those identified in a previous review conducted 10 years earlier [17]. The estimate global number of people who inject drugs is 15.6 million with an overall HIV prevalence 17.8% or 2.8 million, and wide variations across countries and regions. The estimated HIV prevalence rates are 1.1% in Australasia, 3.6% in the Middle East and North

Table 2.3 Percent distribution of new HIV infections, by population group and by region, 2017

Region	People who inject drugs	Men who have sex with men	Transgender woman	Sex workers	Sexual partner of key populations	Rest of population
Eastern and southern Africa	1	6	–	2	8	83
Western and central Africa	10	12	–	2	16	60
Middle east and north Africa	38	17		13	30	2
Asia and the Pacific	14	29	2	4	35	16
Latin America	3	41	6	3	24	23
Caribbean	1	23	1	13	30	32
Eastern Europe and central Asia	39	21		9	28	3
Western and central Europe and north America	7	57		2	24	10
Global	9	18	1	3	19	53

Africa, and 4.5% in Western Europe, with highest rates recorded in Eastern Europe, 24.7%, and in Latin America, 35.7%.

Most sex workers—those who exchange sex for money—are female, although sex workers can be female, male, or transgender. Among female sex workers in low- and middle-income countries the overall HIV prevalence estimated in a recent systematic review was 11.8% [18]. Prevalence of HIV reflect in part the background rates in the general population with the highest rate in sub-Saharan Africa (36.9%), followed by eastern Europe (10.9%), Latin America and the Caribbean (6.1%), Asia (5.2%), and in the Middle East and north Africa (1.7%). Overall, the risk of having HIV infection is clearly higher in female sex workers than in other women in the reproductive age; however, this increase in risk is more marked in countries with a background prevalence up to 1% (odds ratio 24.5) than in countries with HIV prevalence above 1% (odds ratio 11.6) [19]. Criminalization and sexual violence are also important determinant of HIV risk in this population. Prevalence of HIV is also high, above 10%, in female sex workers in the USA [20], while it is generally low, below 1%, in those who do not inject drugs in Western Europe [21]. Men who engage in sex work are also at increased risk of HIV infection. In a meta-analysis of 66 studies, pooled HIV prevalence was 10.5%, 20 times higher compared to the general male population in the same countries. Prevalence rates was highest in sub-Saharan Africa (31.5%) and lowest in Europe (12.2%) [22].

Determinants of HIV risk for transgender persons may be identified at different levels. These determinants include biological factors such as high prevalence of other sexually transmitted infections and hormone-related factors; behavioral determinates including frequent anal condomless sex; a high prevalence of HIV and limited awareness of HIV status within their sexual networks, stigma, and discriminatory laws [23]. Transfeminine individuals appear to be at very high risk, with prevalence rates above 20% in studies conducted in Brazil, Argentina, India, Spain, and the USA. Very little information is available for transmasculine persons; however, some data suggest an increased risk for those who have sex with men [23].

It has been estimated that more than ten million people is incarcerated worldwide on any given day and that in 2013, the number infected was 389,000 with HIV (3.8%) and most of them are injecting drug users. Highest prevalence rates are found in east and southern Africa and west and central Africa, reflecting high rates in general population and in Eastern Europe and central Asia and west Europe, where persons who inject drugs are overrepresented in prisons [24]. Incarcerated persons in key populations are also at increased risk compared to other prisoners. It has been estimated that persons who inject drug, men who have sex with men, and sex workers have a prevalence of HIV 6, 5, and 2 times higher, respectively, than other prisoner populations [25].

In addition to key populations, a number of populations with heightened vulnerability to HIV have been identified, including migrant workers, refugees, long-distance truck drivers, military personnel, miners, and, in southern Africa, young women. These population groups are not uniformly vulnerable or equally affected across different countries and epidemic. Interventions aimed at controlling the HIV epidemic need to be increasingly focused in coming years to key population, and groups identified as vulnerable in specific context [15].

2.8 Conclusions

Today, the new epidemiology of HIV infection shows us the enormous progress has been made in reducing the clinical consequences of the infection, especially in terms of mortality worldwide, particularly in low-income countries, where the international support programs have allowed a massive expansion of the prevention of mother-to-child transmission and the use of ART.

However, the achievement of the UNAIDS 90-90-90-90 objectives will require further

important changes in control strategies directions and how to better target funding in the light of the epidemiological data that have been described in this chapter.

The same preventive biomedical interventions based on the extensive use of ART (i.e., treatment for prevention—TasP, post-exposure—PEP, and pre-exposure prophylaxis–PrEP) are putting a strain on the preventive pressure exerted by behavioral prevention interventions delivered in HIV clinical centers and in point-of-care tests facilities for STI, particularly among MSM living in Western countries. These interventions are leading to risk compensation phenomena that are reflected in a measurable increase in HIV incidence in the populations at increased risk. This informs us of how global efforts have had much less impact on the incidence of new infections than on HIV mortality.

Ending the AIDS epidemic by 2030 will therefore require a drastic change in the ways in which HIV prevention has been pursued over the last 15 years and will require assessing not only the control of the risk of transmission by infected individuals but also the complex set of clinical, behavioral, and social factors affecting the risk of acquisition of HIV in susceptible individuals, worldwide.

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3 An Overview of Social and Behavioral Determinants of STI

Matthew Hogben, Jami Leichliter, and Sevgi Okten Aral

3.1 Introduction

Sexually transmitted infections (STI), including human immunodeficiency virus (HIV), remain an important source of morbidity and mortality in the twenty-first century. The World Health Organization publishes estimates of the global burden of STI, with a 2012 estimate at approximately one million cases per day [1]. Some populations are more at risk than others. Syphilis rates among men who have sex with men (MSM) have been rising: in the United States (US) and Western Europe, numbers of cases among MSM circa 2013 are typically several times higher than a decade earlier [2]. Additionally, estimates of STI prevalence for pregnant women in low and middle income countries, based on systematic reviews, include 1.2–4.6% for gonorrhea and 1.1–4.6% for syphilis among pregnant women in low and middle income countries, depending on the global region surveyed [3]. A rate of 4.6% among pregnant women clearly indicates significant risk for congenital syphilis in a population. Finally, racial, ethnic, and other disparities are pervasive for many STI, regardless of how these disparities are measured [4].

There exists a compelling case for prevention [5]. Antecedent to prevention, however, is an understanding of the determinants of STI. In this chapter, we focus on behavioral and social determinants of STI, which are closely intertwined. Sexual behaviors and sex practices are proximate causes of STI acquisition and transmission. Several other individual-level behaviors are risks for acquisition and transmission (e.g., illicit drug use), and health-management behaviors (e.g., treatment adherence) are contributors to STI maintenance in transmission networks. Social determinants provide the essential context for behaviors; for example, testing behaviors matters little if there are no clinics to provide treatment. Thus, the importance of addressing social and behavioral determinants in this chapter is clear.

In Fig. 3.1, we present an overarching structure for the chapter, expanding upon an earlier model using epidemiologic context to link social determinants and STI [6]. On the right, we have divided primarily individual-level determinants into attributes and behaviors, along with examples. On the left, we have labeled social determinants and the epidemiologic context (prevalence of infections and behaviors measured at the population level). These four categories influence and are influenced by social and sexual network structure, ideally the "intermediate" structural

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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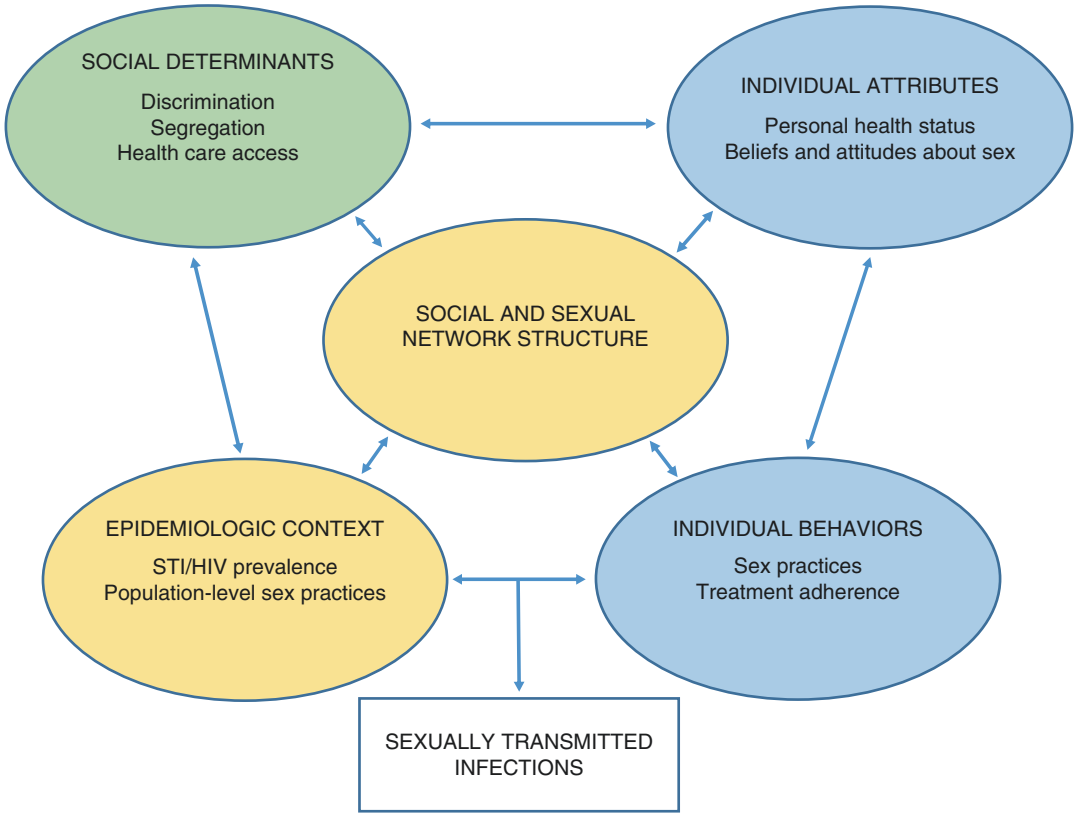

Fig. 3.1 A broad framework for social and behavioral determinants of sexually transmitted infections. *Note*. Behavioral and social determinants combine with epidemiologic context to form social and sexual network structures for STI transmission. Each of these constructs influences and is influenced by each other. The framework is a description of behavioral and social antecedents to STI transmission and is not intended to be read as a causal model

level between the individual and population levels. In an ideal prevention program, we suggest the network structure is the ideal lens through which to assess and reduce transmission. The arrows in the figure are all double-headed as the constructs all have reciprocal influences: STI transmission is not a linear process.

3.1.1 Behavioral Determinants of STI

3.1.1.1 Sexual Behaviors

Sexual Debut Early sexual debut is often intertwined with other determinants of STI, including childhood sexual abuse and sexual violence as precursors, as well as alcohol and other substance use and misuse. Broad data such as the Youth Risk Behavior Survey in the US show that early debut is associated with other subsequent risk behavior [7]: the effects were equivalent for heterosexually identified students and sexual minority students. Students completing the YRBS and reporting early sexual debut (<13 years) were more likely to report not using condoms at last sex whether they identified as sexual minorities or not. In Nepal, a nationally representative sample of adults showed early sexual debut to be correlated with determinants such as multiple sex partners, inconsistent condom use, as well as outcomes including STI history and teen pregnancy [8].

Sex Practices Systematic reviews and other research show that anal sex (receptive more so than insertive) carries the highest risk of HIV transmission (e.g., compared to vaginal or oral sex), while male-to-female and female-to-male transmission rates and their relative magnitudes vary by national income level [9–11]. UK data in the National Survey of Sexual Attitudes and Lifestyles (Natsal) show anal sex experience increasing from approximately 10% in 1990–1991 to 22–24% in 2010–2012 [12]. Nationally representative data from the National Survey of Family Growth (NSFG) 2011–2013 showed that 42% of all US males and 36% of US females (18–44 years) had ever had anal sex [13], estimates that are considerably higher than popular beliefs, although similar to prior estimates [14]. Likewise, 17% of women and 6% of men had same-sex sexual experiences, compared to 1.3% and 1.9%, respectively, who identified as gay or lesbian as a sexual orientation [13]. Even though some other respondents (5.5% of women and 2.0% of men) identified as bisexual, the point is clear that same-sex sexual behavior is more prevalent than estimates of sexual orientation suggest, especially for women.

Number of Sex Partners Large-scale surveys show that increasing numbers of sex partners a person reports is generally associated with an increased risk of reporting an STI [15]. Men and women in the UK show an increase in the mean number of sexual partners across time: 8.6 to 11.7 opposite sex partners for men and 3.7 to 7.7 opposite sex partners for women, with the bulk of these increases occurring between the 1990–91 and 1999–2000 surveys [16]. In contrast, frequency of sex (number of occasions in the past 4 weeks) dropped from 6.4 to 4.9 for men and 6.1 to 4.8 for women, with the drop occurring almost exclusively between the 1999–2000 and 2010–2011 surveys. In the US, NSFG data collected between 2002 and 2013 has shown largely stable estimates for number of partners for both women (lifetime medians of 3 in 2002 and 4 in 2011–2013) and men (lifetime medians = 5 at both periods) [17]. These findings, however, were accompanied by analyses showing changes in distributions of sex partners such that the top 5% of men and women reported more lifetime partners in 2011–2013, compared to 2002. The overall portrait from these two general population surveys is that the riskiness of sexual behaviors as measured by number of partners increased roughly up to 2000 and stabilized or even slightly diminished thereafter.

Concurrent and Non-Monogamous Sex Partners The riskiness of number of partners is compounded by the phenomenon of concurrency, defined as having multiple sex partners with overlapping relationship periods; that is, where the date of last sex for the first partner comes *after* the date of first sex for a second partner [18]. Concurrency is a risk for STD transmission and contributes to STD spread when measured at the population level. One study of adolescents seeking services at a US STD clinic found concurrency to be a risk for STD diagnosis or exposure while controlling for overall number of partners [19]. More US evidence has been shown with syphilis and chlamydia [20, 21], with the latter study addressing concurrency as part of a prospective cohort analysis of racial disparities. In contrast, a cohort study in South Africa found no effect of concurrent relationships on women's HIV acquisition, although there was a small, significant effect for men's lifetime number of sexual partners [22]. Kenyon and colleagues studied partner concurrency (i.e., the individual has a single partner but their partner has concurrent partnerships—partner concurrency is an individual STD *acquisition* risk) in South Africa [23]. In this analysis, partner concurrency was associated with HIV among Black women; prevalence among other groups was too low for further analysis. Clearly, the studies cited here are greatly different in one or more of definition of concurrency, population, geography, and the pathogen of interest; nevertheless, such contrasting results suggest the study of concurrency is complex.

Accordingly, modeling research has examined concurrency across several conditions (e.g., varying timing of relationships, who is originally infected) [24]. The authors concluded that concurrency "saturates" (i.e., the effect reaches

equilibrium) and that reducing concurrency is most likely to affect HIV in situations with *low or moderate* concurrency. However, reducing concurrency as an intervention might be more effective if the infection is presumed to be curable, as with syphilis or gonorrhea. The logic of this hypothesis is that saturation is more difficult to reach if people can be treated and return to a susceptible state. The hypothesis is also consistent with existing data, in which concurrency appears more reliably associated with curable bacterial STI [20, 21].

Associated with concurrency is the "gap," the time between two serial sexual partnerships [25]. Although such relationships are not concurrent, a brief enough period between the termination of one partnership and the beginning of another can allow a person to acquire infection from the first partner and, if untreated, transmit to the next. Data on the gap are hard to acquire as testing the hypothesis requires knowing the dates of partnerships with a relatively high degree of precision. Additionally, mutual, consensual and contemporaneous non-monogamy, or "swinging," is the broadest definition of concurrency. Data on STI and swinging is rare; one Dutch STI clinic collected these data and found swingers made up 12% of their consultations [26]. They also tended to be older than the rest of the clinic attendees and accounted for approximately as many STI as MSM seen in the clinic.

Finally, the Demographic and Health Surveys (DHS) undertaken in many countries contain measures of behavioral determinants of STI history since 1984 [27]. For example, one review of data from 10 countries (in Africa, the Caribbean, and Latin America) identified increasing rates of premarital sex in Latin America and the Caribbean over a decade and a half as well as an increasing median rate of marriage across all surveys [28].

3.1.1.2 Individual Determinants and STI: Other Behavioral Determinants

Technology-Enabled Social Networking to Find Sex Partners Use of social networking sites appears associated with risk behaviors, although there are nuances to this statement. On the one hand, a multi-site study in the US found that use of *both* internet and mobile app methods for sex-seeking was associated with higher odds of chlamydia, gonorrhea, and syphilis for Black and Latino MSM [29]. In contrast, a study of users of Chinese websites suggested no differences in condom use among website-only users, app-only users, or users of both methods, although rates of condomless sex were high: over half of those completing the survey had engaged in condomless sex in the previous 6 months [30]. Other research from the US Southeast showed that men who actually met partners whom they sought online or who had sex with partners they sought online were more likely than other men to report engaging in condomless anal intercourse [31]. A Canadian study found that online sex seekers engaged in more risk behaviors, scored higher on a sensation-seeking measure, and were less likely to identify closely as part of a gay community (as measured by a community identity scale) [32, 33]. Finally, a 6-year series of surveys in China found moderately higher HIV rates (13.2% vs. 10.5%) among internet-using MSM compared to MSM not using the internet (syphilis rates, however, were similar at 11.4% and 12.0%) [34]. On the other hand, a review of four studies (conducted between 2005 and 2008) of MSM using the internet to find sex partners revealed variable results around condomless anal intercourse versus other sex practices. Two studies produced null findings, and the other two were split between protective and risky effects [35].

What might be more salient are the reasons *why* gay, bisexual, or other MSM use online sites. An Australian study reported that men who used "online and offline" approaches to find sex partners had higher numbers of partners and were more likely to report having been diagnosed with an STI than men who used only one approach [36]. That is, part of the added risk found among internet and mobile users in some studies might be due to how much effort study respondents were willing to put into finding sex partners, rather than use of technology per se. In the Canadian study referenced in the previous paragraph [32], men seeking sex online also spent more social time with other gay men and

presumably valued doing so. Moreover, in some studies, internet and app users felt stigmatized by friends and family or unsafe using approaches other than online methods, as was the case in studies in Nigeria and two countries in Southern Africa [37, 38]. In the Nigerian study, men who used the internet were more likely to report being HIV-infected [38]. In the study from Southern Africa, men who sought sex online had higher numbers of sex partners [37].

We found one study looking at online sex-seeking among US veterans (male and female), specifically combat veterans [39]. Veterans who used online sites to find partners were more likely to report diagnosis with an STI (34% vs. 29%). These veterans also had consistently higher rates of mental health issues, including anxiety, depression, alcohol dependence, and post-traumatic stress disorder. The point to be made is that online sex-seeking can possibly be an outlet for people in significant distress and a facilitator of risky sexual behavior, rather than a cause of either.

Substance Use and Misuse Substance use and misuse, including alcohol use, is a correlate of risky sexual behaviors [40] and often clusters with other risks to health [41]. For individuals, substance use is also often correlated with membership in a vulnerable population (e.g., trafficked minors [42]). Natsal data from the UK indicate that illicit drug use is correlated with STI diagnosis as well as with several behavioral determinants of STI (number of sex partners in the past year, lack of condom use by number of partners) [43]. In these data, people using illicit drugs were also more likely to be tested for STI (specifically chlamydia), but the effect size for STI diagnosis was considerably larger than that for testing. Other analyses from the same dataset revealed that binge drinking was associated with having multiple partners and condomless sex [44]. A systematic review of 68 studies with participants from African countries found that MSM in several countries had high rates of alcohol use or dependence, and that alcohol use was generally associated with unprotected sex [45]. In one of the studies reviewed, men who used alcohol before sex were more likely to be diagnosed with an STI [46]. Finally, recent research has examined the role of "chemsex," the sexualization of drug use itself. In one recent study, chemsex was associated with increased rates of sero-discordant condomless anal sex as well as hepatitis C and STI diagnoses [47].

Injection drug use (IDU) is a particular risk for HIV and hepatitis, as demonstrated in the opioid-associated HIV outbreaks in the US in 2015, exacerbated by barriers to care in the largely rural settings of these outbreaks [48–50]. In data from the systematic review discussed above, men who injected drugs most often had more sexual risk than men who did not (even if they used other drugs) [45]. Here, we also note that IDU is also correlated with other sexual risks, for example, exchange of sex for drugs or police protection (an example from Kenya) [51], and condomless anal sex among HIV-infected MSM in the UK [47].

Sexual and Physical Violence This section of behavioral determinants is different from those above in that the topic pertains to the *experience* of violence as a determinant, not the conduct. There are numerous data indicating an association between STI and experience of sexual or physical violence. Global examples include elevated STI rates among women reporting intimate partner violence in national surveys, US family planning clinics, in other primary care settings, and in jails [52–55]. One study specifically measured fear of asking for condom use or refusing sex and found large associations between these imposed risks and forced sex [54]. Other data have also shown that intimate partner violence is associated with lower rates of condom use [56]. Another study of women reporting forced sex also tested whether sexual violence moderated the link between testing and treatment [57]. The authors found that women reporting a history of STI (specifically herpes and warts in this analysis) were less likely than other women to have been treated. Although it is only one study, the findings raise the possibility that sexual violence might be a determinant of STI and sequelae by restricting the likelihood of treatment. In similar fashion, HIV pre-exposure prophylaxis (PrEP)

interruptions were more common among serodiscordant couples in Kenya and Uganda when one partner reported IPV [58].

Childhood sexual abuse (CSA), including via trafficking [42], presents another aspect of sexual violence as a determinant of STI. A prospective cohort in Australia produced consistent associations between CSA and several risky sexual behaviors, especially early sexual debut and multiple sex partners [59]. Cohort data from the National Longitudinal Study of Adolescent to Adult Health revealed an association between CSA and subsequent sex work [60]. Analyses from this cohort also demonstrated that CSA often coexists with other adverse childhood experiences (e.g., parental binge drinking or incarceration), illustrating that analyses of the effects of CSA present complex issues of multiple causation and mediation. Moreover, a review for the US CDC STD treatment guidelines concluded that prevalent STI among victims of sexual assault are a basis for empiric treatment for gonorrhea, chlamydia, and trichomoniasis [61]. Thus, sexual assault can be as much a marker for STI risk as it is a direct determinant because populations in which high rates of sexual assault are reported appear to also have elevated rates of STI.

Finally, although the sexual violence literature has plenty of data on male perpetration of violence (and some on experiences), there is less that connects male violence with STI. One Indian study, however, measured masculine ideologies, intimate partner violence, and genital tract infections (not limited to STI) among men: violent men were more likely to report symptoms of infection and less likely to report a diagnosis [62]. Among MSM, intimate partner violence is associated with HIV prevalence and incidence, suggesting violence as a potential pathway to HIV acquisition [63].

3.1.1.3 Individual Determinants and STI: Preventive Behaviors

Condom Use Condoms, if used correctly and consistently are protective against STI, including HIV, chlamydia, gonorrhea, HSV, and syphilis [64]. The authors make the point that, although protection via condom use is not perfect, even partial protection is enough to affect population rates of STI. What reduces condom efficacy? At the individual level, a study following up reported condom use problems among participants in a risk reduction trial (Project RESPECT) [65] found that user errors and problems (e.g., slippage, delayed application) were associated with STI diagnosis [66]. Studies of efficacy are sometimes confounded by the fact that many people use condoms when they (correctly) believe their risk to be high, and limited in power by STI prevalence in the target population. A second analysis from RESPECT addressed this issue by examining condom use among sex partners of 429 persons diagnosed with STI in a clinic, thus guaranteeing the partners were exposed to infection [67]. Consistent condom use under these circumstances was protective for gonorrhea and chlamydia.

Issues with the effectiveness of condom use are more closely tied to use and other preventive behaviors, such as reduced numbers of partners: preventive behaviors often cluster. An extensive analysis of UN data along with DHS data from developing countries suggests that condom use worldwide has risen over the past two decades, allowing for numerous differences within countries [68]. However, other review data show condom use has dropped among MSM in high income countries, with some evidence of increasing HIV infection (an ecological comparison with differences among countries) [69]. Population surveys indicate that condom use has been stable in the US for women between 2002 and 2015 (at 23–25% at last intercourse) and has risen slightly for men (from 30 to 35%) [70]; equivalent rates for adolescents are much higher and increased over the same time period [71].

In countries with higher HIV prevalence, condom use has sometimes increased while HIV has declined [72, 73]. For example, DHS data in Burkina Faso reveal that condom use rose between 1998 and 2010 by approximately 10–20 percentage points, depending on gender and age group (median rates rose from about 45% to 60%) [73]. HIV also declined over this period, although condom use increases were only one of

several risk reduction practices that increased over the time period. We suggest that many of these global differences reflect different contingencies with respect to HIV prevention and contraceptive choices: condoms are the first line of prevention for many sexually active people worldwide.

Adherence Social and behavioral factors relevant to medication adherence include individual reactions to the medication in question, such as reactions to side effects, safety perceptions, and even size or taste. The large majority of the research concerns HIV, possibly because many STI treatments have single doses. For adherence to ART medications, one review found that experiences of side effects such as fatigue, confusion, taste disturbances, and nausea were associated with lower adherence [74]. In the Vaginal and Oral Interventions to Control the Epidemic (VOICE) RCT of PrEP for women at high risk of infection in South Africa, women were randomized to oral or gel placebo, or to receive either vaginal tenofovir gel or oral PrEP (tenofovir with or without emtricitabine) [75]. The study had null results, with further analyses showing that adherence was likely the salient factor. Participants receiving tenofovir gel who *did* have medication detected in plasma samples were more likely to show a prevention effect, HIV incidence of 3.3 per 100 person-years versus 6.8 in placebo and 8.2 among those in the intervention arm who did not have medication detected in plasma samples [76].

Qualitative data from Uganda also indicates taste can be a factor in adherence for HIV medication [77]. There is minimal research into the effects of taste on adherence to STI medications. The most salient factor for adults in single doses and short regimens of medication appears to be bitterness, although efforts to improve adherence through reducing bitter taste are relatively rare (possibly because economic margins around many STI medications are quite thin). One RCT of metronidazole for trichomoniasis (intravaginal versus oral) noted vomiting and a "metallic taste" as a potential influence on adherence [78]. The authors did mention increased taste-related adverse events for the oral medication arm; nevertheless, the patients were cured, and we found no further efforts to improve the taste to recipients.

Stigma is related to adherence. Stigma around seeking care for STI and HIV also affects acquiring and taking medication or using prevention methods. The effect of stigma on medication use and prevention exists because STI and HIV remain stigmatized conditions, and the experience of stigma affects mental and physical health [79, 80]. Higher levels of internalized stigma reduce adherence, in terms of initiating treatment, taking medications, and staying in care for HIV [81–84]. HPV vaccination presents one example. Aside from safety concerns, systematic reviews reveal parental fears of adolescent sexual promiscuity subsequent to vaccination [85–87]. Evidence from the NSFG revealed no link between HPV vaccine receipt and risky sexual behavior among female adolescents; in fact, sexually experienced adolescents who had received the vaccine were *more* likely to use condoms [88].

The structural influences of stigma upon adherence to care are subtle. For example, cultural perceptions about sexual assault related to stigma and status appear to affect the odds of receiving care related to STI prevention. One study of women who were sexually assaulted and who presented to hospitals found that those assaulted by an intimate partner (current or former) were statistically less likely to have received prophylaxis for STI or been counseled for post-exposure prophylaxis (PEP) for HIV [89]. These effects were despite the greater odds of the women being raped or having sustained physical injuries and serve as an example of how distinctions beyond commonly measured demographic or socioeconomic differences are related to adherence.

3.1.1.4 Constructs Related to Behaviors Associated with STI Risk

Risk Compensation The theoretical causal structure of risk compensation requires an *intentional* comparison. That is, an STI outcome is reduced in susceptibility or severity due to some intervention or change in social conditions,

which individuals recognize. Some of these individuals may "compensate" by tolerating a greater risk of infection via some other causal pathway [90]. Some researchers have theorized that individuals actually are most comfortable at a particular level of riskiness (risk equilibrium) [91, 92].

Because risk compensation can attenuate the effects of efficacious interventions [93], there are numerous efforts to measure the phenomenon, for example, in the context of circumcision trials in several African countries [94, 95]. A population-level analysis of DHS data in 10 sub-Saharan African countries showed no correlation between promotion of voluntary male circumcision and either condom use or increased numbers of partners [96]. Some larger cohort studies of antiretroviral therapy (ART) introduction in sub-Saharan Africa also showed minimal evidence of risk compensation [97], and a later systematic review of risk compensation (the authors used the term *behavioral disinhibition*) in sub-Saharan Africa showed no evidence of the phenomenon during ART expansion in terms of studies included [98]. A systematic review of the effect of ART on risk behavior in fact found an overall protective effect, although heterogeneity among studies was high [99].

ART studies, however, have typically included supportive behavioral interventions in risk reduction and medication adherence [92, 100]. Ecological analyses of demographic surveys during the period of ART rollout found increases is some risky sexual behaviors and STI in many of the same countries in which trials were conducted [98]. A similar case can be made with PrEP studies, in which a randomized trial has shown no risk compensation [101], but a subsequent open-label cohort study showed increases in condomless sex (77% to 86%) and high STI incidence (43%) [102]. Appropriate caution is warranted with ecological analyses and cohorts without comparison groups, but the extent to which complementary behavioral interventions are needed to mitigate risk compensation is surely worth exploration.

Disinhibition Disinhibition is characterized by impulsive behavior, inadequate risk assessment, and frequently disregard for social conventions. We have distinguished disinhibition from risk compensation here in that disinhibition does not require the prior cognitive analysis of an external change (i.e., one does not have to be compensating on the basis of a successful prevention intervention). In their application of a biobehavioral model of feedback and behavioral inhibition via the hippocampal system [103], Hirsh and colleagues posited that intoxication, anonymity, and social power all prevent activation of inhibitory mechanism and are thus causally connected to behaviors that predict STI acquisition or transmission [104]. A recent review synthesized randomized experiments and concluded alcohol use contributed causally to increased sexual risk decision-making, both directly via expectancy and arousal, and indirectly through delayed condom availability [105].

Demonstrating that anonymity increases sexual risk behavior is more complex. First, people might deliberately seek anonymity in order to have sex, and, second, marginalized people might engage in anonymous sex for resources, for example, homeless male youth [106]. That noted, studies of STI and sexual practices have shown correlations between anonymous sex and STI prevalence among MSM [107]. With respect to social power, the most obvious examples are seen in the present exposure of widespread sexual harassment of women, and sometimes young men, by men in positions of power. Both in politics and in the entertainment industry, there is abundant evidence of powerful men seeking or demanding sex from women and using the social power to protect themselves from the appropriate consequences. More generally, research has shown that women who are economically dependent on men are less likely to have sex with a condom [108]. Moreover, social power can be reinforced through social norms. For example, one qualitative study in Tanzania showed how peer norms promoting male decision-making power reinforced intimate partner violence (including sexual violence) [109].

The overwhelming majority of the HIV and STI-related literature mentioning disinhibition treat the phenomenon as completely unhelpful,

but literature from other fields supports the idea that social power can be channeled into prosocial behaviors. For example, a study found that wealthy people who endorsed social inequality (roughly equivalent to social power) were more charitable than those who did not [110]. Other research shows that large-scale charity can actually reinforce disparities in social power [111]. STI prevention interventions aiming to incorporate disinhibition as a prosocial force clearly would have a complex path to tread.

Learned Helplessness The outcome of learned helplessness can be summed up as "nothing I do makes a difference, so why try." [112] For STI prevention, the key individual issue is control over behavioral outcomes. Individual behaviors that have protective value in the general population do not always sufficiently protect members of vulnerable subpopulations (e.g., sexual and racial minorities) [113–115]. The behaviors and outcomes appear functionally equivalent to disinhibition as discussed above; however, the causal mechanism is very different (it is certainly not a question of excessive social power). There is evidence that interventions can alter perceptions of helplessness and consequently behaviors indicative of disinhibition. In one study in Ghana, researchers found that women at high risk for HIV who participated in an oral tenofovir trial reduced unprotected sex, and that the majority of this change was attributable to the women most at risk [116]. Qualitative data accompanying the quantitative estimates showed that women named being in the trial as a reason to use condoms. This polar opposite to disinhibition might have occurred because the women found out they did not have HIV at enrolment and that their odds of acquisition were dramatically reduced (with PrEP) [117]. Similar decreases in sexual risk were shown in Uganda in a population introduced to ART [118]. We note that in both the Ghanaian and Ugandan examples, those receiving medication also received behavioral counseling, and that the Ghanaian women specifically mentioned the counseling in qualitative interviews.

3.1.2 Networks and STI

As we move from individual determinants to social determinants, we give a brief overview of how social and sexual network structure influences the significance of individual behaviors (including partners' behaviors) with respect to STI. Early work in this area was often derived from partner notification programs, especially in countries where public health staff often undertook investigations and kept records [119, 120]. For example, a 1999–2001 Canadian outbreak investigation was managed through adding social network information to standard interviews of infected people about their sex partners [120].

Broad portraits of transmission networks may be inferred from core group or core area research—the principle of concentrated chains of transmission among subsets of a population or in select geographic areas [121, 122]. For example, UK census data from one city revealed that several STI (especially gonorrhea) were concentrated in inner urban areas [123], and US neighborhoods with high rates of illicit drug sales were associated with high-risk sex partnerships and STI diagnoses [124, 125]. These data show that spatial location and relationships to "core group" members are determinants of risk and STI over and above the effect of behaviors and sociodemographic characteristics. In fact, those at risk of STI acquisition are not necessarily core group members (as behaviorally defined) themselves. They are simply "adjacent" to them in social or sexual networks, as demonstrated in one recent analysis of HIV seroconversion among young MSM in a US city: the rate of seroconversion for an individual increased substantially with each HIV-infected person in the individual's network [126].

3.1.3 Social Determinants of STI

It has been long recognized that social determinants of health have an impact on sexually transmitted infections (STI). Research has shown that factors such as imbalanced sex ratios, poverty and income inequality, education, discrimination,

and access to health care have been associated with high rates of STIs [127]. National and regional social and economic changes and related policies can also differentially impact racial and sexual minorities in ways that can lead to a heightened risk of STI acquisition. Thomas and Thomas examined historical data and identified economic practices that disproportionately impacted black persons in that they reduced employment prospects and housing options. This led to poverty, migration, imbalanced sex ratios, and segregation, all of which can influence behaviors associated with STI acquisition and transmission [128]. Indeed, a national study in the United States found that counties with higher rates of poverty and violent crime and lower sex ratios had significantly higher levels of concurrency [129]. Black respondents were also significantly more likely to live in the counties with poorer social determinants [129]. We examine a few specific social determinants repeatedly identified as contributors to STIS—socioeconomic status (SES), discrimination, and health care access—and their role in STIs in more detail below.

3.1.3.1 Socioeconomic Status

Socioeconomic status (SES) is defined as the "social standing or class of an individual or group" and is "often measured as a combination of education, income, and occupation." [130] Marmot's concept, referred to as the status syndrome, draws on SES research to state that social standing, or social class, can greatly impact health, disease, and mortality [131]. An examination of data from numerous countries shows differences in life expectancy and mortality across and within countries by SES and related factors such as education level [132]. At a county level, Murray and colleagues examined differences in health across the United States using an SES-related concept that included but was not limited to race, geographic location, income [133]. They found significant disparities in life expectancy and identified 8 different geographic and subpopulation areas that could not be explained by individual-level factors.

Socioeconomic status has also been implicated as a factor in the acquisition and transmission of STIs. Most of the research base from high income countries has demonstrated that a lower SES is related to disease and poorer health outcomes. A wide-scale geocoding project in the US compiled different measures of SES and examined them in relation to various health outcomes including STIs [134]. Local geographic level SES measures, including economic deprivation measures, were associated with syphilis, gonorrhea, and chlamydia. The authors recommend the inclusion of local measures of SES into existing disease surveillance systems [134]. Lower SES has also been associated with STI risk in middle and low income countries. A study of non-pregnant women in India found that lower SES was associated with acquiring an STI during a 1 year follow-up period [135]. It has also been suggested that a lower SES can place chronic stress on persons that manifest in disease acquisition or poorer health outcomes. One study of black female adolescents living in a higher income country found that SES-related chronic stress was associated with acquisition or reinfection of gonorrhea or chlamydia over the next 3 years; this association was above and beyond many other predictors of STI [136]. However, SES impacts on STIs, including HIV, can differ by countries, subpopulations, and phase of the disease epidemic. One study from Malawi focusing on pregnant women attending an antenatal clinic found that a higher SES, rather than a lower SES, was associated with infection with HIV, but not other STIs [137]. It is possible that as an epidemic grows, persons who were originally at lower risk may become infected at higher rates.

Income Inequality and Poverty A substantial portion of SES-related research focuses on income inequality and poverty. Data from countries across the world show a strong correlation between poverty and health; this indicates that a focus on poverty and income inequality is vital for public health [138]. A national study from the United States found that racial disparities in income was a stronger predictor of chlamydia

and gonorrhea rates than race or ethnicity [139]. Additionally, data from 13 countries showed that lower household income was associated with HIV among female adolescents [140]. Finally, poverty can also play a role in key STI prevention activities. A study in Bangladesh found that persons with lower incomes who had an STI were less likely to refer their partners for STI services than those with higher incomes [141].

Education Level Another measure of SES that has been frequently examined is level of education with lower education often associated with higher risk for STI. A study of adults aged 18–34 years in Kenya found that having an education level that was less than tertiary education resulted in significantly higher risk for STIs including chlamydia, gonorrhea, herpes simplex virus (HSV), and syphilis [142]. Sex workers are at higher risk for STIs, and a study in Russia found that street-based sex workers with lower levels of education were at higher risk for STI [143]. Similar to poverty, level of education can influence STI prevention activities. For example, a study offered free, home-based testing for chlamydia to sexually active women under 26 years of age [144]. Women who had at least a college education were more likely to return the screening test than women with less education [144]. Thus, research findings for SES and its components suggest that alternative approaches to the medical model and behavioral interventions, such as structural interventions, may be needed to curb the STI epidemics.

3.1.3.2 Discrimination

Discrimination that is established within structural systems (e.g., institutional racism) and that is experienced by sexual and racial minorities also has a significant impact on health and STIs. Social norms favoring heterosexuality, referred to as heteronormativity, can influence policies that discriminate against those who do not conform and can result in internalized homophobia [145]. In turn, homophobia, including internalized homophobia, can serve as a barrier to accessing health care services. Using national data, one study found lower HIV testing among MSM who reported that same-sex relations were "always wrong" as compared to other MSM [146]. Another national study found that societal tolerance of same-sex relations was associated with lower HIV among MSM [147].

Structural or Institutional Racism Structural or institutional racism and discrimination has also been associated with poor health outcomes and STI. Institutional racism occurs when racism and discrimination are entrenched within institutions in a society. Various governmental policies, often rooted in historical contexts, can codify and perpetuate racism and discrimination. In turn, this racism and discrimination can establish a context ripe with stressors leading to negative health outcomes, and at times, behaviors that may increase risk of certain diseases. Bailey and colleagues have outlined the ways in which institutional racism can lead to poor health including economic injustice and social deprivation, environmental and occupational health inequities, psychosocial trauma, targeted marketing of unhealthy products, inadequate health care, government-related violence and alienation and maladaptive coping behaviors [148]. In a case study, Thomas and Thomas [128] showed how institutional racism leads to high rates of STI in one county in North Carolina.

At the institutional level, discrimination against LGB persons is apparent in many federal, state, and local laws and policies. WHO identified laws prohibiting discrimination on the basis of gender identity or sexual orientation as a core indicator of sexual health. In the US, there are no federal laws that prohibit discrimination based on sexual orientation or gender identity, and laws and policies differ at the state- and local-levels. Research has found LGB persons experience discrimination in employment, including in limitation of benefits for same-sex partners, and housing [149, 150]. Additionally, research has found an inverse relationship between syphilis, which has more recently been concentrated among MSM, and laws permitting same-sex marriage or civil unions in the United States and Europe [151, 152].

Experienced Discrimination At a micro-level, the lived experiences of persons, including exposure to significant or continual racism and discrimination, can place stress on individuals and can impact their health. One study of persons who use injection and non-injection drugs in a major city examined three forms of perceived discrimination—racial, drug-related, and incarceration-related—and STI/HIV risk behaviors [153]. One-quarter to one-third of study participants reported experiencing each type of discrimination. In adjusted analyses, only racial discrimination was associated with a higher number of sexual and drug-using contacts placing those who experienced such discrimination at a higher risk of acquiring STIs. The effect of perceived racial discrimination and STI risk is not limited to those who live in cities or urban areas. A study of black adults living in rural areas also found a link between cumulative racial discrimination and STI risk [154]. Participants were asked about racial discrimination in a variety of settings, including at schools, work, and the criminal justice system, and a variety of situations including hiring, obtaining housing, medical care, credit, loans, and service at stores. Experiences with racial discrimination were common and frequent with an average of 34 experiences reported by participants who were 29 years old on average. Experienced racial discrimination was significantly associated with increased STI risk behaviors and having an STI in their lifetime [154].

Incarceration Inequalities in criminal justice systems have long been recognized in many geographic regions. Perhaps not surprisingly, inequities in the criminal justice system are often also associated with SES and discrimination (e.g., race, sexual minority). For instance, the US had similar levels of incarceration as other high income countries for much of its history; however, policy changes that began in the 1970s led the US to dramatically increase its incarceration rate which lead to dramatic disparities in risk of incarceration between white and black persons [155]. These policy changes including, those related to the "War on Drugs" and accompanying "Three Strikes" laws, which mandate harsh sentences for convictions of 3 or more drug crimes, disproportionately impact black persons [155]. Increasing incarceration rates have been associated with higher rates of STIs, such as gonorrhea, in the subsequent year [156]. Research has showed at least two potential pathways that incarceration can increase risk for acquisition and transmission of STIs. First, the incarceration can interrupt sexual relationships, causing those involved to find new sex partners [156, 157]. Incarceration can also increase the risk of engaging in transactional sex [157]. Second, incarceration can create imbalanced sex ratios in some communities and the lack of potential sex partners may lead to riskier sex including having multiple sex partners [158].

Residential Segregation Discrimination has also been associated with residential segregation, a situation that can lead to health inequities for STIs. This effect has perhaps been most studied in the US. Black persons in the US are the most segregated subpopulation, even though segregation rates have slightly declined over time [159]. Among black adults aged 15–44 years across the United States, residential segregation was associated with risky sexual behaviors including having multiple sex partners without using condoms [160]. An analysis of surveillance data from nearly 300 geographic areas in the US found that areas with higher residential segregation had significantly higher gonorrhea rates than areas with lower rates of segregation [161]. We should note that residential segregation is also often associated with lower SES in the community.

3.1.3.3 Access to Health Services

Structure of Health Systems The structure of a health care system and access to health services is considered an important social determinant of health by many. The World Health Organization (WHO) has stated that, "access to and utilization of health care is vital to good and equitable health." [162] Health systems can be used to improve both individual and population health, and they can also be designed to promote health equity. However, health systems differ across

countries around the world even when comparing countries with similar levels of development and income. A study of 21 developed countries examined equity in use of health care services measured by visits to physicians including general practitioners and specialists [163]. The study found significant differences in the proportion of the population that had any visit to a doctor across countries which may be indicative of the role that the health system can play in health care access. Additionally, the study identified differences within countries and found that those who were in the top quintiles of income had a 50% increase in visits to doctors compared to those in the lowest income quintiles [163]. Finally, a study that examined health care access in Japan, a country that has had universal health care coverage since 1961, found that wealthier persons still had better access to health care than more impoverished persons [164]. Thus, inequities in access and use of health services exist even in countries with universal health care. We think it is important to consider this information in relation to access to STI services.

Association with Other Social Determinants Access to and use of STI services can be complicated by the relationship of STIs and other social determinants and stigma. For instance, research has shown those living in poverty or with lower household income are more likely to have barriers to accessing health care services. A review of access to STI services among adolescents living in low and middle income countries found that cost of STI services remains a significant barrier to access and use of these important services [165]. Similarly, a study of young men living in a city in the US identified cost as a barrier to sexual and reproductive health services [166]. Additionally, cost of STI services can be problematic at a macro-level even in countries with universal health care [167]. The stigma surrounding STIs can also be a barrier to accessing and using STI services for both females and males in various countries [166, 168]. Stigma may have a differential impact on persons with lower incomes as they have fewer choices for health care and may not be able to travel to a different town or pay out of pocket to avoid the potential for inadvertent breaches in confidentiality, including those related to insurance paperwork. Also, STI-related stigma may lead to an inadequate knowledge of the symptoms, or lack of symptoms, for many STIs. Adolescents in low and middle income countries often did not recognize symptoms of their STI which resulted in delayed or no attempts to seek help or health care for their STI [168].

Differential Access by Subpopulation Access and use of STI-related health services can also differ by subpopulation within a given country. Some populations may be marginalized thereby reducing their access to essential health care services for STIs. Stigma and discrimination against some subpopulations, such as female sex workers, can present barriers to using health care services for STI. The impact of the marginalization of sex workers differs across and within countries. Among female sex workers in Catalonia, the majority had some access to health services and most had an HIV test in their lifetime; however, this differed significantly by country of origin and location of work [169]. Some sex workers reported that stigma and discrimination did impact their health care seeking behaviors. A study of female sex workers in Rwanda found that of sex workers who had STI symptoms, only 27% had tried to seek health care services and many reported that they avoided care given concerns about experiencing stigma and shame from health care providers [170]. A study of health care providers who provided STI services to female sex workers in Laos also highlighted the difficulties sex workers may face in obtaining appropriate STI services [171]. Half of the providers reported that they had not received STI training and many had misperceptions about the causes and symptoms of STIs; 53–68% (depending on type of provider) had negative attitudes toward sex workers.

Female sex workers are not the only subpopulation that may experience inequities in health care access and use for STIs. In countries without universal health services, insurance billing practices, which include sending paperwork to the

insurance policy holder for physician visits and health services used, may influence whether or not an adolescent or young adult would seek STI services. In the US, 13% of sexually active adolescents who were on their parents insurance plan reported that they would not seek sexual and reproductive health services because they were afraid their parents would find out [172]. Sexual minorities, particularly those who are adolescents, also may experience inequities in accessing and using STI services. Among adolescents, sexual minority males report higher levels of unmet health needs than heterosexual males, and sexual minority females had higher reports of receiving a physical or check-up than heterosexual females [173]. Immigrants are another subpopulation that often experiences barriers to accessing STI services. A national study in the US found that Hispanic adolescents who chose to conduct the survey in Spanish (a measure of acculturation) were less likely to report having a regular place for health care or receipt of STI testing in the past 12 months than English-speaking Hispanic adolescents [174]. A study in Canada highlighted the numerous barriers immigrants face in accessing health care including economic, geographic, and cultural factors [175].

Finally, access and use of health care does not always mean that some subpopulations receive recommended STI screenings for asymptomatic infections or appropriate testing or treatment when presenting with STI symptoms. A study of MSM found that while nearly all of the men had seen a medical provider over the last 12 months, nearly four in 10 had not received an STI test in the past 2 years despite recommendations for annual screening [176]. A review of studies of health care providers in low and middle income countries also found that providers experienced discomfort in discussing sexuality-related topics with adolescents and some thought that adolescents should not receive sexual and reproductive health services including contraception [165]. Thus, in many instances, inequities in health care access and the provision of STI services persist for many subpopulations.

3.2 Conclusion

The range of social and behavioral determinants of STI is huge. Several topics in this chapter could be chapters in their own right. Nor have we dwelt on social and behavioral theories underpinning the research in this chapter, although we do note that theory is essential to the work. Briefly, individually centered social cognitive models have informed much of the work in behavioral determinants, with broader social factors incorporated into those models through egocentric measures such as perceived social norms [177–179]. Epidemiologic models of social determinants [6, 134, 180] underpin the logic behind analyses of social determinants and STI.

We conclude by returning to Fig. 3.1. This chapter has covered many of the constructs in the figure directly, with the remainder at least implicitly relevant. (Individual attributes received the briefest attention within the section on behavioral determinants.) Regardless of how one constructs a model of social and behavioral determinants, their product is visible in needlessly vulnerable populations and core areas, as well as transmission networks. These very tangible populations, places, and patterns of transmission are where the work of prevention lies, and where we hope our chapter is most useful.

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Part II

Prevention and Control of STI

Antonio Cristaudo

The prevention of sexually transmitted infections (STI) represents a multifaceted approach that tries to combine the role of behavioural, social and biomedical sciences in a single increasingly difficult effort.

During the last two decades, the effects of this synergy have shown many signs of weakness, often becoming itself a factor for increased STI circulation in specific groups of individuals at risk. The success of antiretroviral therapies in contrasting progression from HIV infection to AIDS and their use as prevention of transmission in infected individuals has led to an increase in risky sexual behaviours, particularly among at-risk populations. Meanwhile, the effort of basic research to reach measurable results in the design of effective vaccines against the main STI pathogens has been successful only for HPV and HBV infection, leaving primary prevention of STIs based only on behavioural strategies of harm reduction.

This part focuses on these two seemingly distant scientific areas: the wide range of behavioural interventions against STI diffusion and the matter regarding where the research for the development of new vaccines is going. Massimo Giuliani has been called, in the first chapter of this section, to discuss the role of the behavioural interventions in the STI control. He will critically address rationales, methodological backgrounds and results of the main strategies and programmes aimed to decrease the risk of sexual behaviour in individuals, groups and communities. In the second chapter, an updated review of the results of basic research in vaccine development for STI will be presented and discussed by Alberto Matteelli and collaborators, who will masterfully present advances, successes and failures of the immunological war against STI pathogens in the last two decades of efforts of researchers and clinicians.

4 Behavioural Prevention Strategies for STI Control

Massimo Giuliani

> Sexual desire is sexual desire and its force, in an individual psychology, is independent of the ultimate Darwinian pressure that drove it. It is a strong urge which exists independently of its ultimate biological rationale.
>
> (Richard Dawkins)

4.1 Human Behaviour and Health

Human behaviours are the result of the complex interrelationship between biological, psychological, social and environmental determinants. In synthesis, most human behaviours can be described as a continuous adjustment of strategies aimed to reach a sufficient balance between individual needs and the *pressure* of the physical and social environment. This multi-step process is multifaceted by the reciprocal influence of individual characteristics, such as personality, culture, skills and the societal norms and barriers, such as roles, educational influences of parents, friends and opinion leaders and, not least, prevailing laws and customs.

Within the great variety of human behaviours, health behaviour refers to all the individual activities and efforts aimed to decrease or avoid the risk of a disease, but also to detect and treat a disease when it becomes symptomatic, seeking appropriate medical assistance. In almost all populations worldwide, a large proportion of causes of diseases and disability are associated with *wrong* behaviours or to a weak ability to maintain health habits over time, particularly during the first decades of life [1]. Large epidemiological studies in developed countries have shown that over half of premature deaths and disabilities can be attributed to modifiable risk behaviours and that the association between socioeconomic correlates and life expectancy is independently mediated through behavioural risk factors [2–4].

The evidence that voluntary behaviour is the main determinant of diseases was brought to the attention of the public health authorities just when the undoubted success of the biomedical sciences and the enhancement of awareness, education and life skills of the populations seemed unable to contrast the burden of most behavioural associated-diseases, such as transport accidents, many tumours, metabolic severe disorders associated with diet and inactivity or relevant systemic infections due to travel habits, hospital care or, not least, sexual activities. Unfortunately, most people can recognize these behaviours as health harming and yet continue to engage in them even when undesired consequences emerge. Thus, many efforts are directed to plan and deliver effective interventions to modify harmful human behaviours through

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the enhancement and transfer of risk-perception attitudes and skills for an "effective and durable" change toward preventive lifestyles.

4.2 The Behavioural Intervention Strategies

Behavioural interventions refer to any strategy set up to modify human behaviours, such as those associated with a higher prevalence of chronic non-communicable diseases and injuries [5, 6]. Therefore, the enhancement of health behaviours remains the cornerstone of the prevention of premature morbidity and mortality, particularly in Western countries.

In general, the efficacy of behavioural change interventions depends markedly on the health target domain and, even in cases where the measured effect is positive, its average tends to be scarce. Some years ago, an interesting meta-synthesis showed that the largest effects were measured for behavioural interventions addressing stress management. Intermediate effects were reached through interventions aimed to modify addictions, eating habits and physical activities, while the smallest effects, although significantly positive, were measured for interventions aimed to change sexual behaviour [7]. These findings seem to suggest that health-harming behaviours are challenging to modify, probably due to the difficulties in intervening on the wide spectrum of the determinants involved in each domain.

However, other reasons can be implicated in the complexity of certain health-harming behaviours. Most of the disease-associated behaviours are biologically-based and complex neurological pathways regulate their expression, such as in case of eating, sex and addictions. In their turn, the neurological pathways and associated physical changes regulate motivated and finalistic behaviours required for the survival of the individual and of the species, such as finding food, as well as agonistic and reproductive behaviours [8].

The biological basis of certain behaviours seems to explain why the prevention strategies aimed to change or limit dangerous behaviours in humans failed to reach measurably effective outcomes, particularly during the mid-long term. The fact that these approaches are often ineffectual suggests that human behaviour is mainly automatic, cued by environmental stimuli, thus resulting in actions that are largely unaccompanied by a conscious approach.

Throughout history, humans have constantly attempted to understand and identify the causes and ways to avoid the risk of diseases by changing habits, modifying the pressure of the surrounding natural environment and setting physical barriers between them and their causes, particularly for infectious diseases. Accordingly, public health approaches have sought throughout the history of civilization (a) to control or coagulate health-related behaviour of individuals, (b) to protect individuals from the behaviour of others and (c) to mobilize group behaviour to influence health-related social and physical environments. In this evolutionary process, human sexual behaviour seems to be excluded, and the efficacy of sex in determining diseases or their biological outcomes has remained almost the same as thousands of years ago. In fact, even today, sexual intercourse represents the most stable and effective biological contact that leads to the sharing of biological fluids and pathogens between two individuals.

4.3 Sexual Behaviour and Health

There are several reasons for the re-emerging interest of researchers and public health authorities in sexual behaviour. Firstly, within human behaviours, sexual behaviour remains one of most effective determinants of disability worldwide. In addition, it represents the first cause of health assistance of the young, due to the two main biological outcomes of unprotected sex: unintended pregnancies and sexually transmitted infections (STI), including HIV [9]. In Europe, sexual behaviour is included in the *top ten* behavioural determinants of disability after transport accidents, smoking and drinking, substance use and misuse, diet and physical inactivity [10].

During recent decades, different investigators from different disciplines have tried to construct a modern concept of *human sexual behaviour* thorough a wide range of theories, models and approaches. Despite the contribution of the many behavioural studies that Alfred Kinsey launched

after the Second World War, "what sex is" is still an important question without a measurable response. The meaning of the term itself, "sex", tends to change relevantly within general population according to gender, age, sexual orientation, ethnicity, educational level and mores. As for other human behaviours, sexual behaviour is the complex result of the interrelationship between different factors, some of which are more evident and measurable because associated with perceptible behaviours, while others are less evident and associated with the wide spectrum of individuals' values and their social and cultural environment.

In fact, what we define as "sexual behaviour" includes a wide set of biological inputs, actions, cognitions, knowledge, skills, perceptions and mores, which constitute its main structural components. These components represent a "personal surrounding environment" where the specific characteristic of sexual behaviour (i.e., frequency of contacts, practices, n. of partners, etc.) manifest themselves (Fig. 4.1). This non-reductionist

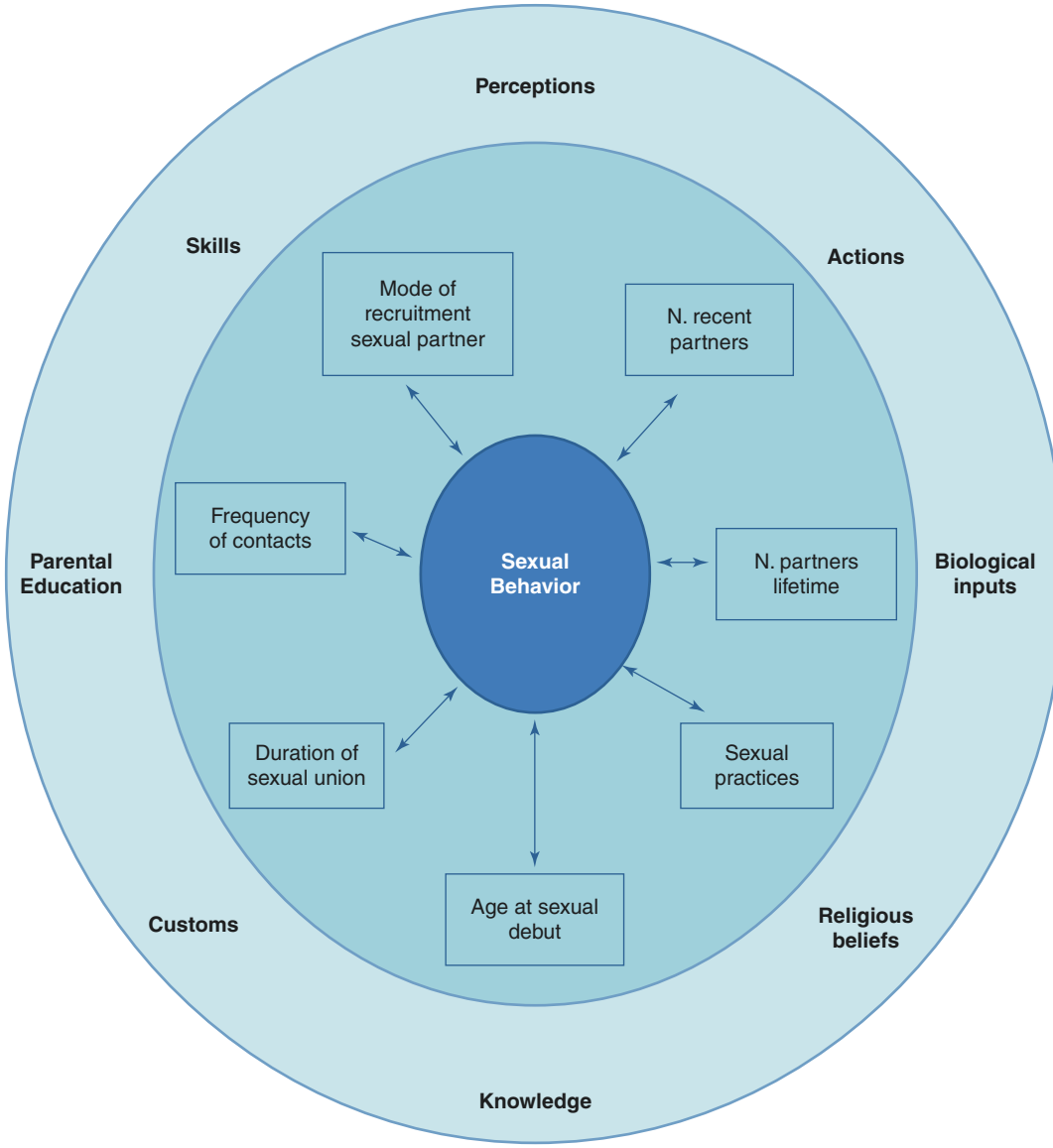

Fig. 4.1 Sexual behaviour as result of the interrelationship between selected proximal and distal individual factors

perspective is extremely relevant when considering sexual behaviour as a target for behavioural interventions aimed to reduce STI risk. Many studies have demonstrated that the behavioural prevention programmes aimed to influence and modify only the explicit characteristics of sexual behaviour would be expected to fail or lead to less effective and durable effects over time than those that provide direct or mediated actions toward the social, economic cultural-oriented environment of the individuals. Moreover, compared to other health domains, where behaviour is generally an individual and self-directed activity, sexual behaviour is hard to modify because it expresses itself in a social relationship, which involves at least another or more persons.

When we refer to sexual behaviour in terms of public health priorities and infectious consequences, generally we consider the behavioural aspects of sex, as a sequence of acts and practices expressed mutually within a sexual couple or group, and where the most relevant deeper determinants are not immediately perceived. Consistently with this approach, sexual behaviour can be partitioned into many specific practices that can vary largely on the basis of age, gender and sexual orientation. Importantly, different levels of risk of STI transmission/acquisition can be attributed to these practices (Table 4.1). This fragmentation is the result of several studies that have emphasized the need to understand the wide variability of sexual behaviour particularly among the individuals of different sexual orientation or gender also in order to plan interventions aimed to enhance awareness on specific STI risk or modify specific practices.

Since the end of the 1980s, another relevant issue that has contributed to boosting a deeper analysis of human sex was the HIV-1 epidemic. This in fact showed how much behavioural prevention strategies were urgently needed to stem the spread of this incurable STI, and at the same time, how knowledge and tools available for such interventions were poor and methodologically weak. Meanwhile, several studies, mostly conducted in western industrialized societies, started to provide unprecedented information on the characteristics, prevalence and distribution of sexual risk behaviours in the population. These studies concurred to define some relevant elements concerning the sexual risks that are currently valid in many Western countries. The two

Table 4.1 Selected unprotected sexual practices, risk level and associated STIs

Sexual practice	STI risk level	Associated STI
Masturbation (self-)	Absent	Not described
Personal use of sex toys	Absent	Not described
Exposition to urine	Absent	Not described
Mutual masturbation	Low	Genital warts
French kiss	Low/ moderate	Hepatitis type B
Physical contact without oral or genital penetration	Low/ moderate	Pubic lice, genital warts, molluscum contagiosum
Cunnilingus	Moderate/ high	Genital herpes simplex infections
Receptive/insertive oral intercourse	Moderate/ high	All STI and HIV-1 infection
Rimming	High	Hepatitis type A, *N. gonorrhoeae* infection
Sharing sex toys and dildos	High	Hepatitis type C, HIV-1 infection, LGV
Fisting	High	Hepatitis type C, HIV-1 infection, LGV
Receptive/insertive vaginal intercourse	High	All STI and HIV-1 infection
Insertive anal intercourse	High	All STI and HIV-1 infection
Receptive anal intercourse	Very high	All STI and HIV-1 infection

STI sexually transmitted infections, *LGV* lymphogranuloma venereum, *HIV* human immunodeficiency virus

principal key-points highlighted by these studies were: (1) the distribution of risky sexual behaviours across populations tends to be very biased. The majority of people has low-risk sexual behaviours, while a small minority engages in high-risk practices; (2) the various dimensions of sexual behaviour tend to be highly correlated. The same people engage in a variety of high-risk sexual practices. The same others in low-risk behaviours [11].

4.4 Why Is Sexual Behaviour Hard to Change?

Reproductive and sexual behaviours are intrinsic, natural phenomena, strictly associated with a fundamental purpose for human existence. Moreover, to ensure optimal functioning, these behaviours are based on two important complex sensations in humans: desire and pleasure. Sensations that have in *craving* and in *reward* their biological analogues, respectively. The desire/craving pathway in mammals can be considered the most important biological component of motivation and goal-directed behaviours and an intermediary process aimed to transform all strategies into action. Evolution has ensured that this action-promoting process is governed by a complex neurobiological pathway that ensures a rewarding experience in sexual and reproductive acts through the pleasure/reward pathway.

However, humans derive pleasure from sex far more than most animals, and the duration of each episode, the frequency and the variability of sexual acts far exceed those of all other animals [12]. During the last decade, many studies have described the neural basis of sexual drive motivation and arousal [13, 14]. Briefly, several brain structures (i.e., orbital frontal cortex, prefrontal areas, pyramidal cells of the prelimbic area) are involved in receiving and interpreting incoming sensory information as well as in sending signals to other important cerebral components (i.e., *nucleus accumbens* and *tegmental ventral area)* where motivation, impulse and craving are generated, so that the person is driven to engage in behaviour to reach the sexual object. Commonly, once gratification has been achieved, the individual perceives excitement, satisfaction and pleasure. This circular pathway generates a strong motivation to engage repeatedly in these reward-seeking activities. Important studies have demonstrated how the chronic activation of these circuits leads, also under the influence of individual variables and operational conditioning, to sexual addiction [15].

4.5 Societal Changes, Sexual Behaviour and STI Risk

Factors associated with the risk of acquiring or transmitting infections during sex depend on many characteristics, some of which are intrinsic to individuals, while others are based on their social interactions and on the norms of the social community to which they belong and reside [16]. The same variation of the frequency measures (i.e., incidence and prevalence rates) of the STIs is often influenced by fast and durable changes in cultural norms and beliefs that influence social visibility and acceptability of sexual rules and encourage or limit sexual activity in a group or population.

After the Second World War, in many developed countries, some important societal changes indirectly influenced the likelihood of the general population to engage in at-risk sexual behaviour. The progressive decrease of the median age at sexual debut observed in most Western countries has contributed to exposing adolescents of both genders much sooner to the risks associated with sexual activity. At the same time, the effects of an early coitarche on STI risk were amplified by a shift toward later marriage that has led to an increase in premarital sex, at levels higher than in developing countries [17].

Before the year 1970, the age at marriage in Western populations commonly overlapped with age at first sexual intercourse and age of childbirth, particularly among women. During the last four decades, the two ages have gradually diverged, in both genders. Consistently, the time-

span between ages at sexual debut, marriage and first pregnancy, the latter two as proxies of a stable sexual relationship, has increased by several years, exposing the individual longer to changing sexual partners and increasing his/her own STI risk. Some observations have shown that women with a longer duration of premarital sex were more likely to report multiple sexual partners and had higher odds of HIV-1, HSV-2 and other STIs [18]. In France, where decennial trends of median age at first sex, at marriage and at 1th childbirth for both genders were available, the median age at first sexual intercourse for women decreased sharply from 1950 to 2010, becoming close to that of men, so that the gap has now narrowed to a few months (17.6 years for women and of 17.4 for men). During the same period, the ages of marriage in both genders and the age at first pregnancy progressively increased, confirming a progressive dissociation between sexual debut and conjugal life. In France, the span between the ages at coitarche and at marriage has increased from 2.8 years in 1970 to 12.3 years in 2010 (Fig. 4.2). Importantly, no effect on this continuous decrease of coitarche in both genders, remained ongoing also after the emergence of AIDS in the mid-1980s [19, 20]. While there has been a decrease in age at sexual debut in the youngest, in recent decades a slow but progressive increase in the age of cessation of sexual activity, in adulthood, has been observed. Today, some relevant social factors have influenced and enhanced the sexual life of individuals in their later ages (Box 4.1). Worldwide, this fact has caused a growth in the population of elderlies who engage in sexual behaviour at-risk for STI and HIV infection [21]. Several surveys conducted over the past 30 years have shown that the self-reported quantity and quality of sexual experiences among 70-year-olds have improved in recent decades [22], that nearly 74% of married men over 60 remain sexually active, that 28% of men aged 66–71 have a sexual intercourse on a weekly basis [23]. Smith et al. reported that, among a small sample of individuals over 70 years of age residing in New York, 18% of women and 41% of men were sexually active [24]. The prolonged sexual life among elderlies has also led to an increase in STI risk. From 1996 to 2008, incidence rates of STI among individuals aged 55–59 were described as increasing in the United Kingdom, Australia and the USA [25–27].

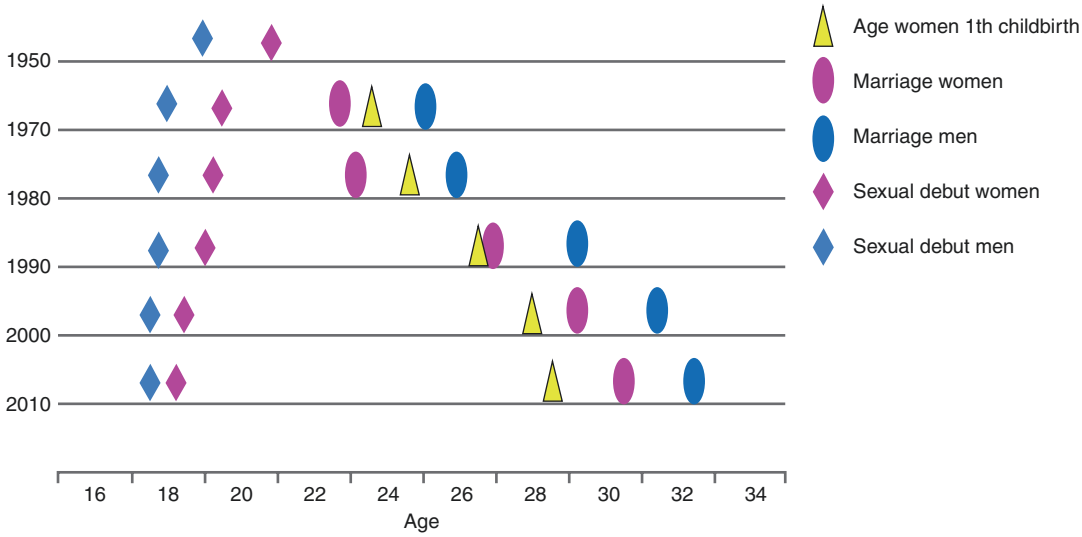

Fig. 4.2 Distribution of median age at sexual debut, marriage and childbirth by gender in selected years (data source: Institut National de la Statistique et des Etudes Economiques (INSEE), 2018 France)

Box 4.1 Selected Factors Associated with the Prolonging of Sexual Activity in Later Ages

- Prolonging of life expectancy due to improved medical care and healthy habits
- Improved physical and psychological well-being
- Lower impact of the chronic diseases care upon sexual performance
- Increased healthy survival
- Availability of treatment against erectile dysfunctions
- Achieved social acceptability about the sexual activity in the elderly

4.6 The Risk of STI Across Populations

It is well known that the likelihood of acquiring an STI is not equally distributed in the general population. Several individual, societal and environmental factors can contribute to confer different levels of risk for different persons or groups in a large population. To date, hundreds of epidemiological studies demonstrated that some population groups show a higher concentration of risk factors and for this reason they express a greater proclivity in acquiring or transmitting an STI. Commonly, these populations also represent the target groups for preventive programs and for participation in studies aimed to evaluate behavioural interventions, where the size and direction of effects can be better evidenced, due to their greater risk level at baseline.

Using the most relevant proxies of risky sex, the groups that have been most frequently included in evaluation studies are adults with current STIs, adults who have multiple sex partners and adults who do not use consistently condoms. Sexually active adolescents are also at increased risk for STIs and have demonstrated to be an appropriate population for evaluation studies of behavioural interventions.

Other examples of population groups at increased STI prevalence rates are: men who have sex with men (MSM), persons with low incomes living in urban settings, current or former inmates, military recruits, persons who exchange sex for money or drugs, persons with mental illness or a disability, current or former intravenous drug users, persons with a history of sexual abuse and patients attending public STI clinics.

Social deprivation and belonging to minorities represent additional factors of risk-increase for STI both in developing geographical areas and in developed countries. In the USA, for instance, within the populations with a particularly high prevalence of STIs, African Americans show the highest STI prevalence of any other racial/ethnic group. Higher STI prevalence rates have also been measured in American Indians, Alaska Natives and Latinos compared to white persons.

In fact, several important social and economic issues influence STI risk particularly in urban settings. Poverty, homelessness, incarceration, limited access to quality health care, lack of medical coverage and other socioeconomic issues create a context that confers considerable risk for all STIs. Stressful life circumstances often eclipse STI as a health concern, with survival needs forcing people into harmful practices and transactional relationships, often based on sex-exchange. Current data from most of the studies did not permit an evaluation of the effect of these social and structural boosters. Nevertheless, it is important to recognize them and their role in STI circulation in many areas of the world.

4.7 Transmission Dynamics of STI and Behavioural Intervention

To date, we know over 35 different pathogens, such as viruses, bacteria, protozoa and parasites, which have been selected during eras to live and transmit within human populations by sex. During the millenniums of history, most of the sexually transmitted pathogens have phylogenetically evolved to circulate durably in human communities through reproductive behaviour and to maintain their incidence rates at adequate levels to avert or limit their extinction. In fact, the trans-

mission dynamics of a STI are not only influenced by sexual behaviour as a set of specific practices or actions, but also by additional factors to support its movement within a human community. Using HIV-1 infection as a reference, in 1997 May and Anderson modelled the influence of these factors, designing a model defined as the *reproductive rate of a STI*. This epidemiological model includes some important parameters that explain how an epidemic is generated from single index infections, when all the individuals of a population are susceptible [28]. Three main factors were identified in this model: the probability of transmission to a susceptible host by a single infecting contact (β), the number of contacts established between infected and susceptible hosts (c) and the duration of host infectivity (D). These factors interact synergistically producing a reproduction infection rate (R_0), according to the formula $R_0 = \beta * c * D$. When the reproduction rate is greater than one, the infection tends to spread, generating new cases. Differently, when the rate is less than or equal to one, the epidemic will tend to end or remain in a state of equilibrium, respectively. It is interesting to note that, in the case of STIs, these three factors have a behavioural dimension. In fact, the probability of transmission is strongly influenced by the use of barrier methods (i.e., male or female condoms) and by the choice of specific sexual practices negotiated with the partner (i.e., masturbation vs. genital intercourse). Similarly, the duration of a host's infectivity depends primarily on their ability to recognize and manage their own infection. Finally, the number of contacts is also the measure of the number of recent sexual partners of the host, and of other characteristics of his/her transmission network [29]. To reduce the incidence of a STI, behavioural interventions must be able to affect this reproductive pathway of infection. In general, the strategies built to limit, directly or indirectly, the size of the factors associated with the risk of acquiring or transmitting an infection can be considered useful to this purpose. The aim to shorten the infectious period, reduce transmission probability and number of sexual contacts all contribute to reducing the R_0 and the STI incidence and all these measures can be identified as quantitative outcomes of a behavioural intervention strategy.

Strategies to reduce the size of the factors implicated in the circulation of STIs are those that preclude or limit the exposition to others by sexual behaviour or reduce infectivity and transmission likelihood. Box 4.2 contains a list of selected behavioural strategies included in most of STI/HIV prevention programs conducted during the last three decades worldwide.

To deliver knowledge, attitude and skills to at-risk individuals, behavioural interventions for STI prevention are based on diversified communication techniques. Box 4.3 shows a list of the

Box 4.2 Selected Behavioural Strategies to Reduce STI/HIV Risk

- Sexual abstinence
- Sexual debut delay
- Monogamy
- Consistent condom use
- Condom use negotiation among women
- Reduction of recent sexual partners
- Decrease in substance use
- Seeking care behaviour
- Adherence to harm reduction programmes
- Adherence to biomedical interventions

Box 4.3 Panel of Different Single-Session Behavioural Techniques Utilized in 20 Intervention Studies Included in Eaton et al. [30]

- Didactic education
- Personalized feedback
- Communication skill building
- Safe sex discussions
- Eroticizing safe sex
- Activities designed to alter perceived social norms
- Condom skill training
- Motivational interview
- Role-playing risk scenarios

most frequent communication strategies, utilized in a series of studies included in a meta-analysis of Eaton et al. focused on evaluating single-session behavioural interventions [30].

4.8 Behavioural Interventions to Prevent STI and HIV Infection

When sexual behaviour is the target for behavioural interventions, the latter can be defined as all strategies aimed to promote behavioural change in individuals, communities and small high-risk groups, by use of a wide range of educational, motivational, peer-led and skills-sharing approaches [31]. In recent decades, the analysis of behavioural interventions in the STI control has been a topic that has raised interest and debate among many research groups around the world.

In the literature, the term "STI control" is frequently used interchangeably with "STI treatment", yet these are quite different things. In this context, it should be pointed out that STI control is a public health action, achieved by implementing strategies composed of multiple synergic interventions, aimed to reduce incidence and prevalence measures. In fact, control of any communicable infection is a public health outcome, while the treatment is a biomedical intervention that usually does not result in lower disease burden.

The epidemic of HIV-1 infection, which exploded in the late 1970s in the Western world, dramatically opened, at the end of the twentieth century, the issue of the dangerousness of sexual behaviour for human health, promoting urgent prevention programmes based on differentiated strategies of change-behaviour and launched randomized controlled studies to measure their effectiveness in groups of susceptible people of different gender, age and sexual orientation.

To date, the large number of programmes aimed to direct sexual behaviours of populations toward safe practices have largely encouraged people to reflect on their behaviours and promoted an enhanced social awareness of the risks of STI for human health. However, in many areas of the world, dominant cultures are still very reluctant to consider sex and sexuality as important issues for public health and the social development of their populations. This attitude has greatly limited the inclusion of behavioural strategies for STI prevention and has contributed, during the last three decades, to the progressive spread of HIV and other STI, particularly in areas such as South East Asia, Africa and India [32].

All behavioural interventions for STI prevention are mainly aimed at reducing the effect of individual determinants that affect the risk of acquiring/transmitting an infection but must also consider the specific transmission dynamics at population level. In other words, effective interventions must address risk control through a dual approach that takes into account two main levels: the individual and the social/cultural level. This is because, in an individual, the behavioural and biological determinants of a STI, defined by Boerma & Weir as "proximate determinants", are in turn strictly influenced by a broad spectrum of demographic, cultural, socio-health and economic factors that modulate their effect in the dynamics of spread of infection in a population [33]. Moreover, despite the evidence of a substantial effectiveness of behavioural interventions directed at the individual, considerable evidence suggests that this approach alone is often not enough to ensure the adoption of safe practices for a long time or their maintenance in the presence of factors of moderation of the effects. Thus, multiple levels of interventions can assure a strengthened efficiency of the outcomes over time due to modifications of over-individual factors. Results from preventive approaches for HIV infection have suggested that there is growing a need to group different strategies together to achieve cumulative effects [34, 35].

4.9 Acceptability of the Behavioural Intervention

The aims of behavioural risk-reduction interventions must be realistic and applicable to be recognized as achievable by the target population.

In particular, the initiatives aimed to modify at-risk sexual behaviour should be tailored to the sexual habits of the susceptible individuals. Otherwise, behavioural programs have scarce possibilities of producing measurable positive outcomes.

Risk-reduction initiatives targeted at adolescents based on abstinence, delay in sexual debut, or monogamy have proven to be inappropriate and ineffective in many settings. A large study that evaluated the role of abstinence coverage in sexuality in the USA education showed that the States with mandates emphasizing abstinence had higher STI rates than States with non-mandates for abstinence [36]. Differently, abstinence-plus interventions that promote sexual abstinence but also encourage safer-sex strategies (e.g., condom use) for sexually active participants seem to achieve better results. Results from a Cochrane systematic review that assessed the effects of abstinence-plus programmes for STI/HIV prevention in high-income countries found a significant protective intervention effect for at least one behavioural outcome and for the increase of HIV knowledge [37].

4.10 The Levels of Intervention

Despite the fact that most of the prevention interventions target STIs, have been carried out on at-risk individuals using one-to-one approaches, we know different levels of delivering a behavioural intervention.

For didactic purposes, at least four main types of behavioural intervention level can be planned and delivered according to the outcomes, times and target group of a preventive programme: (1) individual level, (2) relationship or couple level, (3) family level and (4) community level.

The *individual-level intervention* concerns interventions specifically designed and targeted for single people or patients to promote commitment to change. Commonly, these interventions emphasize motivational factors, provide skill training, and enhance communication style, sexual negotiation, skills in condom use, modification of false beliefs and peer norms. Enhanced screening programmes to improve case finding and awareness (i.e., HIV Counseling & Testing initiatives, point-of-care rapid testing for selected STI, antenatal screening for STI/HIV, etc.) among asymptomatic individuals can be also considered individual-centred behavioural interventions.

The *relationship or couple-level intervention* concerns actions targeted for sexual partners, mainly in a stable relationship where one of them or both have a STI (i.e., HIV-discordant couple, STD patient and susceptible partner, etc.). Some studies have revealed that this approach is useful for adolescents, particularly in teenage girls that are in a power-imbalanced relationship with male partners. These interventions aim mainly to facilitate the disclosure of risk within the couple and promote care-seeking behaviour among partners.

The *family-level intervention* is commonly aimed to promote increased communication between parents and adolescents about sexuality, sex and STI prevention, increase parental monitoring and perceptions of the young regarding parental control as increased family support. Recently, this intervention has proved to be useful to promote vaccination willingness among parents of adolescents eligible for HPV immunization.

The *community-level intervention* tends to build and promote shared rules and social norms that sustain and promote safe sex and health practices in a well-defined population group identified by a common social status or characteristics, such as age, sex, ethnicity, sexual orientation, work place, etc. This type of intervention can provide advantages in the achievement of preventive objectives because it exploits mechanisms of imitation and social support between peers. Differently, current cultural attitudes, social norms and perceptions about STI in a specific community can affect the feasibility of a community-level intervention and limit the exposition of a large part of susceptible population to a preventive strategy.

4.11 Theoretical Framework of Behavioural Interventions

Behavioural interventions are based on a spectrum of psychological models which support their conceptual frameworks, characteristics of

delivery (i.e., face-to-face, group sessions, role-play) and their specific key elements and tools (i.e., video, written materials, practical exercises). The principal models represented in most of the intervention protocols and evaluation studies are: the Theory of Reasoned Action and Self Efficacy [38, 39], the Social Cognitive Theory [40], the Health Belief Model (HBM) [41], Information, Motivation, and Behavioural Skills Model (IBM) [42] and the Theory of Readiness to Change [43].

The different theories that try to explain the determinism of human behaviour and predict the steps of its changes suggest that there are a limited number of critical factors underlying an individual decision to plan or refuse a given behaviour. Thus, the different approaches seem to suggest that STI preventive behavioural interventions, in order to be effective, must target a single behaviour-to-change, identified on the basis of a careful assessment of an individual's sexual history. However, the change of behaviour is usually a slow, multi-step process during which the individual must achieve a new life prospective, resist previous behavioural inputs, organize new choices and be able to sustain over time this new balance through the constant recognition of clear and measurable individual and social benefits.

Jemmott and collaborators in a 20-min one-time intervention used Social Cognitive Theory as a conceptual framework for a Sister-to-Sister Project where a behavioural counselling intervention was delivered in a culturally sensitive and gender-matching way during a routine medical consultation. The intervention was designed to maximize empowering and educating women in condom use and negotiation with partner [44]. Differently, Crosby et al. used the theory of Information, Motivation, and Behavioural Skills Model (IBM) to prioritize correctness and consistency of condom use among men by a one-time, 45- to 50-min session [45]. The RESPECT Project, based on the Theory of Reasoned Action and Social Cognitive Theory, was an individual-level, client-focused intervention based on brief, 20-min interactive counselling sessions and the use of a "teachable moment" to increase patients' concern about their vulnerability and promote risk reduction [46, 47].

4.12 The *Teachable Moment*

A mechanism that plays an important role in determining the effectiveness of behavioural interventions, particularly when they are brief, is what is referred to as the " teachable moment" [48]. Across several approaches, from social sciences to medical disciplines, the "teachable moment" has been proposed as an event or circumstance which can lead an individual to be prone toward a positive behaviour change. The term can have a conceptual or an operational meaning. In scientific literature, the use of the term falls into three main categories: (1) the "teachable moment" is synonymous with "opportunity" (81%); (2) represents a specific "context or setting" that leads to a higher than expected behaviour change (17%); (3) represents a phenomenon that involves a cueing event that prompts specific cognitive and emotional responses (2%). Moreover, in conditions of real-life health assistance, the term concerns a special setting-interaction where the clinician can easily transfer to the patient knowledge and attitudes to enhance awareness and self-vulnerability perceptions. In fact, interventions to promote healthy behaviours benefit from a greater awareness of the target population and their increased perception of vulnerability toward the disease identified as preventable. The "teachable moment" is extremely important also in interventions aimed at smoking cessation, in those used for the treatment of alcoholism, the reduction of cardiovascular risk and cancer prevention, where significant changes in lifestyle are required to prevent, in addition to a disease, a serious threat to life.

It is therefore necessary to make use of these moments of learning. For example, the willingness to change risk behaviours is more likely to occur after a STI diagnosis and this may partly explain the positive effects of behavioural interventions observed with patients in the clinical setting, even in single sessions. Receiving a diagnosis of STI can represent "per se", for many people, a powerful motivation to change their risky sexual behaviour. This makes it an important window of opportunity for an effective intervention of behavioural change for the future.

A descriptive exemplification of the *teachable moment* can be represented using the dynamic interaction between a provider and client when

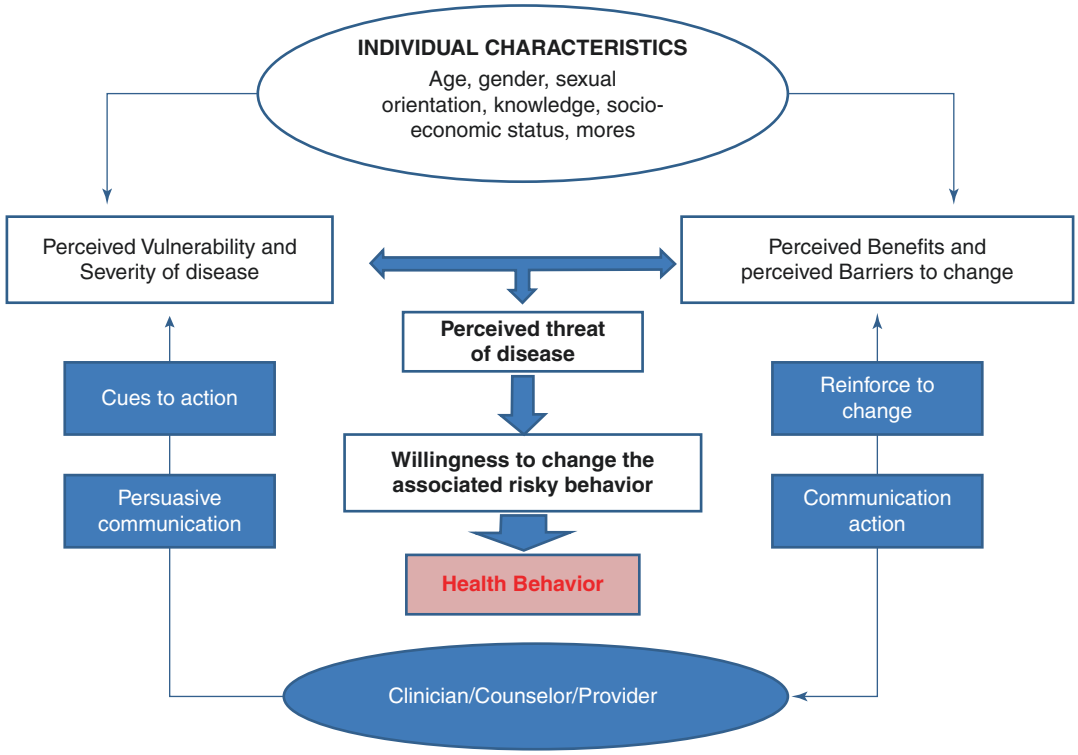

Fig. 4.3 Promotion of behavioural change during a dual circular communicative relationship according to the Health Belief Model

the purpose is to promote a health behaviour by leveraging the client's knowledge and attitudes. In the communicative relationships, the characteristics and perceptions of the client should be positively affected by the communicative and persuasive action of the provider. If the pressure of the relationship is able to influence the perceived vulnerability of the individual as well as its perceived severity of disease, and meanwhile to promote awareness of benefits minimizing the difficulties, then the willingness to change a behaviour towards better health will be reached (Fig. 4.3).

4.13 The Structure of a Behavioural Intervention

Generally, most of the behavioural interventions aimed to change at-risk sexual behaviour in individuals or groups are based on three different synergic phases that represent their own three different cognitive and pro-active areas towards which the contents and objectives of the preventive strategy must have a positive influence to reach effective outcomes. These phases are frequently defined in different ways, but they can be generally categorized in a first phase during which the intervention actors help the individual *to recognize the STI risk*, in a second phase aimed *to elicit a commitment for change* and in a third phase which is fundamental *to transfer realistic preventive skills* to at-risk people. Each phase, in turn, can be split into different actions supported by different communication methods, contents, materials and techniques. Commonly, the three phases are delivered using one of the above-mentioned standardized approaches, within a one-to-one or group relationship and through effective communication techniques.

To our knowledge, there are no examples in the literature that have provided a detailed list of the content that should be delivered during the three phases of a behavioural intervention for STI

prevention. Only Shain et al. seem to have collected and presented a good example of contents, materials and instructional techniques for reducing sexual risk through an individual or a group relationship, although the behavioural intervention was aimed to prevent HIV infection among minority women in the USA [49].

4.14 Multi- vs. Single-Session Behavioural Interventions

Although behavioural interventions have provided positive evidence in reducing the incidence of STI, many of these are organized in several phases and sessions, and thus have a considerable impact on the patients and in terms of the care resources to be deployed [50, 51].

Moreover, one of the major difficulties of multi-session behavioural interventions is that they must be conducted and adapted according to the current procedures of health assistance in clinical services. In recent decades, these services have continued to experience significant reductions in resources and staffing, along with a general lack of tools to retain patients for the duration of an extended intervention [52]. Limited resources, in terms of funds, time and staff often make multi-session interventions impracticable or lead clinicians to modify their structure and power.

Many observations seem to suggest that any intervention aimed at STI prevention that is conducted in the routine of clinical services must be short, targeted and effective. Today, single-session behavioural interventions are particularly interesting since they appear to have the best characteristics to be successfully included in service activities. There are enough experimental studies with outcome data that make it possible to determine whether interventions based on a single session can lead to significant reductions in the incidence of STI compared to the standard of care alone.

In a meta-analysis of 20 primary experimental studies using biological outcomes of effectiveness, such as STI incidence, a control group and a case follow-up up to 2 years for a total of 52,465 participants were included that were subjected to 29 different single-session interventions [30]. Six of the interventions were conducted with adolescents and 23 with adults. Women represented 37% of the overall study sample. Median age of participants was 29.5 years. Most of the studies were conducted in groups of individuals living in the USA. The studies included varied greatly in design, session duration and components. The length of the interventions was between 140 and 250 min, with a median duration of 79 min. The average follow-up time was 58 weeks. The most frequently used interventions were didactic education and skills development strategies. In a smaller number of interventions, the motivational interview was the methodological framework of reference. Overall, the interventions used succeeded in reducing the incidence of STI in the target population. The average weighted risk was 0.65 (95% CI = 0.55–0.77). The meta-analysis showed how significant reductions in the incidence of STI were obtained particularly among female workers, among American adolescents, and among STI patients. These results demonstrate that single-session behavioural interventions are effective in various scenarios and in different risk groups and that interventions based on minimal costs can lead to clinically significant outcomes.

4.15 Moderating Factors Related to the Effectiveness of the Intervention

The final effect of a behavioural intervention can be affected by specific factors not strictly associated with the characteristics of the delivered programme, but intrinsically linked to the target population or its social environment.

In a meta-analysis published in 2011, Scott-Sheldon et al. found that ethnicity could moderate the efficacy of the intervention [53]. The meta-analysis examined how some characteristics of the intervention and of the target population may influence the amount of the STI prevention effect. The most effective interventions are those conducted with non-white participants, with African Americans, those of longer

duration, those that included *ad interim* evaluations close to the completion of the intervention, those compared with the waiting list and control groups and those that were conducted at individual and group level, compared to those conducted at community level (i.e., mass media). On the other hand, characteristics such as age, gender, risk group, the year of publication of the study, the quality of the study design or the presence or absence of a control group do not seem to significantly change the size of the effect of reduction of STI incidence [53].

The results of the studies show that behavioural interventions in a single session can have a significant impact in terms of clinical outcomes on a wide range of population groups. The measured reduction in STI incidence among participants in intervention groups is close to that achievable with biomedical prevention technologies. This suggests that focusing on one-session interventions, especially for the purpose of conducting visits during the routine, is practical and efficient and can lead to savings in case handling costs.

In addition, it is possible that interventions in a single session, repeatedly exposing patients to change behaviour pressure during routine care, may limit the decrease in effectiveness as follow-up time increases, playing a role in stimulating and improving effectiveness in the long term.

The interventions of a single session can also contribute to solving the problems of partial effectiveness, risk compensation and non-adherence if associated with the new biomedical strategies of HIV prevention [54].

A recent web-based survey conducted on 580 MSM showed that high financial hardship can be included in the list of moderating factors. Financial hardship was associated with an increase of at—risk sexual practices, such as condomless receptive anal intercourse (ARR: 1.34, 95% CI 1.07–1.67) and condomless insertive anal intercourses (ARR: 1.30; 95% CI 1.07–1.67) [55].

Regarding the long-term results of the interventions, a reduction in the effectiveness of any type of strategy over time is generally observed. Both behavioural and biomedical interventions require high adherence by individuals to maintain risk-reduction strategy or regimes and their effects tend to dilute over time. In fact, studies based on mathematical models have shown that the combination of prevention interventions is a necessary strategy to combat the HIV epidemic [56, 57].

Mathematical models that have evaluated HIV-1 prevention interventions have also shown that even small increases in the risk of behavioural transmission are able to reduce the benefits of a reduction in the risk of transmission obtained with biomedical strategies, such as early antiretroviral therapy for newly diagnosed individuals [58].

In fact, biomedical strategies alone showed low rates of effectiveness, slightly over 50%. Male circumcision seems to reduce HIV risk by 55% [59] and pre-exposure prophylaxis by 44% [54]. The behavioural interventions could improve these rates of reduction achievable with these biomedical forms of prevention, because also the interventions to assess the effectiveness of pre-exposure prophylaxis in preventing HIV have shown that a behavioural dimension such as the "non-adherence" represents a critical factor affecting the efficacy in practice [54].

4.16 Designing and Measuring a Behavioural Intervention Against STI

One of the basic questions of any behavioural intervention aimed at STI prevention is the reason why some individuals are more prone to change their behaviour at risk, whereas others are not. This is because the success of an intervention programme is determined not only by the robustness of the method through which it will be delivered, but above all by the understanding, which of the proximal or distal determinants need to be influenced to ensure a greater probability of behaviour change in a target population. For example, the increase in the use of condoms could be promoted by increasing their availability, also by increasing the perception of individual risk, promoting its

use among peer leaders or limiting the social or cultural barriers to its use. The choice of one or other of these options could make the behavioural intervention effective, weakly effective or ineffective.

Thus, before the program is designed, the nature and complexity of the behavioural problem must be well understood, also through the collection of data and knowledge about individual, interpersonal and social factors of the target population/group (i.e., awareness, values, beliefs, practices, motivations, social networks, barriers, etc.). Obviously, this approach is totally situation specific and suggests that a behavioural intervention that has proved effective in a specific setting or population cannot necessarily be exported to another social, geographical area or group to reach the same results.

A programme for STI prevention based on behavioural interventions should be planned and designed according to a sequence of seven operational steps. Each step, in turn, should be accurately described in terms of: (a) specific definition of the problem; (b) source of data and knowledge useful for its definition; (c) examples from previous evidence; (d) stakeholders involved (or potentially) in step definition and support; (e) additional needed knowledge to complete the framework of the step.

Table 4.2 is a summary of the methodological cues from guidelines and studies published to date. It synthetically describes the main operational steps involved in the design of a preventive behavioural intervention aimed to prevent STI. The contents of the table might not be exhaustive, due to the wide range of key choices in the field, but it can be useful to identify a structured methodological pathway to build a study/programme protocol, from the first identification of the problem to the assessment of the outcomes.

As is required in any evaluation of the effectiveness of an intervention in the biomedical field, the studies that evaluate the effectiveness of behavioural interventions are also required to use rigorous methodologies to control the true amount of their impact on the outcomes and to design standardized procedures to minimize the effect of specific biases on the measures and avoid false measures and conclusions.

Generally, the behavioural intervention evaluation studies based on self-reported data from participants and designed without a random assignment to control biases and effects are considered to be weaker than those with experimental design and based on biological and measurable findings. Thus, randomized control trials (RCTs), which represent the gold standard for evaluating the impact of any intervention or procedure in the biomedical field, seem to the best choice also to evaluate preventive behavioural interventions. However, large debates are still ongoing regarding the appropriateness of RCTs for evaluating BI and some criticisms have been forwarded following the analysis of several studies (Box 4.4). The debates are the expression of the general difficulty in including behavioural sciences "*tout court*" inside the methodological framework of biomedical trials. Regarding this matter, Watters argued that current experimental methods are often inapplicable to study sexual behaviour and cannot be used to assess particularly community public health programmes [60]. It has also been emphasized that RCTs are not appropriate for assessing behavioural programs because they are not able to take into consideration the complexity of behavioural and psychosocial interventions.

Box 4.4 Main Criticisms to Application of RCTs in the Evaluation of Behavioural Interventions

- Difficult in blinding the intervention
- Withholding of controls
- Poor representativity of real-life conditions
- Scarce generalizability of the results and dependency on the setting
- Excessive dependency on biological outcomes
- Dependency of the effect-size on changes in social/community norms about outcomes
- Difficult to design studies targeted for large samples of high-risk individuals

Table 4.2 Main selected steps required in planning of behavioural interventions (BI) for STI prevention

Order	Step	Definition	Sources	Examples	Stakeholders	Additional needed knowledge
1	Assess the problem	Identify a specific problem associated with STI circulation	National STI surveillance data, local studies from STI clinics or health facilities, studies on selected at-risk groups, population surveys, etc.	Elevated STI incidence or prevalence. Scarce attendance at local STI clinics. Lack of access to consistent condom use by at-risk groups. High rates of unintended pregnancies	Public health authorities, STI researchers and clinicians, municipalities	Reliability and updating of epidemiological data. Resource-requirement evaluation Temporal length of intervention to reach outcomes
2	Describe target population or audience of BI	Identify the population or group/s that must to be reached with the BI	Case-control studies. Socio-behavioural studies on STI vulnerability of population and subgroups	Adolescents, school children, MSM, commercial sex workers, IDU, STI patients, pregnant women, general population etc.	Target population, health workers, NGO, parents, educational agencies	Knowledge of interpersonal factors of target population/group (i.e., awareness, values, beliefs, practices, motivations, barriers, etc.)
3	Assess the organization of BI	To evaluate the capacity of your organization to develop the BI also with others	Structural (funds, spaces, staff, technical equipment, media support etc.) and process resources (staff expertise, administrative officers, peer support, etc.)	Enough no. of providers by expertise, high level of agreement of intervention with clinical practice, adequate funds etc.	National funding authorities, patronages, awards agencies, private research companies	Fund-raising ability of the staff in scientific and clinical research, level of compliance of social authorities with the BI
4	Define measurable, specific and achievable objectives and outcomes	Identify the objective and outcome preferring biological outcomes vs. self-reported findings	Previous trials on same (or similar) target population, published studies based on experimental and biological outcomes	Reduction of 20% in STI incidence rate, increasing by 25% in uptake HIV testing, reduction of 15% of unintended pregnancies, increase in access to consistent condom use, etc.	Providers of the BI trial, health authorities	Availability of baseline data pre-intervention Impact evaluation scenario
5	Choose educational models and theoretical bases of BI	Identify a theory/model that endorse the BI and its specific tools	Scientific evidence of robustness and cost/effectiveness of model from meta-analyses and meta-synthesis	Health believe model, self-efficacy theory, social cognitive theory, theory of reasoned action, etc.	Providers of the BI trial	Current social and community norms on sexual behaviour

6	Implementation of BI	Planning by a large committee that includes target population, the operational steps of BI including: Procedures, educational and training materials, tools, budget and time line	Staff, peers, external scientific consultants	Study protocol, protocol of procedures, ethical guidelines	All (or the majority) the stakeholders identified in previous steps	Ethical clearance from ethics authorities and committees
7	Assess effectiveness	Identify techniques, timing and procedures to assess differences in outcomes between intervention vs. non-intervention participants	Clinical, behavioural and other relevant data collecting during the intervention trial	To measure significant differences in: n. new STI episodes; no. of recent occasional partners; increase of age at first intercourse; decrease no. of USI per temporal unit	All providers of BI, external evaluation agencies, NGO, communities, national and local health authorities	Expertise in evaluation trials of preventive BI, assessment of potential moderators of the effects Advocacy of the results

STI sexually transmitted infections, *BI* Behavioural intervention, *NGO* Non-governmental organizations, *USI* unprotected sexual intercourse

Thus, to consider the RCTs as the only effective way to measure the size of effects in healthcare means not recognizing these limits when they are to be designed for studies in the behavioural health area.

Behavioural trials clearly differ compared to clinical trials, where the final efficacy of a drug is decided according to the result of positive observations (i.e., safety, doses, administration way) in specific evaluation consecutive phases, and where the true direction and size of effect seem progressively clearer. Differently, we agree with Stephenson and colleagues when they defined a behavioural intervention as a "black-box" where the "active ingredient" cannot be identified a priori [61].

For this reason, it is not easy to disentangle the weight of the different elements that concur, for example, regarding the effectiveness of a cognitive behavioural approach in reducing sexual risk behaviour. Trying to do so, also by a well-designed RCT, could prove to be particularly tiring and not cost-effective. However, despite the undoubted differences between behavioural and biological/clinical interventions, the possibility to establish, using measurable parameters, whether or not a specific "ingredient" does more harm than benefit cannot be altered by his intrinsic complexity. For these reasons, the attempt to use any form of measurable assessment must be pursued also to ensure that behavioural interventions also have the most reliable level of assessment.

4.17 Measurable Versus Self-Reported Outcomes

One of main steps in enhancing the reliability of a behavioural intervention (BI) trial target to avert STI is to identify measurable outcomes and to limit the use of self-reported measurements to assess the effects of the programme. Measures such as condom use rates or the number of recent sexual partners, which may not always be true and may be vulnerable to memory biases should be discouraged or attenuated favouring events which were assessed with objective measures. Thus, if a trial's efficacy in terms of condom use and numbers of partners reflects reality, then a decrease in the new STI episodes or unintended pregnancies among exposed participants should also take place. In fact, regarding the consequences of sexual behaviour, the STIs and the unintended pregnancies can be considered two important biological correlates useful to this aim. The STIs represent effective markers of sexual risk behaviour and assessing differences in their incidence rates between exposed- and non-exposed participants makes it possible to use strong proxies to evaluate the size effect of the intervention. This is particularly true when acute STI are considered, such as syphilis, gonorrhoea or first episode of genital herpes, which represent proxies of recent unprotected sexual intercourses. Another benefit in the use of acute STIs as behavioural outcomes is that, differently from HIV-1 infection, these infections show higher incidence rates, particularly in the individuals and groups commonly involved in programmes of behavioural interventions. Therefore, a well-designed controlled study may show a weak impact of a behavioural intervention on HIV incidence but positive effects on STI rates measured among exposed participants.

Furthermore, choosing strong outcomes also in behavioural intervention trials makes it possible to control efficiently the social desirability associated with self-reported data [62]. To measure rates of unprotected sexual intercourse or number of recent partners through the self-reporting of participants can lead to an overestimation of the positive effects of intervention, due to the propensity of the BI-exposed participants, to "optimize" their behaviours when reporting to researchers, differently from the not-exposed controls or those commonly exposed only to standard-care. This is because it is generally difficult to allocate the inaccurate reporting due to the social desirability bias equally across comparison groups [63]. Today the social desirability bias can be softened by procedures of data collection based on the use of technical digital devices such as computers, smartphones or tablets. Since the end of twentieth century, the studies conducted with computer-assisted self-interviews

(CASI) have started to show that the disclosure of sensitive or stigmatized behaviours was largely easier than in face-to-face interviews [64]. Today, it is well known that the reliability of biomedical data collected from study participants using digital devices is not inferior and is of higher validity than those collected with conventional pencil and paper questionnaires (PAPI) [65]. A validity of the digital devices used in evaluation trials was confirmed also for the collection of informed consent and for the comprehension of research study procedures [66].

Most of the results from the evaluation studies pertain only to efficacy rather than effectiveness. RCTs are highly controlled to assure strong interval validity, but they usually include conditions that are not ordinarily present in a local community in real life (e.g., incentives to return for multiple sessions, free delivery of condoms, etc.) [67]. The challenge is how community evaluation trials can take what is known to be valid from intervention trials and translate these strategies into policies and organizational plans. Unfortunately, when the results are transferred to real life, there may be significant variations. The challenge, for the researchers involved in studies of evaluation of behavioural intervention, is to design a pragmatic methodology that mimics real-life conditions where the intervention will be generalized, if effective, after the trial.

4.18 Behavioural Counselling Interventions

Counselling sessions represent the most common setting and framework where the three phases of a behavioural intervention are delivered. In healthcare settings, the term *Counselling* commonly refers to a flexible but well-structured relationship, between a provider and a client, usually a clinician and a patient, during such a relationship, the provider helps the client to better understand the implications and needs of a new health condition or disease (i.e., new diagnosis), to facilitate adjustment to changing life circumstances and to cope efficiently with the consequences [68]. The counsellor's role is to facilitate the client's work in ways that respect the client's values, personal resources and capacity for self-determination. The counsellors draw from several theoretical approaches, including those that are cognitive, affective, behavioural and systemic. These theories may be applied to individuals, groups and families. When a counselling approach is utilized for prevention purposes it is generally defined as Behavioural Counselling, Preventive Counselling or Educative Counselling and aims to promote in an individual or group with health risky behaviours, enhanced awareness, motivation and commitment to change habits (i.e., smoking, addictions, sedentary and risky sexual behaviour, etc.).

The counselling centred-relationship is commonly conducted in a specific face-to-face encounter of different length, named; *session*. A counselling intervention can be organized in more than one session, according to the characteristics, setting, target population or outcomes of the behavioural intervention.

Several meta-analyses conducted after the year 2000 found adequate evidence that intensive behavioural counselling interventions can reduce the risk of STIs in sexually active adolescents and in heterosexual adults at increased risk. Lower, weak or negative effects have also been demonstrated in some high-risk groups, such as men who have sex with men (MSM) and commercial sex workers (CSWs).

When positive, the benefit of behavioural counselling is of moderate magnitude, and certainly meaningful in practical terms. In general, the positive effect is the result of the evidence that behavioural counselling interventions reduce at-risk sexual behaviours and increase consistent barrier-method use (i.e., condom) and other protective sexual habits or practices.

A key characteristic of counselling interventions is the length of sessions, defined as *intensity*. The intensity can differ according to different delivery settings (i.e., primary care facilities, STI clinics, family planning centres, etc.), different framework theories upon which the counselling is based (i.e., Social Cognitive Theory, theory of reasoned action, etc.) or specific characteristics of the target population

(i.e., STI patients, out-reach-groups, inmates, etc.). Most published counselling interventions range in intensity from 30 min to 2 or more hours of contact time between the provider and client. Meta-analyses aimed to summarize the results from large RCTs, which assessed the effects of Counselling, showed that the evidence of benefits increases with intervention intensity. High-intensity counselling interventions (defined in the review as contact time of ≥2 h) were the most effective, moderate-intensity interventions (defined as 30 to 120 min) were less consistently beneficial and low-intensity interventions (defined as <30 min) were the least effective. According to the general framework of the objectives and contents of a behavioural intervention against STI's risk, most successful counselling-based approaches also aim to provide basic information, to assess the person's risk level, to transfer preventive skills and increase motivation and commitment to safe practices. Moreover, successful behavioural counselling programmes tend to use a targeted approach based on the age, gender, sexual orientation and ethnicity of the group in question. Intervention methods included face-to-face approaches, frequently supported by videos, written materials and telephone contacts. Many variables are yet to be supported by enough evidence to be related independently to effectiveness, such as: group versus individual format, counsellor characteristics, setting or type of control group.

4.19 Evaluation of Behavioural Counselling Interventions

Since the mid-1990s, the studies aimed to assess the effectiveness of behavioural counselling interventions (BCI) can be considered the first examples of evaluation of behavioural interventions as a consequence of the need to contrast with standardized preventive conducts the spread of the HIV-1 epidemic and later, the re-emergence of some bacterial STIs (i.e., syphilis, gonorrhoea). These studies were the first to contribute to bringing the behavioural sciences closer to biomedical trials and the evaluation of outcomes of health practices.

Table 4.3 includes selected examples of effective BCI evaluated in large experimental studies. These five selected programmes were characterized by some common key elements, which provided each intervention with specific advantages in terms of effectiveness. All the studies, which were assured dissemination of the interventions through a network of specific health facilities (i.e., STI clinics, women health clinics, community clinics), used biological outcomes to evaluate the effect, were based on a robust theoretical framework and were targeted for high-risk populations [44, 46, 47, 69–73].

Most recently, a critical systematic review of reviews identified 18 reviews which included 48 primary studies which evaluated behavioural interventions using biological (i.e., incidence, prevalence, HIV testing uptake) or condom use measures to support the adoption and maintenance of prevention behaviours through counselling-based strategies. Twenty-five studies described effective use of counselling sessions alone or in association with HIV testing (HTC) to promote preventive attitudes and behaviours. Six reviews contributed strong evidence of effectiveness from ten studies, describing couples-based counselling interventions. One observational study assessed the effect of couples-based counselling on HIV incidence with findings in support of the intervention (level of evidence: C1). Nine studies, including three randomized control trials (RCTs), assessed self-reported condom use after couples counselling and data from these studies supported the behavioural interventions with a high grade of evidence (A1). Many of these counselling interventions to reduce HIV risk behaviours were delivered in health settings through face-to-face relationships or in community sites [74].

From two reviews specifically aimed at HTC intervention, 12 studies were selected, including four RCTs, and 3 showed observation data in support of the effectiveness of interventions, 8 a large, but not consistent, effectiveness and only one ineffective effect in HIV reduction [75, 76].

Table 4.3 Effective behavioural counselling interventions for STI prevention and control

Intervention	Study population	Setting	Aims	Duration	Providers	Methods	Ref.
SISTER TO SISTER	Inner-city African American women	Inner-city women's health clinics	Eliminate or reduce sexual risk behaviours and prevent new STI episodes	1 session; 200 min for the group format and 20 min for the one-on-one format	African-American female nurses with >10 years of nursing experience and working with target population	Demonstration, exercises, games, group discussions, lectures and teaching, practice, printed materials, role-play, and video	[44]
Project RESPECT	Heterosexual, HIV-negative patients	Public STI clinics	Eliminate or reduce risky sexual behaviours and reduce STI incidence rates	Brief counselling: Two 20-min sessions delivered over 7–10 days. Enhanced counselling: One 20-min and three 60-min sessions (200 min total) delivered over 3–4 consecutive weeks	Trained HIV and STI counsellors	Counselling, exercises, goal setting, printed materials and risk-reduction supplies (condoms)	[46, 47]
HORIZONS	Heterosexual, sexually active African-American adolescent girls seeking sexual health services	Public community clinics	Reduce STIs, increase condom use, increase communication with male partners about safer sex and STIs and increase male partners' use of STI services	Two 4-h group sessions on 2 consecutive Saturdays, followed by 4 (15-min) telephone contacts approximately every 10 weeks over 9 months	African-American female health educators	Discussion, exercises, games, practice, printed materials, role-play, telephone reinforcement and vouchers for STI services	[69]
VOICES/ VOCES	African-American and Hispanic patients	Inner-city public STI clinics	Prevent new STI episodes and increased condom use	One 20-min video followed by one 25-min group discussion session	Gender-matched facilitators	Video, group discussion, risk-reduction supplies (condoms) and printed materials	[70–72]
EXPLORE	Men who have sex with men (MSM)	Public community clinics	Prevent HIV infection and STIs	Ten one-to-one 20-min counselling sessions delivered within 4–6 months of randomization	Trained (40 h) HIV counsellors	Counselling, goal setting, motivational interviewing	[73]

STI sexually transmitted infections, *HIV* human immunodeficiency virus

A wide systematic review of randomized, controlled trials and non-randomized, controlled clinical trials conducted to evaluate the benefits and harms of behavioural counselling interventions in primary care was published in 2014 [77]. The review included interventions targeting adults and adolescents of any sexual orientation and studies conducted in developed countries that recruited participants from primary care settings, mental health clinics, reproductive health clinics, including STI clinics. The studies included reported biological outcomes (i.e., STI incidence), behavioural outcomes (i.e., changes in measure of sexual behaviour) or adverse effects of sexual risk-reduction counselling (i.e., care avoidance, shame, guilt or stigma). The studies included had to have at least 3 months of post baseline follow-up for all outcomes and were based on interventions at high-, moderate-, and low-intensity. Using 31 trials for a total of over 70,000 participants, the review concludes that high-intensity (>2 h) interventions reduced STI incidence in adolescents (7 trials; OR = 0.38 [95% CI, 0.24–0.60]) and adults (19 trials; OR = 0.70 [CI, 0.56–0.87]). Differently, lower-intensity interventions were generally not effective in adults, and moderate-intensity interventions may be effective in adolescents (2 trials; OR = 0.57 [CI, 0.37–0.86]. No consistent evidence was found that sexual risk-reduction counselling was harmful [77].

An additional recent review aimed to assess the role of behavioural counselling in STI prevention programme settings using seven restricted inclusion criteria showed that prevention counselling is likely to benefit STD clinic attendees other than MSM. The cost-effectiveness of this intervention appears unclear, but feasibility would be improved when counselling approaches are implemented in multi-level interventions and intervening in this way may exercise a valuable role through partnership [78].

Session intensity seems to have a different effect on the outcomes if applied through face-to-face counselling than if delivered in-group sessions. An old trial showed that two 20-min counselling sessions were as effective as four 1-h group sessions in reducing risk behaviour and STI incidence, particularly in patients attending STI clinics [79].

Similar emphasis on a drastic reduction in number of sexual partner or frequency of sexual intercourses, particularly in groups with elevated concurrency and mixing partners, such as MSM, has led to weak reduction rates of STI and HIV infection [80] or as well as in the Project RESPECT follow-up, to an uninspected increased risk of STI at 6 months [81].

Differently, tailored programmes based on moderate and progressive reduction of risky behaviours seem to produce positive results particularly through enhanced condom promotion programmes.

Behavioural counselling interventions (BCI) are generally included into other initiatives of secondary prevention, such as targeted screening or partner notification. In these contexts, behavioural intervention is commonly delivered in STI clinics, with a face-to-face approach and with individuals diagnosed with a new STI episode or with at-risk participants in periodical STI/HIV screening.

Most studies that assess the effectiveness of BCI target high-risk groups recruited in STI clinics from the attendees. The African-American heterosexuals [44, 45], tested positive individuals for STI at enrolment [46], people with history of drug use or exchanging sex with money or drugs [53] or sexually active adolescents, represent the most frequently groups examined to measure the effectiveness of a BCI. Differently, additional researches were needed to make conclusions about the efficacy of BCI for serodiscordant couples and individuals at low to moderate risk for HIV [82].

BCI approach was found to be feasible and effective in STI clinics and HIV-1 centres where high rates of repeat infections are expected, particularly in high-risk populations. However, some constraints were described when behavioural counselling techniques were delivered in a clinical setting. Time expenditure is the first constraint reported by clinicians involved in STI centres, when behavioural counselling activities must be added to the time of a patient's visit. This problem is even more relevant considering

that high-intensity behavioural counselling is the one that has revealed the greatest effectiveness. For this reason, evidence has been reported that the time of visit and waiting list in public facilities represent the principal barriers to counselling activities in routine health assistance.

Another constraint on BCI concerns the need of expertise of the facilitators. Positive effects of a counselling session depend on the capacity of the provider to manage both the relationship of effective communication and the theoretical framework that surrounds the intervention. This expertise is not part of the curriculum of the doctor or nurse's studies and requires additional costs for training courses of the personnel or to engage external trained facilitators. In many programmes, the providers are nurses with several years of nursing experience [44] or trained STI/HIV counsellors or clinicians [45, 46]. Particularly when lay health advisors were selected from the target community to administer the intervention, part of the funds and resources of the programme had to be provided to assure an adequate level of expertise to providers [44, 47].

Finally, the costs of counselling interventions are difficult to measure. In most evaluation studies, cost data were not assessed or reported. However, the fact that for counselling to be effective, it should be intensive, and organized in multiple sessions and delivered by expert providers, means inevitably it is expensive and time-consuming. One study reported that the median cost estimate per patient counselled in intervention was around 56 US dollars [81].

4.20 Condom as a Behavioural Preventive Tool

The male condom is one of the oldest contraceptive methods and the earliest method for preventing HIV infections [83]. In developed countries, condoms are inexpensive and widely available and data from the United Nations show that the overall prevalence of use is around 18.0% [84]. Despite the fact that in 1997, in vitro evidence has confirmed that latex and polyurethane, which are currently used to produce and test condoms, are impenetrable to pathogens which determine STIs, also if viruses [85], the history of condom as an effective tool to change the risk in sexual intercourses is still disputed. Today condoms represent the first line of prevention for many sexually active individuals worldwide. Although protection via condom use is not perfect, even partial protection is enough to affect population rates of STI.

The first strong evidence regarding condom effectiveness at population level originates primarily from two large European longitudinal studies conducted among heterosexual HIV-discordant couples. In the early 1990s, Saracco et al. showed that among 343 women with a stable HIV-infected partner, the incidence rate was 7.2 per 100-person year (p/y) among those who did not always use or never used condoms and 1.1 per 100 p/y among those who always used them [relative risk (RR) 6.6, 95% confidence interval: 1.9–21.9] [86]. In the same years, among 256 couples who had had stable sexual relations for more than 3 months, only in those who used condoms consistently for vaginal and anal intercourses ($n = 124$), none of the seronegative partners became infected with HIV-1, despite a total of about 15,000 episodes of intercourse [87].

Just a few years before, some cohort studies in the USA conducted in men who have sex with men (MSM) also showed significant reductions in risk behaviour and in the incidence of HIV and other STIs because of extensive interventions to promote consistent condom use [88–90].

In 2001, the Department of Health and Human Services of the National Institute of Health (NIH) issued a technical report which cited evidence that the condom is effective in preventing HIV transmission and female to male transmission of *N. gonorrhoeae* but point out that, no empirical evidence was available to affirm that the same risk reduction can be reached for *C. trachomatis* infection, syphilis, genital herpes and HPV [91]. After this criticable position of the NIH, several studies promptly provided further evidence of condom effectiveness and stressed the importance of the studies' methodologies to evaluate the true protective role of condom use in STI prevention [92, 93]. After the publication of some

strong evidence that demonstrated considerable protection of condom use also against HPV and its carcinogenic effects [94] in most developing countries with generalized high rates of STI and HIV, condoms have been actively promoted for all the active population despite the presence of social, cultural and economic barriers to their consistent use [95]. For some authors, the effectiveness of consistent condom use can be as high as 95% [96]. Similarly, elevated was the estimate of effectiveness (76%) proposed for the consistent condom use in anal sex between MSM, a protection level that could be increased by the combined use of lubricants in anal intercourses [97].

Through a meta-analysis of 14 longitudinal studies, Weller et al. in a Cochrane Systematic review aimed to estimate condom effectiveness in reducing heterosexual transmission of HIV. From the comparison of 13 cohorts of "always" condom users vs. 10 heterogeneous cohorts of "never" users, they estimated a proportion of HIV seroconversion reduction associated with consistent condom use of approximately 80% (HIV incidence estimate of 1.14 per 100/p-y. vs. 5.75 per 100/p-y among always- vs. never-users, respectively) [98].

Behavioural counselling interventions have also been shown to be effective in increased condom use. In the large meta-analysis of O'Connor et al. the odds of condom use increased after high-intensity interventions (OR, 1.29, 95% CI: 1.13–1.48) and moderate-intensity interventions (OR: 1.21, 95% CI, 1.00–1.46). Many included primary studies seem to suggest that treatment effects of behavioural counselling interventions show variations in size for different population characteristics. In fact, trials and subgroup analyses targeting adolescents were highly likely to be effective, with most showing at least a 50% decrease in the odds of acquiring an STI after behavioural counselling. Differently, no consistent evidence of differential effectiveness was measured by sex or race/ethnicity, low-income setting; mental illness; or history of sexual, physical, or intimate partner abuse. However, these groups were generally poorly represented in available studies, such as low-risk populations, adolescent boys, and MSM [77].

Overall, single-session behavioural interventions showed positive effects on sexual risk, both in terms of reducing the number of unprotected sexual intercourse and increasing condom use. In 20 studies included in a meta-analysis and conducted primarily on patients or clients in a clinical setting and STI clinic, evidence of positive results in favour of behavioural intervention was observed in 15 studies. The overall effectiveness was $d = 0.22$ (95% CI = 0.06–0.37) and six out of the 15 studies had an effectiveness ranging from $d = 0.40$ to $d = 0.93$. The evaluated behavioural interventions were based primarily on: one-to-one information, motivation interviews and skills improvement [30]. This effect demonstrates a significant improvement in condom use between participants in intervention groups compared to control group participants. Despite a certain heterogeneity between the studies, none of these showed a *reversal of effect* with less use of condoms among those exposed to single-session behavioural intervention. Most of the selected studies showed reductions in sexual risk between intervention participants compared to controls. In all other studies, the similar reduction in sexual risk was observed at follow-up in both cases and controls [30].

The results of an intervention of condom promotion are closely linked to the policy applied to deliver the messages. A meta-analysis focused on nine RCTs analysed the effects of structural and community-level interventions in developing countries to promote condom use, defined as those actions improving accessibility, availability and acceptability of any given health programme/technology. The meta-analysis concluded that there was no clear evidence that the intervention influenced neither HIV nor STI rates when comparing the intervention group and controls. Only knowledge about HIV and other STIs seem to have improved in the intervention- group participants (RR 1.15, 95% CI 1.04–1.28, and RR 1.23, 95% CI 1.07–1.41, respectively) [99].

That behavioural interventions reduce sexual risk behaviour and avert STIs and HIV infection, were also the conclusions of the meta-analysis by Scott-Sheldon et al. using 42 RCTs and

quasi-experimental studies (N = 40,665; Mean age = 26 years; 68% women; 59% black) with biological outcomes. The results showed fewer incidence of STIs (d = 0.16, 95% CI = 0.04, 0.29; k = 62), including HIV (d= 0.46, 95% CI = 0.13, 0.79; k = 13) associated with increased condom use in intervention participants [d = 0.17, 95% confidence interval (CI) = 0.04, 0.29; k = 67], compared to the control [53].

In a 2013 Cochrane Systematic Review, seven programmes based on educational or counselling approaches, (i.e., randomized or non-randomized studies) to encourage or improve condom use among heterosexual men and women, were evaluated in a 2013 Cochrane Systematic Review [100]. The review regarded studies that provided strong biological data on: pregnancy, HIV/STI, or presence of semen after sex. Outcomes were measured at least 3 months after the behavioural intervention started. Data from seven eligible RCTs, four conducted in Africa, two in the USA and one in England, showed no significant difference between the intervention and non-intervention group for pregnancy or HIV, but favourable effects were evident for some STI incidence rates, such as for HSV-2 (ARR = 0.65, 95% CI 0.43–0.97), syphilis (ARR = 0.58, 95% CI 0.35–0.96) and gonorrhoea (ARR = 0.28, 95% CI 0.11–0.70). Differently, a negative effect on gonorrhoea for young women in the intervention group versus the control group (ARR 1.93; 95% CI 1.01–3.71) was also recorded in a study [100].

4.21 Behavioural Interventions Targeting High-Risk Populations

To date, a small number of studies are available to fully explore how behavioural strategies impact target populations when they are represented by individuals at risk for STIs, such as female sex workers (FSW), socially fragile women, MSM, who are HIV-infected, whose who engage in transactional sex or inject drugs. There have been few studies to date that have focused on these subgroups, and samples from primary studies have had relatively small proportions of individuals with these characteristics. Nonetheless, a large part of studies has encouragingly shown that protective behaviours increase markedly following behavioural interventions that sample individuals at higher risk for STI or those who living with HIV.

4.21.1 At-Risk Females

Behavioural interventions are effective in reducing HIV and the incidence of STIs among female sex workers (FSWs) and clients living in low- and middle-income countries. Data from 13 RCTs and quasi-RCTs trials collected by Wariki et al. on 8698 participants confirm a positive effect of a behavioural intervention based on cognitive social theory (CST) in reducing HIV transmission and STIs [101]. Greater intervention efficacy was observed in studies that specifically targeted African-American females in primary care settings, used gender- or culture-specific materials, used female deliverers, addressed empowerment issues, provided skills training in condom use and negotiation of safer sex, and used role-playing to teach negotiation skills. The cited review by Jemmott et al. covering 37 primary studies published from January 1988 to June 2007 and showed that behavioural interventions had a significant impact on reductions in HIV risk sex behaviours and sexually transmitted infections [44].

The protective effects of behavioural interventions in reducing risky sexual behaviours and incidence STIs have also been shown among Latina women in the USA by Althoff and collaborators in 2015. The effects persist for as long as 2 years following the intervention and are of near equal magnitude regardless of whether the intervention is implemented in women with STI at baseline or community-based populations, indicating that these interventions may be useful in a variety of settings [102].

4.21.2 Men Who Have Sex with Men

Men who have sex with men represent the population at highest risk for STI, worldwide. In this group, the characteristics of partner recruitment,

the frequency of sexual intercourses, the median number of recent and lifetime sexual partners associated with a wide age-mixing partner and spectrum of sexual practices, represents the key elements for a dramatic circulation of STI. For this reason, the MSM who live in developed countries are the target of the main part of conducted behavioural intervention for STI and HIV control, particularly after the year 2000 when a re-emergence of some classic STI were observed in this population. To date, in Europe, MSM continue to be the population most affected by HIV infection and the number of new infections is increasing faster than among other populations [103].

Among MSM, some harm reduction strategies, such as negotiated safety, partner examination, withdrawal before ejaculation, strategic positioning, and serosorting are used as imperfect self-managed means to reduce HIV risk, despite limited evidence that such strategies decrease the likelihood of seroconversion. Furthermore, the increased proportion of gay males unaware of their HIV-1 infection observed in recent years in Western countries affected the advantages of serostatus assortative behaviour and transformed the reliance on their own and partners' serostatus into a fallacious strategy [104].

Behavioural interventions to reduce unprotected sex among MSM range from individual-level interventions (i.e., one-on-one counselling, peer education, counselling and testing, relationship training) to group level programmes (i.e., sexuality education, risk-reduction skills training) and community-level interventions (i.e., empowerment activities, chat room involvement, mass media campaigns) [105, 106]. The effectiveness of HIV/STI preventive interventions targeted at MSM has been assessed, with conflicting results, in various publications mainly from the USA.

The first trial that assessed the effectiveness of behavioural intervention targeting MSM in preventing HIV-1 infection was a multisite two-group randomized controlled phase II-b trial conducted in the USA to test the effect of a programme in preventing HIV seroconversion. The study population was 4295 MSM randomized in two arms. The intervention consisted of ten one-to-one 20-min counselling sessions followed by maintenance sessions every 3 months. The control condition was based on two annual counselling sessions (i.e., RESPECT Project). The rate of HIV acquisition in intervention participants was 35.0% lower than in the control group, which is consistent with a significant reduction (20.5%) in the occurrence of unprotected receptive anal intercourse (UAI) in intervention participants compared to the control group [73].

Two meta-analytic reviews published after 2004, using data from 54 and 33 studies on MSM, estimated that behavioural interventions were associated with a significant decrease in unprotected anal intercourse from 17% to 27% and in the number of sexual partners (by 15%) with a significant increase in condom use during anal intercourse. Theoretical models, which included interpersonal skills training, incorporated several delivery methods, and which were delivered over multiple sessions characterized the interventions associated with positive effects among MSM [105, 107].

In Europe, another important review of reviews, which assessed the effectiveness of behavioural interventions among priority populations, identified some primary studies that were relevant to MSM. These studies, which reported 12 different intervention evaluations, concluded that group- results from another six European randomized controlled trials, involving over 4000 MSM at enrolment and conducted in the post-cART-era, showed a clear reduction in size effect of the behavioural community- and individual-level interventions, to produce positive effects in changing sexual risk behaviour [108]. Berg in 2009 in a systematic review found that the estimate pooled effect is scarce and suggested that MSM who participate in HIV/STI prevention initiatives were 10% less likely to report unprotected anal intercourse (RR = 0.90, 95% CI: 0.83–0.96) [109]. The role of phenomenon of "risk compensation" as a response to reassurance introduced by the biomedical interventions for HIV infection, seem to explain a

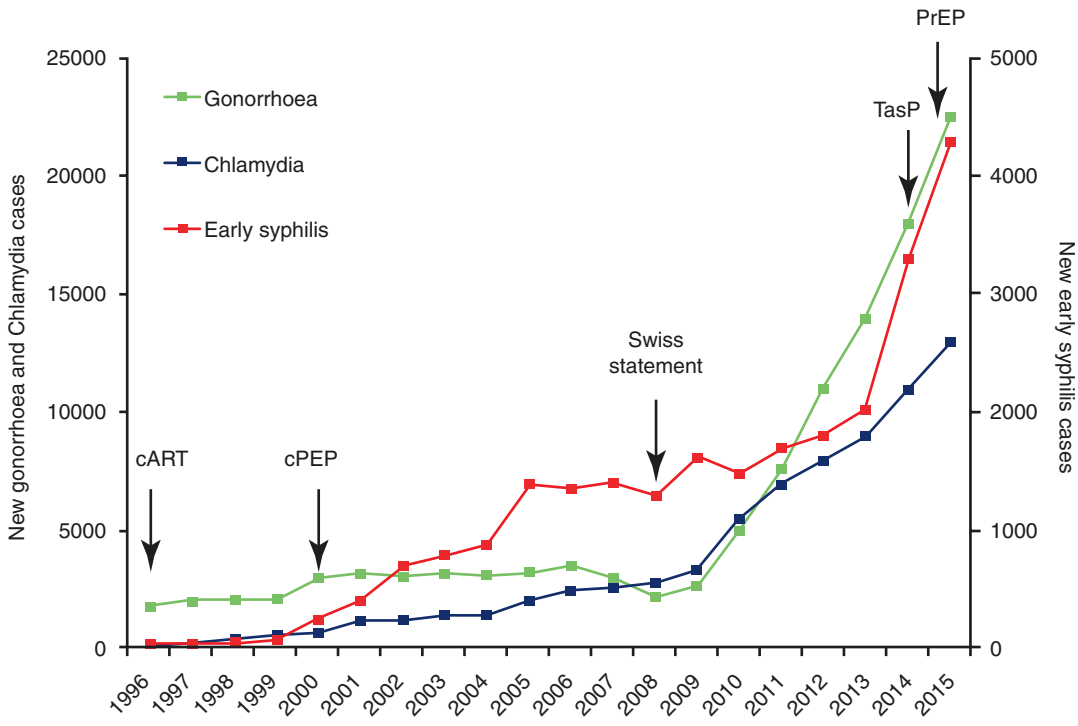

Fig. 4.4 Trend of new diagnoses of early syphilis, gonorrhoea and Chlamydia trachomatis infection from 1996 to 2015 in men who have sex with men (MSM) in England, according to the introduction of the combination antiretroviral therapy (cART), post-exposure prophylaxis (cPEP), Swiss Statement, cART for Prevention (TasP) and pre-exposure prophylaxis (PrEP). (Reproduced with permission from [116])

drastic reduction of commitment to primary prevention strategies among MSM and the decrease in effects of the classical behavioural intervention [110]. Recently in the USA., Metsch and collaborators seem to confirm this dramatic decline when they find an increased risk of STI among MSM counselled than among controls in the AWARE randomized clinical trial [81].

Since the cART became available in the mid-1990s has changed the course of HIV infection from a deadly disease to a chronic condition. From the introduction this therapy, national surveillance data have progressively shown significant changes in sexual behaviour of MSM toward at-risk practices [111–113], and a consistent dramatic increase in incidence of bacterial STIs [114, 115]. In England, since the cART for short-term prophylaxis in the form of combined post-exposure prophylaxis (cPEP) was introduced, the trends in incidence of bacterial STI have increased sharply and have continued to rise following the Swiss Statement and since the cART for prevention (TasP) and pre-exposure prophylaxis (PrEP) was disseminated [116] (Fig. 4.4).

Moreover, data from behavioural studies showed that the proportion of condomless sex with occasional and stable partners had also recently increased among HIV-1 infected MSM in Switzerland and England [117, 118]. The PrEP was investigated as a determinant in risk compensation in MSM in two recent open label RCTs where the participants allocated to immediate PrEP showed increases in condomless sex with 10+ partners more frequently than participants allocated to deferred PrEP (PROUD trial, 21% vs. 12%; p = 0.03) [119] and in STI incidence [120]. Unfortunately, also an inaccurate HIV risk perception by MSM can affect the uptake of PrEP and become a barrier to HIV prevention, as seen also in the results of the PrEPARE2 trial [121].

The responses of gay men to risk post-cART with a range of different strategies, which are not

altogether safe, to reduce harm, need to be understood and addressed to produce a rapid reinterpretation of the preventive pressure that must be utilized. A preventive pressure that takes what social analysis has to say seriously by a synergic action between the traditional, the "modern" epidemiological/clinical and the "new" social or socio-cultural public health. The same risk avoidance strategies adopted by gay men suggest a way forward by turning our attention to the ways in which medicine is taken [122].

4.21.3 Workers

Despite the fact workplace interventions to prevent HIV are feasible, there is moderately quality evidence that they can produce measurable effects on the uptake of HIV testing, on HIV incidence, or in self-reported sexually transmitted diseases and a decrease in risky sexual behaviour. These are the conclusions of a systematic review, based on eight randomized control trials (RCTs) with 11,164 participants, to assess the effects of behavioural interventions for reducing HIV and risky sexual behaviour when delivered in an occupational setting [123].

4.21.4 Prevention with Persons Living with HIV-1

In 2005, the journal *AIDS* dedicated a monographic issue titled "Prevention with positives" which focused on preventive studies targeting persons living with HIV-1 (PLWH) [124]. Since then, a lot of articles have presented results about the effects of behavioural intervention in reducing at-risk behaviours in these patients. Despite the fact that, in January 2008, the Swiss National AIDS Commission issued a statement (i.e., Swiss Statement) that an HIV-infected person on effective combined HIV treatment (cART) cannot transmit HIV through sexual contact [125], the reduction of sexual risk remains the cornerstone of HIV prevention also among PLWH because this strategy avoid the STI that it is one of the three conditions that make the Swiss Statement true. To underline the relevance of BI addressed for PLWH, UNAIDS and WHO, also, in response to the Swiss Statement, stated that "…it has not been proven to completely eliminate the risk of transmitting the virus […]". More research is needed to determine the degree to which the viral load in blood predicts the risk of HIV transmission and to determine the association between the viral load in blood and the viral load in semen and vaginal secretions" [126]. A relevant amount of evidence suggests that PLWH practice sexual risk-reduction strategies to protect themselves from STIs and their partners from HIV and other STIs. A series of studies examining unprotected anal sex in HIV-positive MSM found that most men practiced "safer sex", using condoms consistently or employed strategies such as serosorting (only engaging in sex with other HIV-positive people) and strategic positioning (engaging in receptive anal intercourse only) to avoid transmission [127].

Despite concerns that the use of combined antiretroviral therapy (cART) might increase sexual or drug-injection risk, recent available research suggests that unprotected sex is reduced among HIV-infected individuals on treatment. The reasons for this are not yet clear, although self-selection and mutually reinforcing effects of HIV treatment and prevention messages among people on cART are likely [128].

However, the residual proportion of PLWH who still engage in risky sexual practices represent a cause for concern and can be taken into consideration as a target of BI interventions also in the era of the *Swiss Statement*. Moreover, factors associated with irregular condom use also vary according to sexual orientation and gender in PLWH.

Some additional factors seem to increase sexual risk among PLWH including drug and alcohol use, greater importance of sex in one's life, greater sexual desire, greater sexual frequency, use of erectile dysfunction medication as well as mood disorders and multiple partners [129].

Some evidence suggests that among the over 50 HIV-positive MSM, 18% percent reported unprotected anal sex with a partner of unknown or serodiscordant status in the previous 3 months, whereas

34% had had unprotected sex with an HIV-positive partner in the previous 3 months [130].

Five randomized controlled trials that examined the effects of behavioural interventions on increasing condom use among 725 HIV-infected females showed no significant effects at 3, 6 and 12-month follow-up visits [131].

Recently, new observations evidenced by 2014, 50% of all PLWH in the USA would be 50 years of age or older and that from 13% to 30% continue to engage in risky sexual behaviours. In a systematic review of 12 studies, Negin et al. identified two RCTs focused on reduced sexual risk behaviour in PLWH [132]. The two RCTs suggested positive effects of telephone-administered motivational interviewing and behavioural skills training, on the number of unprotected sexual acts with partners of unknown or negative serostatus [133, 134].

Two reviews for a total of seven selected primary studies assessed prevention counselling, showing a mixture of beneficial and ineffective or harmful results from PLWH, particularly from studies conducted in developing countries [131, 135]. In the past, two different reviews published in 2006, showed conflicting results. Data on 3234 PLWH from 15 studies showed that intervention participants exhibited lowered sexual risk relative to control participants on condom use but not for number of sexual partners. Importantly, the interventions were more successful at increasing condom use if the sample included fewer men who have sex with men (MSM) or younger participants and when interventions included motivational and skills components [136]. Differently, Crepaz and coll. in a meta-analytic review of controlled trials showed that interventions significantly reduced unprotected sex and decreased acquisition of STI, particularly when they were: based on behavioural theory; designed to change specifically HIV transmission risk behaviours; delivered by health-care providers or counsellors; delivered to individuals; delivered intensively; delivered in settings where PLWH receive routine services or medical care; provided skills building, or addressed a myriad of issues related to mental health, medication adherence, and HIV risk behaviour [137].

4.22 To Combine Behavioural and Biomedical Interventions

Some authors have stressed the evidence that demonstrated the lack of effective results from preventive programmes based on single levels of intervention.

After 2010, the value of risk-reduction interventions in the prevention of STI/HIV was re-examined in the light of over two decades of behavioural study results and a consensus of experts have expressed, in different documents, the need to shift efforts toward the design and evaluation of new "combined prevention packages". Some evidence that has supported this methodological shifting was that; (1) the results of the trials showed that there can be no single strategy to contrast STI epidemics; (2) different interventions with modest levels of effect might lead to more substantial efficacy if combined; (3) and many human behavioural factors tend to affect the efficacy of biomedical interventions, when available, these evidence should be taken in consideration to strengthen the outcomes of socio-behavioural programmes [138].

Integrated programmes, which are the result of a synergic combination of behavioural, social and medical interventions have recently been proposed as the best way to reach measurable outcomes to contrast and control the circulation of sexually transmitted pathogens. This strategy appears to be particularly appropriate when the main preventive outcome is to contrast the circulation of a STI such as HIV-1 infection.

In fact, the epidemic of HIV infection has highlighted the value of synergic integration between initiatives aimed to change the sexual behaviour of the single individual with interventions that improve social justice and human rights (i.e., in at-risk communities, in stigmatized groups, in hard-to-reach populations) and with biomedical interventions aimed to reduce vulnerability in susceptible populations (i.e., male circumcision, vaccine availability, vaginal or anal microbicides) or infectiousness in index cases (i.e., prompt STI treatment, treatment as prevention/TasP and susceptibility in partners (i.e., Post

exposure prophylaxis/PEP and pre-exposure prophylaxis/PrEP) [54, 139–143].

In this regard, over 10 years ago, King Holmes coined the definition of Highly Active HIV Prevention (HARP) using an integrated model of Coates where the *behavioural change* is included in a preventive paradigm with other three different levels: social justice and human rights, biomedical strategies and treatment for STI and HIV [31, 144].

Interventions to reduce behavioural risk can play a relevant role in these integrated programmes, particularly if they are designed to be included in the routine activities of assistance services also when concentrated in a single session. In fact, the effectiveness of biomedical technology-based preventive measures can be seriously undermined by risk compensation which was applied to sexual behaviour to explain why increases in condom use were not reflected in reductions in STI or HIV-1 incidence rates [145]. Risk compensation occurs when an intervention prevents an adverse outcome, paradoxically making risk-taking behaviour more attractive; compensatory increases in risky behaviours then result in a failure to reduce the adverse outcome. As already described for MSM, the risk compensation hypothesis explains why trends in STIs and risky sexual behaviour since the mid1990s have increased in the context of continued developments and improvements in antiretroviral therapy for HIV treatment and prevention.

4.23 Present Challenges and Future Prospective

Today, the continuous epidemiological changes in STI diffusion patterns observed in the last two decades have moved the behavioural interventions into a critical position. The modest overall effect-size on STI prevalence measured in different populations by large multicentre studies, particularly in the post-cART era, is proving to be a weak weapon against the challenge of an unstoppable increase in STI incidence rates, particularly in high-risk groups, such as MSM.

Among MSM, biomedical interventions for HIV prevention, such as TasP, PEP and PrEP, are putting a strain on the preventive pressure exerted by behavioural prevention interventions delivered in STI/HIV clinical centres and in point-of-care test facilities in many Western countries.

In addition, other relevant cognitive phenomena in this vulnerable population, such as the optimism of the cure, safer-sex fatigue and the consequences of HIV infection on the mental health of patients, have reduced the threat from HIV infection, the need for safer sex and decreased the occurrence of protected receptive anal intercourses [146–149]. Moreover, recreational poly drug use continues to be of significant concern in MSM particularly when it is "sexualized" and has become a relevant risk factor for recurrent unprotected sex and harmful practices. One quarter of a sample of MSM drawn from 20 sexual health clinics in England report using three or more recreational drugs in the previous 3 months [150]. There are additional concerns about sexualized drug use (SDU) or "chemsex behaviour" because it can affect the medication adherence in HIV-1 patients and the use of antiviral drugs as prophylaxis (i.e., PEP and PrEP) in at-risk individuals, and was associated with increased rates of serodiscordant condomless anal sex, STI diagnoses and transmission risk of blood borne viruses, as well as HCV [151, 152]. The same use of social networking sites to maximize sexual encounters in adults appears associated with higher odds of bacterial STI in minority MSM [153].

All these facts suggest that the future of behavioural intervention to avert STIs, particularly those targeting higher risk groups, will be enhanced with biomedical methods, despite the fact biomedical methods to prevent HIV have, however, contributed to increased proportions of STIs within MSM as a result of risk compensation. Thus, it will be crucial to improve the study design and methodology of interventions, also to better distinguish between strategies aimed at STI prevention and those to contrast HIV-1 diffusion. Today, the goals achieved for one are not automatically successful for the other.

Innovative behavioural initiatives will have to be increasingly available in the vaccination programmes currently available against some STI (i.e., HPV, HBV, HAV and meningococcal infections) and for those that will become available in the future, on which the negative perceptions of the susceptible individuals and of a large part of civil society currently weigh, with expensive consequences on the level of coverage and on the herd effect. More and more in the future, sexual health clinics (SHC) and STI centres will represent strategic sites which provide an opportunity to vaccinate STI patients and MSM, who do not readily recognize their own risk or disclose their sexual orientation to the general practitioner or to operators of health facilities where vaccines are delivered. Forty-five percent of MSM in a national survey in Britain between 2010–2012 had attended a SHC in the last 5 years [154].

Behavioural interventions will become increasingly important also to implement and ensure the effective use of Point of Care tests (POC) to maximize STI screening in low- and middle-income countries and to deliver attitudes and skills to higher risk individuals and groups in the POC activated in the urban areas of the high-income countries, away from the barriers to access that still exist in the Western STI centres today.

Behavioural sciences aimed to change risky sexual behaviour will soon have to include digital technologies to contrast the use of Internet as a emerging risk environment for STIs [155]. Recently, some educational programmes were based on the use of automated text messages or *serious games* delivered by smartphones and tablets. These experiences, which especially target MSM communities, were designed to improve HIV test-uptake and HPV vaccination willingness, and are showing promising, if not conclusive, results [156, 157].

4.24 Conclusions

Today, the increased rates of diseases associated with harmful human behaviour suggests the difficulties of prevention programmes in changing human conducts. This is particularly true for sexual behaviour, which is determined by various factors and serves many individual needs. During the past two decades, the medical and social sciences have interacted together to design effective behavioural interventions to contrast STI diffusion and the HIV-1 epidemic, obtaining much success but also measurable defeats. The research on behavioural preventive strategies has identified numerous models, ranging from the individual-level to those able to influence public policies and social values. However, despite the amount of evaluated positive effects from large targeted interventions, none of what has been done to date should be considered sufficient in itself, because the "*novel epidemiologies*" of STI and of HIV-1 infection are showing unexpected patterns of inter and intra-population diffusion, which challenge the *moderate effectiveness* of the preventive tools at our disposal today. Moreover, powerful risk compensation mechanisms are emerging in the most vulnerable groups, mainly after the introduction of new effective biomedical HIV prevention tools. Furthermore, the massive use of synthetic drugs in association with sex in the MSM population is further weakening the abilities of at-risk individuals to resist the preventive fatigue and so increasing their behavioural recidivism.

Only the interventions that will be able to consider all these novel key elements of diffusion of STI can be candidates to reach measurable positive results.

All studies design methods and theoretical frameworks which have shown positive results in large culturally tailored behavioural interventions must be critically collected and used as the cornerstone of most effective and targeted strategies in the future. However, it is still necessary to resist the temptation to consider behavioural interventions as effective results that can easily be exported to different social or geographical contexts as if they responded to the valid paradigms of the biological sciences. This concept, which equates behavioural interventions with vaccines or drugs, must be definitively abandoned.

Finally, it is also necessary to follow new paths that develop what many studies have

indicated as peculiar to the effectiveness of behavioural interventions, but also hard to guarantee, such as: to predict interaction of multiple forces in the intervention delivery; to make intervention capacities for responsive changes according to unpredictable modifications in the social context and to assure enough resources to sustain both intensity and long-term durability of strategies to produce measurable and stable effects in the different target populations.

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5 Vaccines for STIs: Present and Future Directions

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5.1 Introduction

The prospects for new STI vaccines are promising. Antimicrobial resistance for gonorrhoea and new syphilis outbreaks represent new urgencies in STI control, and a boost for the development of these vaccines. Global efforts in improving sexual and reproductive health and in reducing vaccine-preventable diseases, together with advances in scientific research, are the optimal occasion for making these vaccines a reality. Twenty-five years ago, the outlook for development and implementation of the first STI vaccines (against HPV and hepatitis B) seemed challenging, but these vaccines are nowadays core elements for global public health strategies. Following on these successes, development of a new generation of STI vaccines is forthcoming.

Figure 5.1 shows the current status of the development pathway for STI vaccines. For some diseases (syphilis and gonorrhoea), vaccine development is at earlier stage, but renewed commitment to these pathogens could result in new candidates over the next years. Additionally, a novel vaccination strategy which uses *N. meningitidis* vaccine is being evaluated for gonorrhoea, with promising results. Genital chlamydia vaccine development was in the preclinical stage until 2016, when the first phase I human clinical trials started, and others may soon follow. So far, HSV vaccine candidates are furthest along in the pathway, with several candidates in phases I and II, and possibly a vaccine will be available in the next 10 years. HPV vaccines are already a reality, but novel prototypes for therapeutic vaccines are at preliminary phase of development, and they could represent additional tools to address HPV epidemic. Surely, HIV vaccine development has concentered major efforts, with some promising results being available in the next years.

In this chapter, we will review major advances in the development of vaccines for syphilis, HSV, chlamydia, gonorrhoea, HIV, and HPV (therapeutic). We will describe the most promising prototypes and strategies, their mechanism of actions, and the stage of research development.

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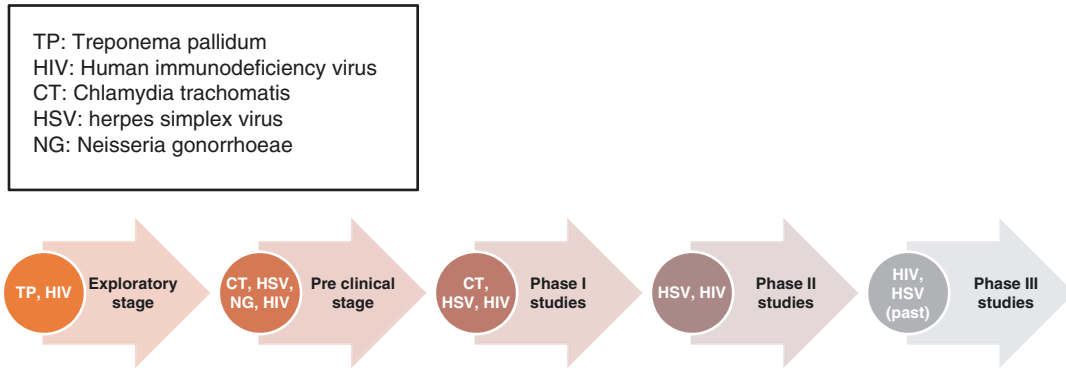

Fig. 5.1 Development pathway for STI vaccines

5.2 Syphilis

Ideally, syphilis represents a disease that can be eradicated: human is the only natural host [1], and the diseases can be easily diagnosed and treated [2]. Despite that, there are few limitations to reach the goal, represented by the various clinical presentation [3], the need of repeated visit to confirm diagnosis and to guarantee adequate treatment [4], and the difficulties in diagnosing reinfections (due to the tendency of antitreponemal antibodies to remain detectable after treatment) [5]. All these factors highlight the central role that a vaccine could have in eradicating the disease [3].

Only a limited number of research groups are currently working on syphilis vaccine development [6], and many aspects of the biology of *Treponema* and of the host–pathogen interactions are still to be understood [7].

Major challenges in the basic research on *Treponema* are represented by its inability to be cultured in vitro or to be genetically manipulated [1]. Moreover, its outer membrane is located within a cytoplasmic membrane-proximal layer [8, 9], making it extremely labile and easily disrupted by experimental manipulations. Additionally, *Treponema* has extremely low OMP (outer membrane protein) content [9–11] and, as consequence, conventional OMP identification methods present low sensitivity.

A crucial point to understand is the host–pathogen interaction in syphilis: *Treponema*, if untreated, is able to determine a non-sterile immunization, which means that people treated are less likely to develop chancres upon re-inoculation (chancre immunization), but they are still able to become infected [12].

As stated by Cullen et al. [2], a major goal of the hypothetical vaccine is to elicit sterile immunization, as the development of a vaccine to prevent disease but not infection could raise ethical issues (despite reducing morbidity and mortality). Indeed, it could increase rates of transmission of the disease and facilitate the progression in asymptomatic, undiagnosed patients to late-term complications.

Recently there have been few advances in understanding the natural history of the diseases and the interactions with the human immune system. In primary lesions CD4+ T-cells, macrophages, and natural killer cells predominate in cellular infiltrates, whereas in secondary ones CD8+ T-cells tend to prevail [13]. The production of Th1-related cytokine promotes the development of a delayed-type hypersensitivity response that is crucial for the elimination of the pathogens from the lesions [14]. Th1-cytokines activate macrophages, but phagocytosis requires the opsonization of the *Treponema* (opsonophagocytosis) possible by a strong cross-talk with the humoral immune response [15]. Despite that, the pathogen shows an elusive nature, and a subpopulation of *Treponema* can evade it [16], maybe because of different antigen presentation or variation [17]. Finally, the systemic response

is thought to prevent the escape of *Treponema* into secondary sites during the haematogenous dissemination, by the identification of proteins expressed by the pathogen [3].

The ideal vaccine should prevent chancre development, treponemal dissemination, persistence, and reinfection. It should aim at eliminating symptoms within an infected individual and at interrupting the disease transmission [3]. This could be reached through the elicitation of a delayed-immune response, which targets the *Treponema* within the vascular system (avoiding the dissemination in secondary sites) and addresses the escape sub-population. In addition, it should provide cross-protection, to avoid reinfection due to different strains, and, more importantly, it should be a recombinant subunit vaccine, to permit the administration in immunocompromised patients (i.e., HIV), which are one of the target risk groups for vaccination.

The first advocacy for a vaccine against *Treponema* date from the nineteenth century [18], but a first partial achievement was reached only in 1969.

At that time, Metzer et al. demonstrated that rabbits exposed to the injection 4-times/week for 7 weeks of a strain of *T. pallidum* with reduced virulence (obtained by short-term storage at 4 °C) avoided clinical manifestations after the inoculation of pathogens, and in some of them there was no transfer of pathogens in lymph nodes [19]. Unfortunately, the intramuscular administration showed a less effective response [20] and treated rabbits developed a latent infection, as it was a non-sterile immunity.

So far, the unique demonstration of elicited sterile immunity is due to Miller et al. They inactivated by γ-irradiation whole bacteria, which became non-infectious and non-proliferative, but still retained surface antigens and their motility. The proposed schedule was long (37 weeks), with high dose daily inoculations, but determined a full protection, which lasted at least one-year post-treatment. It determined both chancre immunity and sterile immunity, as demonstrated by the absence of bacteria in lymph node transfers from the exposed rabbits [21]. This schedule was unpractical for human administration and did not confer protection against the strain Haiti B, which was considered at that time as being *Treponema* subspecies *pertenue*, and not *pallidum*.

From these two experiences derived important concepts for future researchers: a sterile immunity can be elicited in rabbit model, but to reach it an intact surface is needed, which is challenging because of the pathogen fragility in being manipulated.

Several other experiences followed in more recent years. They focused on the selection of individual antigens tested in rabbit models: *Treponema* protein (Tp) Tp92 (BamA) [22, 23], TprK [24, 25], TprF [26], TprI [27], TmpB [28], Gpd [22], 4D [29], endoflagella [30], TpN15 [31], TpN47, Tp0155, Tp0483, and Tp0956 [23]. Other antigens displayed a partial, non-sterile immunity, determining the development of attenuated lesions: Gpd [22], Tp92 (BamA) [23], TprF [26], 4D [29], and endoflagella [30]. In one of the cases of TprK antigenic protein, the elicited partial protection was associated with the N-terminal region of the protein [32]. Unfortunately, some of these antigens determined divergent results: Tp92 (BamA) [23], TprK [33], and Gpd [33].

All these studies suggested that a single antigen is not sufficient to elicit immunity, and supported the development of a vaccine using a cocktail of antigens [6], considered critical both for chancre development [25, 34, 35] and for treponemal dissemination [36–38]. Finally, to address pathogen dissemination, a novel Treponema protein, Tp0751, has recently shown promising results in determining sterile immunity [39]. In future other novel technologies, such as reverse vaccinology and subtractive genomics, could fill the gap of identifying vaccine candidates for syphilis [40] and will possibly be implemented.

5.3 HSV Viruses

Among sexually transmittable infections for which a vaccine is not yet available, herpes simplex virus (HSV) is the pathogen with more candidates already identified, and with few of them at advanced stage of development [41].

There are two serotypes of HSV, both being transmittable through sexual intercourse, but with a different preference. HSV-2 is mostly sexually transmitted and represents the leading cause worldwide of genital ulcer disease (GUD), while HSV-1 is transmitted by oral contact, causing oral-labial herpes. If this is certainly true in low-income countries (LICs), where generally HSV-1 infection occurs in childhood, in high-income countries infection during this age is declining [42]. As a consequence, adolescents remain susceptible to HSV-1 infection at the moment of sexual debut, and HSV-1 is becoming the leading cause of GUD in Americas, Europe, and Western Pacific [43], generally with a mild natural history and few recurrences [44, 45]. At the same time, HSV-1 does not seem to prevent the acquisition of HSV-2, but it reduces the risk of acquiring symptomatic HSV-2 infection [46]. This demonstrates that the two serotypes have complex immunological interactions. In both cases, reactivations may be silent in up to 84% of cases, and most of the HSV-2 transmissions is thought to occur through asymptomatic viral shedding [47, 48].

Moreover, HSV-2 infection increases the risk of acquiring HIV-1 of two- to threefold both in women and men [49, 50], and the enhanced risk does not seem to be related only to breaks of the mucosa caused by ulcers, but it appears to be the consequence of an increased density of CD4+ T-cells in the genital mucosa of HSV-2 infected people, regardless of the presence of GUD [51].

For this reason, the development of a vaccine against HSV-2 could have an impact also on the epidemiology of HIV, reducing the related excess risk of acquiring HIV-1 in HSV-2 infected people [52]. All these epidemiological considerations make a vaccine against HSV-2 more needed than one against HSV-1, despite a combined protection could be particularly important in high-income countries [42].

There are two possible approaches in the development of vaccine against HSV: prophylactic (to prevent the infection) and therapeutic (to treat or modify the disease).

A model on the impact of a prophylactic approach on public health predicted that a vaccine with an efficacy of 75% in sub-Saharan Africa, given a 50% coverage of 14 year olds and catch-up vaccination through age 29, could reduce HSV-2 incidence of about 50%, and a reduction of 10% HIV incidence in 10 years [53]. Prophylactic vaccines should be administered before sexual debut and could benefit from existing vaccine-delivery infrastructures. Protection could last at least during young adulthood (the highest HSV-2 incidence period), and a booster could be required later. The possible coverage against HSV-1 has implication on the target population, as the acquisition of HSV-1 infection in early childhood would require vaccine administration largely earlier than adolescence.

Conversely, a therapeutic vaccine would have a different target population, given by patients with recognized GUD. However, if a vaccine induces CD4+ activation in genital mucosa, there could be the risk of increasing target cells for HIV infection, despite the reduction of mucosal ulcerations [54]. Additional concerns are related to virologic assays for the diagnosis, that may be not available, while the widely available serologic assays are limited by poor specificity [55].

For all these reasons, both strategies are considered to be effective in a public health perspective, but a prophylactic vaccine could be ideally the most appropriate in low-income countries and in setting with high HIV prevalence [42].

Despite these evaluations, development of prophylactic vaccines is currently low represented. Indeed, they require very large trials in high-income countries, which rise several technical issues. Additionally, it is currently hard to distinguish hypothetical vaccinated people from infected whole-virus vaccine prototypes, as diagnostic procedures are lacking [42].

So far, all prototypes available induce neutralizing antibodies against at least one of the 11 envelope proteins of HSVs, including glycoprotein D (gD) and/or B (gB). The first one has been included in all vaccine candidates, while gB was a component in earlier vaccines [56, 57].

Unfortunately, previous clinical trials on prophylactic vaccines failed to demonstrate adequate efficacy. Two phase III clinical trials were conducted on a gD2-based vaccine with alum and

monophosphoryl lipid A adjuvant. The first one reached 73% efficacy in preventing GUD, but only in HSV-1 seronegative women [58]. The second clinical trial (Herpevac) was conducted among HSV-1 seronegative women. It failed to determine protection against HSV-2 but shown 35% efficacy against HSV-1 infection, and 585 (95% CI 22–80) against GUD HSV-1 related [59]. Importantly, the protection against HSV-1 demonstrated a correlation with anti-gD2 antibody titers [60].

From these experiences in prophylactic, antibody-response approach there were two main achievements: firstly, clinical studies, but even preclinical pig model, demonstrated a diminished protection against HSV-2 infection in HSV-1 seropositive persons [58, 61]. Secondly, new candidates with deleted gD2 HSV-2 virus have shown protection against lethal infection in animal models, demonstrating that probably gD2 is an inappropriate entry protein target. In support of that, experimental animal models highlighted that the addition of other glycoproteins (gC or gE2) enhances elicited protection [61, 62]. However, new T-based vaccines are giving promising results in animal models, even using innovative ways of administration (intranasal vaccination or intradermal laser adjuvant-assisted peptide vaccine) [63, 64].

In the last years, there have been several signs of progress towards the development of a therapeutic vaccine, and all candidates currently in a clinical phase are therapeutic. The first reason is that available antiviral drugs are not completely effective in suppressing shedding and avoiding recurrences [65]. Secondly, clinical trials for therapeutic vaccines are easier, less expensive, and shorter compared to trials needed for prophylactic ones [52]. Indeed, shedding rate is an accepted surrogate for transmission risk and diseases severity [66, 67], and patients represent controls of themselves, performing daily self-sampling before and after vaccine administration.

At least four therapeutic vaccines are in clinical development. One will likely enter in phase I study by the end of 2018 [42], and a vaccine against HSV-2 will probably be licensed within 10 years [52]. Type, structure, and study phase of each vaccine in clinical development are summarized in Table 5.1.

All of them elicit both humoral and cellular responses [41], and it is currently unknown if they induce these responses also in genital mucosa, as there are several methodological difficulties in developing adequate studies to assess that. As a consequence, it is also unknown whether therapeutic vaccines could reduce the risk of HIV-1 infection or increase it [52],

Table 5.1 Therapeutic HSV vaccines in clinical trial phase (adapted from http://www.who.int/immunization/research/meetings_workshops/16_Deal_Gottlieb_HSV.pdf)

Vaccine	Company	Study phase	Type	Structure	Trial results	References
GEN-003	Genocea	Ready for phase III	Subunit with adjuvant	HSV-2 gD2 plus a truncated infected cell polypeptide 4 with Matrix M-2 adjuvant	Phase IIb: Good safety profile; 40% reduction in shedding; 52% reduction in lesion rate; 50% reduction in recurrence frequency; 33% reduction in recurrence duration	[68–71]
COR-1	Admedus	II	DNA	Two plasmids, coding for full gD2 and for truncated gD2 (codon optimized with a ubiquitin tag)	Phase I: good safety profile; T-cell responses in 19 out of 20 subjects; no increases in antibody response	[72, 73]

(continued)

Table 5.1 (continued)

Vaccine	Company	Study phase	Type	Structure	Trial results	References
VCL-HB01	Vical	II	DNA with adjuvant	DNA coding for UL46 (tegument protein VP11/12), and gD2, plus Vaxfectin® (a lipid based adjuvant)	Phase I/II: good safety profile; 57% reduction in lesion rate at 9 months	[74–77]
HSV529	Sanofi Pasteur	I completed NCT02571166 ongoing (phase I)	Live genetically modified	Replication defective HSV-2 with genes UL5 and UL29 deleted	NCT01915212 completed (phase I) Data pending	[78–80]
G103	Immune Design Corp	Preclinical, phase I scheduled in 2018	Subunit with adjuvant	Trivalent: gD2, UL19 (capsid truncated VP5) and UL25 (structural, DNA packaging protein), plus GLA-SE (glucopyranosyl lipid A in stable emulsion) adjuvant	Not applicable	[81]
HerpV	Agenus	Phase II completed, development appears to have been discontinued	Subunit with adjuvant	Thirty-two 35-mer peptides plus human HSP 70 heat shock protein) with QS-21 adjuvant	Phase I: Good safety profile; all participants receiving QS-21 adjuvant vaccine (7 subjects) demonstrated HSV-2 antigen specific CD4+ response after vaccination; CD8+ T-cell response detected in most participants; phase II results not available	[77, 82, 83]

and more in general, there is lack of model evaluation of the impact of therapeutic vaccines in a public health perspective regardless of HIV-1 epidemiology.

5.4 Chlamydia

Genital chlamydia infection is a concern in all world regions, with an estimated 131 million incident cases globally in 2012 [84], and it affects mainly adolescents and young adult under 25 years [85]. Without treatment, chlamydia can ascend to the upper genital tract in women to cause acute pelvic inflammatory disease (PID), which can lead to longer-term complications including tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Due to the global burden of chlamydia-related sequelae, there is a pressing need for an effective vaccine [41].

A genital chlamydia vaccine would ideally target adolescents before sexual debut to maximize immunity during the period of the highest transmission risk. Complete immunity to infection would be the optimal target, but it may be difficult to achieve. However, even a partially

protective vaccine that inhibits upper genital tract infection and damage or reduces ongoing transmission could have a significant impact and provide benefits on an individual and population-level [86]. An effective chlamydial vaccine would have public health benefits in both high-income countries (HICs) and low-middle-income countries (LMICs). Nevertheless, a chlamydial vaccine would probably provide the greatest benefits in LMIC settings, where lack of medical infrastructure and resources preclude *Chlamydia* screening programs and the burden of chlamydia-associated sequelae is higher.

Currently, there is no licensed vaccine for *Chlamydia trachomatis*, but evidence from animal models and human studies suggests that a vaccine is feasible. Animal challenge studies, including mouse, guinea pig, and non-human primate models, demonstrate that partial and sterilizing natural immunity can develop from a primary infection; however, this protection is short-lived and not sufficient to provide long-term immunity [87]. In animals, partial immunity can reduce bacterial burden and duration of secondary infection but does not prevent upper genital tract pathology. In humans, epidemiologic studies reveal a decreased prevalence of infection and decreased bacterial load with increasing age despite continued exposure [88]. In addition, in a prospective study of 200 women in the US, those whose chlamydial infections cleared spontaneously between testing and treatment were less likely to become re-infected on follow-up [89]. The ability of natural infection to induce partial immunity is promising for vaccine development [90].

The immunologic mechanisms against *Chlamydia* infection are not fully understood [91]. Interferon-γ (IFN-γ) producing CD4 T-cells play a critical role: they might confer protection against *C. trachomatis* and are enough for resolution of the infection, but the involvement of IL-2 and TNF-α seems to be essential for bacterial clearance [92]. Tissue-resident memory (TRM) T-cells could have an important role for vaccine development, as the intensity of mucosal CD4 T-cell responses is a correlate of protective immunity. TRM T-cells are long-lived non-circulating memory cells able to respond to infection independent of systemic T-cells [93].

From recently acquired data, antibodies also play an important role, with different possible mechanism, including enhancement of Th1 effector responses and direct pathogen neutralization [94–96].

An ideal vaccine should elicit both cell-mediated and humoral immunity; in particular, the interaction between these two defense mechanisms plays a central role. If both arms of the immune system are promoted by a vaccine, the primary role of neutralizing antibodies will be to reduce the initial infectious load; once they are intracellular, remaining bacteria can be targeted by a bactericidal cell-mediated immune (CMI) response [94]. In order to be effective, a genital *C. trachomatis* vaccine likely needs to elicit tissue-resident T-cells. The majority of preclinical vaccines evaluated mediate protection predominantly through T-cells with no neutralizing antibodies [97–100]. More recently, studies supporting the effectiveness of neutralizing antibodies also became available [94–96].

In a study by Olsen et al., a recombinant chlamydial major outer membrane protein (MOMP) subunit vaccine candidate promoted strong neutralizing antibody titers and Th1 responses and showed protection against vaginal chlamydial infection in mini-pigs and against upper genital tract disease in mice [94, 95]. This vaccine is the first candidate entered in human phase I clinical trials in 2016 [101]. While the chlamydial MOMP has long been the focus of vaccine development, several new candidate antigens (e.g., polymorphic membrane proteins [PMPs]) are emerging and are showing great promise, in both mouse and primate models. Combination of MOMP with PMPs identified by immunoproteomics represents a promising approach [6, 102]. A major advance in the field has been the ability to generate vaccine-induced seeding of genital mucosa with CD4 tissue-resident memory T-cells, which was the key to long-lived protection against chlamydial infection in mice. This was achieved using mucosal immunization with UV-inactivated *C. trachomatis* combined with a novel nanoparticle-based adjuvant [93].

Overall, thanks to new discoveries in the immunological field and to new technics, the development of anti-chlamydial vaccine seems to become increasingly feasible. After the first one entered in phase I, other vaccine candidates are likely to enter clinical evaluation in the coming years.

5.5 Gonorrhoea

More than 106 million new cases of gonorrhoea are estimated to occur yearly worldwide and the incidence is expected to continue to rise in the next years [103]. The management of gonorrhoea infection is based mainly on antimicrobial therapy, combined with prevention, rapid diagnosis, partner notification, and epidemiological surveillance. However, there are now multiple reports of *N. gonorrhoeae* strains that are resistant to the last remaining first-line treatment option for gonorrhoea, the extended-spectrum cephalosporins [104–106]. There is, therefore, a pressing need for the development of new interventions against gonococcal disease, but an effective vaccine is probably the only sustainable solution for controlling gonorrhoea and substantially reducing the global public health burden. This is due to the rapid emergence of resistance to all introduced therapeutic antimicrobials, the predominance of gonorrhoea in resource-limited settings where syndromic treatment of symptomatic patients dominates, and the lack of symptoms in many patients. Unfortunately, the few clinical trials of gonorrhoea vaccines that have been performed (all before 1990) were disappointing because those vaccines did not provide significant protection from infection.

Many biological challenges exist to gonococcal vaccine development. There is no naturally acquired immunity to the infection; *N. gonorrhoeae* has a highly antigenically variable surface and is well adapted to evade host responses, and robust animal models to study the infection are limited. Multiple potential gonorrhoea vaccine targets have been identified based on their relative antigenic conservation and stability among strains, but these have not led to feasible vaccine candidates yet [41, 107, 108].

The greatest problem in the development of a gonococcal vaccine is represented by the host restriction of these bacteria, which makes it difficult to establish gonococcal infection in laboratory animals. Recently, more sophisticated mouse models have been created providing further information about the human immune response to infection. Liu et al. found that normal gonococcal infection elicits an inflammatory Th17 response that precludes development of adaptive immunity by mechanisms dependent on the regulatory cytokines TGF-β and IL-10; if cytokine or other adjuvants are used to shift this to a Th1-based response, mice become immune to repeat infection [109, 110]. The most recent study of this group shows that intravaginal treatment of gonococcal infection in female mice with microencapsulated IL-12 induces persisting anamnestic immunity against reinfection with *N. gonorrhoeae*, even if antigenically different strains, dependent on T-cell production of IFN-γ and B-cell production of antibodies [111].

A new promising field for development of preventive gonococcal vaccine is represented by reverse vaccinology, which includes genome and proteome mining [41]. This technique has been very successful in the discovery of vaccine candidates against many pathogenic bacteria. Progress with this approach for a gonorrhoea vaccine remains in its initial phase, but it could lead to new scenarios. Proteome mining uses bioinformatics to select proteins with desired characteristics from large datasets, thus narrowing the search for promising antigenic targets for gonococcal vaccine.

Recently two studies by Zielke et al. enabled the identification of potential gonorrhoea vaccine targets, using a comprehensive proteomic platform. Initial characterization of five novel vaccine candidate antigens that were ubiquitously expressed under different growth conditions demonstrated that homologs of BamA (NGO1801), LptD (NGO1715), and TamA (NGO1956), and two uncharacterized proteins, NGO2054 and NGO2139, were surface exposed, secreted via naturally released membrane vesicles, and elicited bactericidal antibodies that cross-reacted with a panel of temporally and geographically different isolates. In addition,

analysis of polymorphisms at the nucleotide and in amino acid levels showed that these vaccine candidates are highly conserved among *N. gonorrhoeae* strains. Finally, depletion of BamA caused a loss of *N. gonorrhoeae* viability, suggesting it may be an essential target [112].

Another promising development for gonococcal vaccine relates to existing vaccines against another *Neisseria* species, in particular the group B meningococcal vaccine using the outer membrane vesicle (OMV) antigen presentation strategy. *N. gonorrhoeae* and *N. meningitidis* share 80–90% homology of primary sequences and thus some level of cross-protection is plausible. In a recent report, Petousis-Harris et al. provide a proof of principle for vaccine protection against gonorrhoea. The researchers assessed the effectiveness of the outer membrane vesicle (OMV) *N. meningitidis* serogroup B vaccine (MeNZB) against gonorrhoea in a retrospective case–control study that included young adults attending sexual-health clinics in New Zealand. MeNZB was administered to >1 million New Zealanders in response to a meningococcal epidemic during 2004–2008 and 81% of the population aged <20 years were vaccinated in 2004–2006. The vaccinated individuals were significantly less likely to have been diagnosed with gonorrhoea compared with controls (adjusted OR 0.69, 95% CI 0.61–0.79; $P < 0.0001$), particularly in the years during and immediately after vaccine administration. The effectiveness of MeNZB against gonorrhoea was estimated to be 31% (95% CI 21–39%), after adjustments for ethnicity, geographical area, and sex [113]. The level of effectiveness might not initially seem impressive; however, mathematical modelling suggests that a vaccine with 30% efficacy could decrease gonorrhoea prevalence by more than 30% within 15 years, if population coverage is sufficiently high and protection lasts over the highest risk period (i.e., most sexual partner change) among young people [114]. MeNZB is no longer available, but the licensed, four-component meningococcal serogroup B vaccine (4CMenB—BEXSERO® GlaxoSmithKline) includes the same OMV as MeNZB and three recombinant vaccine antigens (neisserial heparin-binding anti-gen (NHBA), factor H-binding protein (fHbp), and *Neisseria* adhesin A (NadA)). Consequently, widespread administration of the 4CMenB to adolescents might also reduce gonorrhoea prevalence owing to cross-protection.

5.6 HPV

We only mention vaccines against human papillomavirus (HPV), for which a dedicated chapter in Sect. 5.5 is reserved.

Vaccine against HPV was the first STI vaccine that became available. After its introduction in 2006, important results in terms of HPV reduction rates in countries when vaccine coverage is high have been observed [115].

Human papillomaviruses (HPV) are non-enveloped DNA viruses with an icosahedral capsid and they specifically replicate in epithelial cells of the skin, genital and oral mucosa. The capsid is composed by the two viral proteins L1 (major capsid protein) and L2 (minor capsid protein) and represents the viral component that can be recognized by the host's immune system [116].

The currently licensed vaccines are prepared from purified L1 protein that self-assembles to form type-specific HPV virus-like particles (VLPs). These VLPs closely resemble the outer surface of HPV virions. VLPs contain no viral DNA and are therefore non-infectious. The vaccines are designed for prophylactic use and have not been found to effectively clear existing HPV infections or treat HPV-related diseases [117, 118].

The quadrivalent vaccine (Gardasil) was first licensed in the United States in 2006. The L1 proteins for each type are expressed via a recombinant Saccharomyces pombe (type of yeast) vector. It contains VLPs derived from the L1 protein of HPV types 6, 11, 16, and 18 [116, 117].

The bivalent vaccine (Cervarix) was first licensed in 2007. The L1 proteins for each type are expressed via a recombinant baculovirus (type of insect cell) vector. It contains VLPs derived from the L1 protein of HPV types 16 and 18 [116, 117].

The newly approved (in 2014) version of Gardasil, V503 or Gardasil9, comprises VLPs

from HPV 31, 33, 45, 52, and 58 in addition to the quadrivalent vaccine [119].

For Gardasil and Cervarix, the way of protection is presumed to be the induction of virus-neutralizing antibody responses. Current HPV vaccines induce mainly type-specific immune responses, although some degree of protection against non-vaccine types has been observed. For future vaccine generation it could be important covering a broader range of high-risk HPV types; in this context it is worth noting that the N-terminal part of L2 protein contains a region highly conserved among different HPV types and this region could be a promising target for future HPV vaccines [116].

Another developing and promising field is represented by therapeutic vaccination. In contrast with prophylactic vaccines, therapeutic vaccines are designed to treat patients after the appearance of HPV-related lesions [120]. They aim to treat preexisting HPV infections by stimulating dendritic cells (DCs) and T lymphocytes response against tumour antigens. Therapeutic vaccines target oncoproteins E6 and E7, that are the main responsible for the malignant transformation of HPV-related lesions [121] and that are generally consistently expressed in precancerous and cancerous lesions.

Several phase I and II trials tested the in vitro and in vivo efficacy of therapeutic vaccines, but more complete data have to be provided.

5.7 HIV

In the 1980s the etiological agent of the acquired immunodeficiency syndrome (AIDS) was discovered. Since then, the introduction of combination antiretroviral therapy (cART) has dramatically decreased the AIDS (48%) related deaths between 2005 and 2016 [122].

Mathematical models estimated that the introduction of a vaccine with an efficacy of 70% and 5 years of protection in 2027 could reduce annual new infections by 78% within 2070 [123].

One of the major goals for HIV vaccine development is the prevention of HIV-1 acquisition at mucosal surfaces. The most difficult challenge in vaccine biotechnology is the development of an effective HIV vaccine, especially because the causal mechanisms of protection from HIV infection have not been definitively established [124, 125]. Additionally, the extreme diversity of HIV strains belonging to different subtypes can differ by up to 35% in their envelope (Env) proteins [126, 127].

Thus, pilot studies of recombinant HIV-1 Env glycoprotein subunit (rgp120) vaccines conferred protection in chimpanzees from intravenous and mucosal challenge with homologous and heterologous HIV-1 strains [128, 129]. Therefore, initial HIV-1 vaccine approaches focused primarily on the generation of neutralizing antibodies (nAb).

Since 1987, hundreds of vaccine candidates have been clinically tested as HIV-1 vaccines, in the table below a summary of HIV-1 vaccine efficacy trials are reported (Table 5.2).

Table 5.2 Summary of HIV-1 vaccine efficacy trials

Trial ID	Vaccine composition	Response
AIDSVAX B/E (VAX003)	Two clade B and one CRF01_AE gp120 antigens in alum	No protection
AIDSVAX B/B (VAX004)	Clade B recombinant gp120 antigens in alum	No protection
HVTN502 (STEP)	MRKAd5 HIV-1 Gag/Pol/Nef	Halted at interim analysis For futility; early transient Increased infection In vaccines
HVTN503 (*Phambili*)	MRKAd5 clade B Gag/Pol/Nef	No effect, late increased HIV infection in unblinded male vaccines
RV144	ALVAC-HIV vCP1521, AIDSVAX B/E rgp120 in alum	31.2% protection
HVTN505	DNA, rAd5 (A, B, C)	No protection

Ad Adenovirus, *gp* glycoprotein, *HVTN* HIV Vaccine Trials Network, *MRK* Merck, *MVA* modified vaccinia virus Ankara, *NCT* National Clinical Trials identifier, *vCP* canarypox vector, *CRF* circulating recombinant form

5.7.1 VAX003 and 004

In 1999, in Thailand 2546 injection drug users (IDU) were involved in the randomized, double-blind, placebo-controlled efficacy trial of AIDSVAX B/E (VAX003). The AIDSVAX B/E vaccine contained two recombinant gp120 HIV Env antigens (CXCR4 lab-adapted clade B strain and a CCR5 primary subtype CRF01_AE isolate) adjuvanted in alum [130].

Instead, in North America and Netherlands a double-blind randomized trial Env-based, named AIDSVAX B/B, VAX004 was administered to 5403 men who have sex with men (MSM) and women at high risk for heterosexual transmission of HIV-1. The VAX004 was a bivalent vaccine composed of subtype B rgp120 from strains MN and GNE8 [131]. The overall vaccine efficacy estimated was 6% [131]. VAX003 and VAX004 recombinant Env-based vaccines failed to demonstrate any level of protection, despite being immunogenic.

5.7.2 STEP and Phambili Studies

The STEP study used the MRKAd5 HIV-1 Gag/Pol/Nef vaccine in subjects at high risk of infection, HIV-1 seronegative women, and MSM. This phase II, double-blind, randomized, placebo-controlled trial enrolled 3000 individuals from North America, the Caribbean, South America, and Australia [132].

On the other hand, in South Africa the *Phambili* trial (phase II) involved MRKAd5 clade B Gag/Pol/Nef administered to 801 of a scheduled 3000 heterosexual men and women [133].

Since the STEP trial revealed an increased incidence of HIV-1 acquisition in male vaccines versus placebo recipients, both studies were stopped, even though both vaccines were immunogenic and well tolerated. Therefore, despite the failure to protect and being stopped early, the STEP trial MRKAd5 HIV-1 Gag/Pol/Nef vaccine was the first to place a selective pressure on the infecting virus.

5.7.3 RV144

Sixteen-thousand-four hundred and two healthy individuals at heterosexual risk of HIV acquisition were divided into vaccine and placebo arms, for the RV144 "Thai Trial" enrollment. The vaccine efficacy was 26.4% [134]. The production of non-neutralizing IgG against the V1/V2 region of Hiv-1 Env was attributed as a protective mechanism [135].

5.7.4 HVTN505

Lastly, the HVTN505 trial with double-blind reported lack of efficacy. The trial enrolled 2504 men and transgender women who have sex with men to receive either vaccine (n = 1253) or placebo (n = 1251). The DNA vaccine of this study contained clade B gag/pol/nef and clade A, B, and C env while the recombinant Ad5 expressed clade B gag-pol and clade A, B, and C env. Overall, 41 individuals became HIV+ in the vaccine arm, while 31 became infected in the placebo arm, supporting a lack of vaccine efficacy [136, 137].

In conclusion, out of the above described six HIV-1 vaccine efficacy trials [138, 139], only the RV144 study has demonstrated a modest reduction in HIV-1 infection rates using a modified intention to treat protocol. A rich HIV-1 vaccine clinical trials pipeline, along with the initiation of HVTN702, a repeat of the RV144 trial in South Africa, provides much hope that additional correlates of protection will be elucidated and that an HIV-1 vaccine might yet become a reality.

The newly developed HIV vaccines are mainly based on viral vectors, which are the best delivery tools because of their intrinsic adjuvant capability and unique cellular tropism. The recently studied vectors are adenovirus serotype 26 (Ad26), poxvirus, CMV vectors [140, 141]. The recent best effort in HIV vaccine has been the development of two viral vector-based vaccine trials: *Imbokodo* and HVTN 702.

The HVTN 702 Phase 2b/3 clinical trial will be conducted in South Africa, enrolling 5400

healthy, sexually active men women aged 18–35 years old. The HVTN 702 study is based on the RV144 protocol [141], consisting of two experimental vaccines: a canarypox-vector based vaccine called ALVAC-HIV and a two-component gp120 protein subunit. In order to stimulate a more robust immune response, the adjuvant MF59 is administered too, differently from the adjuvant used in RV144. Results from the study are expected in late 2020.

Imbokodo is a vaccine made of "mosaic" immunogens aiming to induce a protection against the diverse global HIV strain [142]. Subsequently, two early-stage human clinical trials named APPROACH and TRAVERSE, were developed, showing a good tolerance and a HIV specific immune response in the vaccines [143]. The APPROACH study enrolled 400 participants between 18 and 50 years old, who have been administered with different regimens containing Ad26.Mos.HIV, Modified Vaccinia Ankara (MVA)-Mosaic, and/or HIV-1 Clade C gp140 drug product (gp140 DP) components. This study has started on Dec 2014 and will end on April 2019 [144, 145].The TRAVERSE study is a double-blind clinical trial phase I/II conducted in the United States and in Rwanda from June 2016 and May 2018, enrolling 198 subjects [145]. Two well-tolerated different vaccine regimens have been used: at first the trivalent Ad26.Mos.HIV and boost versus the trivalent Ad26.Mos.HIV and Clade C gp140 plus adjuvant; afterwards the tetravalent Ad26.Mos4.HIV and boost with Ad26.Mos4.HIV and Clade C gp140 plus adjuvant were used [143]. The *Imbokodo* trial evaluates the quadrivalent mosaic vaccine based on the TRAVERSE Study enrolling 2600 HIV-negative women in sub-Saharan Africa and results are expected in 2021.

Another adenovirus vector-based HIV mosaic vaccine trial has been administered in the double-blind phase I/II clinical trial, named ASCENT. The primary purpose of the study is to assess safety/tolerability and Env-specific antibody responses of two different mosaic-based vaccine regimens: the first Ad26.Mos4.HIV vaccine has been administered at Week 0 and 12, followed by Ad26.Mos4.HIV vaccine + Clade C glycoprotein 140 vaccine containing protein mixed with adjuvant (aluminium phosphate) at Week 24 and 48. Alternatively, Ad26.Mos4 HIV vaccine has been routed at Week 0 and 12 followed by d26.Mos4. HIV vaccine and a combination of Mosaic gp140 and Clade C gp140. Results from ASCENT are expected in early 2019 [145].

5.8 Conclusion

About 30 years ago, the development and the implementation of STI vaccines appeared very limited [146]. Yet during the last 30 years, the introduction of vaccines against HBV and HPV radically changed the history of both diseases and represent nowadays a powerful weapon in the global control of these STIs. Moreover, the appearance of two new urgencies, antimicrobial resistance for gonorrhoea and new syphilis outbreaks, strengthens the need for vaccine development and dedicated vaccination strategies. The roadmap launched by WHO in 2014 [6] provides a comprehensive guide for research and together with the global health sector strategy on Sexually Transmitted Infections 2016–2021 published in 2016 [147] supports the collaboration and the commitment needed to make STIs vaccines a reality. The previously described vaccine candidates for STIs let us look to the future with confidence, especially with regard to therapeutic HSV vaccines (those at more advanced development stages) and HIV, for which results from at least three promising trials are expected to be available in the next 2–3 years. For other diseases (syphilis and gonorrhoea) vaccine development is at an earlier stage, but renewed and supported commitment could result in new candidates over the next years.

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Part III

Laboratory and Diagnostic Techniques

Vittorio Sambri

Particularly in the countries that can afford an aetiological diagnostic approach, the laboratory plays a much greater role in terms of diagnosis of specific STI pathogens and in the determination of antimicrobial susceptibility. Laboratory activities also play a key role in STI surveillance and in research programmes within both resource-poor and wealthier countries.

This part of the handbook focuses on the laboratory methods and tests which are potentially powerful contributors to the management and control of the modern STIs. Today, the technological progress reached in the biomolecular detection of sexually transmitted pathogens and the conspicuous number of assays offered by the market, makes the choice of the best test for clinical purposes, increasingly difficult. A cost-effective choice is strictly dependent mainly on the availability of resources, on prevalence of the target infection, on which population we decide to screen, on where (e.g., in a hospital-based lab or in point of care test) the test will be used, and not at least, on sensitivity, specificity and predictive value of the test.

All these issues will be discussed by Vittorio Sambri in an important chapter focused on the laboratory methods for the diagnosis of bacterial infections. In the second chapter, novel methods for the diagnostic approach to viral infections by the team coordinated by Manola Comar will be analysed. These two chapters describe testing procedures largely oriented to the modern aetiological approach to STI diagnosis but also basic and inexpensive techniques that have been used successfully in resource-poor settings. Experts experienced and skilled in different fields of diagnostic medicine update the two contributions to ensure that the diversity of methods available for the diagnosis of sexually transmitted infections (STIs) is captured and made as current as possible in this rapidly changing field of infectious diseases.

6 New Diagnostic Approaches to Viral Sexually Transmitted Infections

Manola Comar, Francesco De Seta, Nunzia Zanotta, Serena Del Bue, and Pasquale Ferrante

Among standard and molecular approaches currently used for diagnosis of sexually transmitted infection (STI), sensitive high throughput techniques (HTS) based on the random amplification of genomes including microarrays and high throughput sequencing have enabled significant contributions to multiple areas in virology, including virus discovery, molecular epidemiology, pathogenesis, and studies of how viruses to escape the host immune system and antiviral pressures. By overcoming conventional methods of viral identification, metagenomics, which gives access to all nucleic acids present in a given sample, allows the description and characterization of biological sample viral communities including unknown or variant of viruses associated with several human diseases. Although the application of viral metagenomics to clinical samples is made difficult by the fact that viral sequences represent a very low proportion compared to host DNA sequences. Leading to the requirement of high depth of sequencing and intensive bioinformatics analyses to increase the probability of virus detection, new and more affordable deep sequencing-based assays are now being implemented in clinical laboratories. Here we focused on the use of new approaches to viral STI diagnosis including the current deep sequencing platforms, based on the recent available data.

6.1 Human Papillomavirus (HPV)

*Human papillomavirus*es (*HPV*) have been identified as the cause of approximately 5% of all cancers worldwide [1]. *HPV* infection is associated with virtually all cervical cancers and a significant proportion of anogenital (vulvar, vagina, penile, and anal) and oropharyngeal cancers [2]. *HPV* infection is also associated with other skin and mucosal lesions, such as warts and benign papillomas [3]. The majority of *HPV* infections does not cause symptoms or diseases and are cleared within 12–24 months post infection. Only a small fraction of those infections that persist or progress to a preneoplastic lesion results in cancer. Each infection stage and behavior may be influenced by environmental, host, and viral factors, the knowledge of which is essential to understand the natural history of the *HPV* infection and to develop new tools for improving the management of *HPV*-positive lesions, their

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prevention, detection, and treatment. *HPV* infects both men and women, although the burden of attributable disease is much larger in women because of the high susceptibility to *HPV* infection of cervical cells [1, 4].

Even though most *HPV* infections are asymptomatic and clear spontaneously, persistent infections with "high-risk" (oncogenic) mucosal *HPV* cause approximately 5% of all cancers worldwide. These include almost all cases of cervical cancer—with annually over 500,000 newly diagnosed cases and over 260,000 cervical cancer deaths worldwide—as well as a large proportion of other anogenital carcinomas and oropharyngeal tumors in both women and men [5]. The overall burden of *HPV*-related disease is difficult to estimate, but it is believed that approximately 600,000 annual cases of cervical, anal, penile, vulvar, and vaginal cancers combined are attributable to high-risk *HPV* [5].

6.1.1 HPV Transmission

The majority of anogenital *HPV* infections are acquired through sexual contact, and acquisition is strongly determined by the accumulation of sexual partners and their respective sexual behavior [6]. A new infection can be detected soon after the first sexual contact with an infected partner, and most of them will be detectable within 1 year of exposure [7]. As a result of this high frequency of transmission, *HPV* infections are very common in young women depicting the peak in prevalence, which generally takes place around 20–25 years of age. An abrupt decline follows as a result of frequent clearance and lower exposure to new partners. This pattern is consistent in populations all over the world and confirms the sexual transmission as the main mode of transmission. Variations to the left/right of the peak age will relate to the average age of the sexual initiation in a given population. The intensity of the peak will be modulated by the average number of sexual partners interchange in men and women. The shape of the curve could also be affected by screening practices. Infections leading to cervical intraepithelial lesions may be detected through screening and thus its natural evolution be modified by treatment. After this period, prevalence of infection remains relatively stable at the range of 5–10%. In some countries, a second peak is observed after menopause. Reasons for this late increase are not well understood [8]. *HIV* has always been associated as a major co-factor with *HPV* to induce cervical cancer. The mechanism has been largely associated to the immunosuppression conferred by active *HIV* infection and not by a direct effect of *HIV*. A large number of *HIV*-infected women are now following antiretroviral therapy (ART), and therefore, it is expected that a good compliance with treatment will be followed by a reduction of cervical lesions and cervical cancer [9]. A recent analysis compiling the existing literature shows that women complying with ART in a prolonged time lower their risk of acquisition of high-risk *HPV* types and lower the incidence of intraepithelial lesions and their progression [10]. In some settings, like in Eastern and South Africa, the high prevalence of *HIV* infection in women alerts of the need to fully control ART compliance to prevent additional damage due to *HPV* infection and its consequences.

6.1.2 HPV Classification

More than 200 *HPV* genotypes have been identified in the last century and grouped in different genera (Alpha-, Nu-/Mu-, Beta-, and Gamma-papillomavirus) according to the viral genome structure and tropism to human epithelial tissues [10]. The Alpha genus includes genotypes that have been described to cause cancer, while Beta- and Gamma-papillomavirus infection courses are generally asymptomatic, but immunosuppression states (i.e., organ graft, *HIV* infection, epidermodysplasia verruciformis disorder among others) can trigger these types to produce cutaneous papilloma or increase the predisposition to skin cancer [11].

Twelve types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), also known as high-risk types, have been classified as carcinogenic to humans according to the International Agency

for Research on Cancer (IARC). Low-risk types, including *HPV*6 or *HPV*11, generally cause benign diseases such as genital warts [12], while other types, classified as probably or possible carcinogenic, are rarely found in large series of cancers or are associated with additional factors, so their oncogenicity remains to be clarified [13].

6.1.3 HPV Life Cycle and Genome

Despite the papillomaviridae family represents a remarkably heterogeneous group of viruses, the different *HPV* share the same genome structure and organization [14]. A circular double-stranded DNA genome of approximately 8 kb is structured into three main regions: (1) The early region (E), encoding genes that are necessary for the viral cycle and with an important role in cell transformation (E1, E2, E4, E5, E6, and E7). E6 and E7, together with E1, E2, E4, and E5, expression is essential for the viral genome replication and virion synthesis and release, but it also plays a key role in cell transformation [11]. (2) The late region (L), encoding the L1 and L2 capsid proteins. (3) The upstream regulatory protein, referred to also as the long control region, a noncoding region containing the replication origin and transcription factor-binding sites that contribute to regulate DNA replication by controlling the viral gene transcription.

HPV life cycle begins with the infection of the basal layer through microtraumas that compromise the epithelial barrier [14]. *HPV* genome is maintained at low copy number in the infected host basal cells. Upon differentiation of epithelial cells, the virus replicates to a high copy number and expresses the capsid genes (L1 and L2), resulting in the production of new progeny virions that are released from the epithelial surface. For persistence, *HPV* needs to infect basal cells showing stem cell-like features that are still able to proliferate [15]. This phenomenon is far less common in *HPV* low-risk types. The epithelial transition zones, such as the endo-/ectocervix and anorectal junctions, are regions more susceptible to carcinogenesis by high-risk *HPV* types [16]. High-risk types are more prone to activate the cell proliferation in basal and differentiated layers promoting the transition from a productive infection to an infection, which is unable to complete the viral life cycle, but is able to activate several pathways leading to the epithelial transformation. One plausible explanation of the increased oncogenic capacity of the high-risk types and particularly of the *HPV*16 type resides in the activity of the E6 and E7 oncoproteins. Although the E6 and E7 activity is present in both high- and low-risk types, their role in low-risk types is limited to the increase of the viral fitness and viral production and it is largely insufficient to trigger the development of preneoplastic lesions and cancer [17]. *HPV* E6 and E7 early proteins conduct their main role in the carcinogenic process through the inhibition of p53 and pRB tumor suppressors [18]. E6 functions include also the activation of the telomerase activity and deregulation of pathways involved in immune system response, epithelial differentiation, cell proliferation, and survival signaling. Besides cell cycle deregulation and proliferation, E7 enhances genomic instability and promotes the accumulation of chromosomal abnormalities. The deregulation of the cell cycle, the activation of the telomerase activity, and genomic instability create a favorable environment for the epithelial cell transformation. *HPV* integration can also drive the carcinogenic process through the inactivation of the E2 expression, the main inhibitor of E6 and E7, and the disruption of host genes because of the viral sequence insertion [19]. The carcinogenic process, initiated with the E6 and E7 activation, needs to be complemented by the accumulation of additional alterations in the host genes to lead to the invasive cancer phenotype. The integrated genomic analysis conducted by The Cancer Genome Atlas Consortium identified genes that were significantly mutated in cervical and head and neck *HPV*-associated tumors [20]. Additionally, high-risk *HPV* types have developed several mechanisms to avoid host immune response, which is important for viral persistence and progression to *HPV*-associated neoplastic diseases [21]. The *HPV* cycle is exclusively intraepithelial and not lytic; therefore, it prevents the associated proinflammatory signal. As a result, the recruitment

of antigen-presenting cells such as Langerhans cells (LC) and the release of cytokines that mediate the immune response are absent or very low after the *HPV* infection. Other mechanisms of *HPV* immune evasion include the regulation of the interferon signaling, inhibition of LC by the E6 and E7 activity, inhibition of adherence molecules such as the CDH1, and modulation of intracellular signaling pathways.

6.1.4 HPV Persistence and Progression to Cervical Intraepithelial Lesions and Cancer

Persistent carcinogenic *HPV* infections clearly predict the risk of cervical cancer in women [22]. Persistence is not homogeneously measured but is a very relevant concept as many populations undergoing screening using *HPV* tests will rely on measures of persistent infection. Muñoz et al. defined persistence as those infections that last more than the median duration but this concept is relevant for natural history studies. Others define persistence as those having two positive *HPV*-DNA consecutive tests with undetermined time interval [23]. Time interval between two measures affects the persistence estimate as many infections will be cleared by year 2. Marks et al. compared 12 versus 24 months in defining a persistent infection and showed substantial increases in the specificity of the combined two *HPV* tests using 24 months for the detection of cervical lesions [24]. In many screening programs, a 12-month test repetition is recommended among those who are *HPV*-positive at first screening with a negative cervical cytology. Although 12 months may be a too short interval, it may provide some "safety window" as first infection may have a long-standing prevalence. For newly acquired infections, a longer interval may be more efficient. Irrespective of the time window, major determinants of *HPV* persistence are *HPV* type and viral load at first detection. It remains unclear whether age is a key element for persistence. However, in the prospective study by Muñoz et al. previously described [23], median duration of *HPV* incident infection was higher for high-risk *HPV* types than for low-risk types and for *HPV*16. Women younger than 30 years had a longer mean duration for *HPV*16 infections of 16.6 months, while women >30 years had a significantly shorter duration of 9.5 months. Others suggest that persistence increases with increasing age [25]. The presence of more than concomitant *HPV* infections, a phenomenon very common in younger populations, is reported to not influence the duration of infection [26]. The low proportion of women who are not able to clear the infection are those at risk of developing cervical cancer. Soon after the *HPV* infection is established, cellular changes can be observed in the cervical exfoliated cells. Persistent infections can result in different grades of squamous intraepithelial lesions that ultimately can lead to high-grade lesions and cancer in an average of 5–14 years if undetected and untreated [27]. Within the types involved in the carcinogenic process, persistent infection with *HPV*16 is most commonly associated with a faster progression to cervical lesions and invasive cervical cancer. It is noticeable that progression in *HPV*16 infection is not affected by strong-acting DNA variations of the virus [28]. *HPV*16 is involved in over 60% of all cervical cancers but also in other *HPV*-related cancers [29]. Other types including *HPV*18, 45, 31, and 33 are also commonly detected in cancer specimens but their lower contribution suggests a different natural history as the one of *HPV*16. As a consequence of these observations, detection of *HPV*16 in women aged 30+ is considered in many settings an indication of immediate colposcopy for screening of cervical cancer [30], while detection of other high-risk types may be followed by a second triage test. In addition to the viral characteristics, environmental or exogenous factors have long been identified as modifiers of the natural history of *HPV* infections leading to cervical cancer. Many of the early case–control studies were conducted in populations with low coverage of screening for cervical cancer. Most relevant co-factors identified at the time to increase cervical cancer risk were long-term smoking, multiparity [31], and long-term use of hormonal [32] contraceptives with an average

increased risk of 1.5 to 2-fold in *HPV*-positive women [33]. Interestingly, a recent analysis of a large cohort of 308,036 women recruited in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study, with an average follow-up of 9 years, confirmed that increasing number of full-term pregnancies was positively associated with increased risk of cervical intraepithelial neoplasia grade 3/carcinoma in situ (CIN3/CIS) and that duration of oral contraceptives use was associated with a significantly increased risk of both CIN3/CIS and cervical cancer with hazard ratios of 1.6 and 1.8, respectively, for ≥15 years versus never used [34]. Identification of persistent *HPV* infection in smokers should alert women of the increased risk and reinforce the message to quit smoking. In *HPV*-positive long-term oral contraceptive users, a closer surveillance for cervical disease may be advisable.

6.1.5 HPV Vaccine

Three vaccines targeted against *HPV* have been developed. Gardasil (Merck, Kenilworth, NJ), the original quadrivalent vaccine that targeted *HPV* types 6, 11, 16, and 18, was licensed in 2006. Cervarix (GlaxoSmithKline, Brentford, UK), licensed in 2009, is a bivalent vaccine against types 16 and 18. In 2014, the Food and Drug Administration (FDA) approved a nonavalent vaccine, Gardasil-9 (Merck), directed against *HPV* types 6, 11, 16, 18, 31, 33, 45, 52, and 58, which together cause almost 90% of cervical cancers and cervical cancer precursors. Gardasil-4 stock is still available; however, Gardasil-9 is currently the only *HPV* vaccine on the market in the USA. Several randomized controlled trials assessed both the safety and efficacy of the *HPV* vaccines, including FUTURE I, FUTURE II, PATRICIA, and the Costa Rica vaccine trial [35, 36]. These vaccine trials demonstrated that both the bivalent and quadrivalent vaccines prevented 90–100% of new *HPV*-16 and -18 infections and associated high-grade cervical intraepithelial neoplasia (2+) in women not infected with *HPV*-16 or -18 at the time of vaccination. An efficacy and immunogenicity study of the nonavalent vaccine showed non inferiority of the immune response to anti-*HPV*-6, -11, -16, and -18 compared with the quadrivalent vaccine [37]. These and subsequent trials have demonstrated that *HPV* vaccines are extremely immunogenic, with no current evidence to suggest waning protection at least 10 years after administration.

6.1.6 Diagnostic Techniques for HPV

Nowadays, the *HPV* infection is mainly diagnosed by the identification of the viral nucleic acid using molecular techniques. Molecular techniques are usually based on hybridization followed by signal amplification or the amplification of nucleic acid before hybridization to specific complementary probes [38]. Current state-of-the-art approaches for the diagnosis of *HPV* are discussed below.

6.1.6.1 Colposcopy

Colposcopy is the only accessible way to examine cervix for potentially complex pre-malignant lesions that are usually missed during Pap screening. It gives a magnified view of the cervix through which normal and abnormal cells can be differentiated. It also helps to take direct biopsies for pathological examination, thus enabling the physicians to improve triage for abnormal Pap smears according to the severity of lesions [39]. A study conducted on 1850 patients demonstrated that sensitivity and specificity of colposcopy ranges between 52–98% and 45–87%, respectively [40]. This figure was also in agreement in another cohort study performed in 24 different hospitals of British Columbia in 2001 [41].

6.1.6.2 Histopathology

Histological grading of CIN relies on features such as differentiation, maturation, stratification of cells, and nuclear abnormalities [42]. The accuracy of the histopathological diagnosis depends on appropriate sample collection by colposcopy. Moreover, proper macroscopic depiction, technical processing, microscopic interpretation, and quality management are other

key factors for accurate histopathological diagnosis [43]. Diverse changes in cellular morphology related to inflammation, pregnancy, and atrophy make histological diagnosis complicated and prone to variability [44].

6.1.6.3 Adjunctive Biomarker Assays for *HPV*

The low specificity of histopathological techniques has instigated the researchers to devise some improved methods for *HPV* testing. So, it has been suggested that expression analysis of some cellular and viral proteins can serve as an excellent strategy to diagnose the pre-cancerous and cancerous stages in the cervical cancer patients. Therefore, host cell proteins such as $p16^{INK4A}$, minichromosome maintenance protein 2 (*MC*M2), topoisomerase II alpha (Topo II-A), and telomerases have been investigated for their role as biomarkers in *HPV* testing. Similarly, the expression of some viral proteins including E6, E7, E4, and L1 can also be used as indicative of the onset or the progression of the cancer [45]. These biomarkers showed low sensitivity and specificity values when used separately. However, their performance is enhanced greatly when applied along with cytology and histopathology methods. Consequently, the biomarkers based assays are recommended as adjunctive tests for *HPV* testing. The validated and commercially available methods for routine use are described below.

6.1.6.4 Immunohistochemical Staining of $p16^{INK4A}$

The $p16^{INK4A}$ is a cyclin-dependent kinase (CDK) inhibitor. At normal concentrations of $p16^{INK4A}$ in the cell, CDK binds to cyclin-D and regulates the G1 cell cycle checkpoints. Upon *HPV* infection, the level of $p16^{INK4A}$ is abruptly increased [45]. The intensity of immunohistochemical staining gives the measure of expression of $p16^{INK4A}$ indicating the number of tumor cells in the sample. The clinical studies revealed that this technique has 88–94% sensitivity and 68–96% specificity for diagnosis of ASC-US and LSIL [45].

6.1.6.5 ProExC™ Assay

This method relies on detection of enhanced levels of cervical cancer specific S-phase marker proteins; *MC*M2 and Topo IIA. A persistent infection with *HPV* causes an increased expression of both of these proteins which induce aberrant S-phase in the epithelial cells. ProExC™ which is developed by Becton-Dickinson (Franklin Lakes, NJ) uses mixture of antibodies to detect the over expression of S-phase markers. In a comparative study of eight different tests for *HPV* cervical cancer, it was shown that the sensitivity and specificity for ProExC™ assay ranges from 63.2% to 88.6% and 90.3% to 92.4%, respectively [46].

6.1.6.6 Molecular Techniques

Non-amplifying Hybridization Assays

Hybridization tests are based on the principle of complementation and are considered sensitive methods for the detection of *HPV* infection. These tests use isotope or non-isotope labeled probes. Southern blot hybridization (SBH) was in common use for a long time but nowadays it is an obsolete method because of its laborious procedure. Another hybridization technique, "in situ hybridization (ISH)" allowed the detection of *HPV* inside the nuclei of infected cells [47]. Although non-amplifying hybridization assays are excellent tools for the detection of *HPV*, low sensitivity, laborious procedures, and requisition of large amount of pure DNA have made them clinically unacceptable.

Polymerase Chain Reaction (PCR)

The PCR is the most sensitive and commonly used method among several other molecular techniques. Three different classes of primers have been designed against *HPV*-DNA; consensus primers, group-specific, and type-specific primers. The primers that amplify all mucosal and anogenital *HPV* types are classified as consensus primers. They amplify the conserved L1 capsid gene in all *HPV* genotypes. Group-specific primers are designed for the detection of high- or low-risk *HPV* groups, whereas type-specific primers

identify the individual *HPV* type involved in the infection. The type-specific primers target long control region of L1 or E6/E7 viral genes [48]. PCR working with consensus primers is highly sensitive as it can detect even 10–100 copies of *HPV*-DNA in the specimen. It has very low specificity due to involvement of more than one *HPV* types in the infection. On the other side, group- or type-specific primers can detect *HPV*-DNA in the sample with much higher sensitivity and specificity ranging between 90% and 100% [48].

6.1.6.7 HPV Genotyping

While PCR-based genotyping is also carried out widely, as discussed in the preceding section, a number of other genotyping assays have also been devised. The common *HPV* genotyping assays that are described here are reverse line blot (RLB) hybridization, microarray, restriction fragment length polymorphism (RFLP), and multiplex PCR [49].

Reverse Line Blot (RLB) Hybridization Assay

Most of the available genotyping assays are based on RLB hybridization technique which works by PCR amplification of the *HPV*-DNA with biotinylated primers [50]. Fundamental methodology for all the RLB based genotyping tests including INNO-LiPA® *HPV* (Innogenetics), linear array *HPV* (Roche), digene® RH (Qiagen), EasyChip® *HPV* (*HPV* Blot Kit; King Car, Taiwan), and REBA-*HPV*-ID® (Catch by Gene; Gangwon-do, Korea) remains the same. They slightly vary from one another with respect to primers and length of amplified region [51]. Comparative studies with other *HPV* testing methods (HC2, PCR) reported that these assays are highly sensitive and specific for diagnosis of *HPV*. However, the validation data for all these assays is quite scarce [51].

Restriction Fragment Length Polymorphism (RFLP)

The genotype of *HPV* can be investigated by restriction endonucleases such as *Bam*HI, *Dd6e*I, *Hae*III, *Hin*fI, *Hpy*CH4V, *Pst*I, and *Rsa*I. After the restriction digestion of PCR product, fragments are resolved on agarose gel and specific banding patterns are analyzed for respective *HPV* types. Along with some in-house RFLP based assays only one test is commercially available (BIOTYPAP Kit (Biotools, Nave, Spain). It is a robust test and can identify 31 *HPV* types simultaneously. However, no data are available in peer reviewed literature that may help to conclude the sensitivity or specificity of BIOTYPAP or other RFLP based methods [51]. Moreover, RFLP results for multiple *HPV* infections are difficult to interpret which is avoidable in RLB based tests.

Microarray

Similar to RLB genotyping assays, microarray is based on the principle of reverse hybridization. It slightly differs in a way that the product obtained after PCR is bound to microarray chip containing *HPV*-specific oligonucleotide probes and analyzed for viral DNA using chip scanner. It is an efficient technique that can be used simultaneously for multiple samples and equally effective for both *HPV* detection and genotyping [52]. Moreover, microarray is much more sensitive (100%) as compared to conventional methods [53].

Multiplex Real Time PCR (RT-PCR)

The multiplex RT-PCR offers a good alternate for simultaneous detection, genotyping, and quantification of *HPV* [54]. It is useful to detect almost 14 different types of *HPV* involved in cervical infections with high sensitivity and specificity. Two tests based on RT-PCR; Abbott real-time PCR and COBAS® 4800 *HPV* (Roche) are available in the market. Recently, COBAS® 4800 *HPV* has been approved by FDA for routine use. A clinical study carried out on 558 women from different hospitals in Ireland proved this test for its comparable value to HC2 for detection of CINII [55]. Another comparative study including 406 samples to evaluate performance of COBAS® 4800 *HPV* (Roche) demonstrated that it is comparable to other tests (HC2, Linear array) in terms of efficacy with very low chances of false negativity [56]. Few studies evaluating the clinical performance of Abbott

real-time PCR and COBAS® 4800 *HPV* (Roche) reported consensus sensitivity value between 94% and 98%. The specificity value was also reported between 90% and 94% [57].

Loop-Mediated Isothermal Amplification Method (LAMP)

The LAMP is an alternative method for the identification of viral genotypes involved in cervical carcinoma. In contrast to conventional PCR, it is performed at 63–65 °C for 1 h without the use of thermocycler. The amplification of target DNA produces magnesium pyrophosphate in the solution as a by-product. Hence the gene amplification can be visualized by the appearance of turbidity. It can also be detected in the form of color change when SYBR Green dye is employed in the procedure. LAMP has sensitivity and specificity comparable to PCR and it is also superior in terms of simplicity and cost-effectiveness [58].

6.1.6.8 The mRNA-Based Diagnosis

Until now, *HPV* diagnosis was focused on the detection of viral DNA. These methods were not very specific to differentiate between active and transient state of the virus. However, RNA based techniques can measure the expression of viral oncogenes, enabling a better differentiation of *HPV* infections [42]. Therefore, mRNA testing is considered appropriate for *HPV* diagnosis thus avoiding unnecessary treatments. Conversely, large scale clinical trials are required to validate the accuracy of *HPV*-mRNA testing in diagnosis and prognosis [59]. So far, commercial mRNA detection assays are based on Nucleic Acid Sequence Based Amplification (NASBA) technology and Transcription Mediated Amplification (TMA). Both of these assays are sensitive and provide qualitative measurement of viral load in the specimen [60]. The NASBA is based on reverse transcription and subsequent amplification of *HPV*-RNA, while TMA amplifies both DNA/RNA targets using respective enzymes. In some clinical studies commercially available tests that work on NASBA principle were proved to be 74% sensitive and 68% specific [61]. Similarly, commercially available TMA based assay for the detection of E6/E7 mRNA from 14 different types of *HPV* worked with 95.2% sensitivity and 42.2% specificity.

6.1.6.9 Recent Next-Generation Sequencing Advances in *HPV*-Associated Cancers

The development of technologies utilizing massively parallel sequencing has resulted in a revolution in the way that genomes can be rapidly and comprehensively characterized. A single lane on an Illumina HiSeq 4000 can generate 400 million DNA sequence reads, and this is sufficient to analyze genomes, transcriptomes, and even methylomes. There are different sequencing strategies that can be employed to characterize genomic alterations and also the physical status of viruses within a cancer genome. For example, whole-genome sequencing (WGS) provides the most comprehensive characterization of the cancer genome, as it can discover the full range of genomic alterations, including nucleotide substitutions, indel structural variations, copy number alterations, and viral integrations. Unfortunately, this requires considerable sequencing, which leads to problems with both data analysis and storage, and it is still very costly. Exome sequencing, which involves analyzing only 2% of the entire genome offers an alternative at a lower cost and has been an effective approach focusing on DNA sequencing of just the coding genes. This approach is suitable for mutation discovery in cancer samples and for both somatic and germline analysis; however, exome sequencing is less suited for characterizing the physical status of a virus, unless that virus has integrated next to one of the exons of a gene. Transcriptome sequencing, also called RNA-seq, is a sensitive and efficient approach to detect changes in gene expression profiles of both human and viral-specific genes and in addition can detect novel transcripts, changes in the expression of isoforms from different alternative splicing, as well as intra- and inter-genic fusions. This strategy could prove useful but only if the *HPV* integration event led to the generation of a novel fusion transcript between human and *HPV* sequences.

The sequencing company Illumina produces a 5 kb library construction kit (Nextera) that can be used to generate libraries which are directly

suitable for paired-end next-generation sequencing (NGS). By sequencing the ends of fragments which were originally 5 kb in size, one can obtain detailed genomic information with significantly less sequencing than WGS (WGS usually requires 100 Gb of sequencing, while MP-Seq can be done with just 5 Gb of sequence data). The Biomarker Discovery Program at the Mayo Clinic has developed powerful algorithms to analyze these data [62]. These algorithms readily reveal the physical status of *HPV* in any cancer genome and can also be used to determine changes in copy number throughout the human genome independent of the physical status of *HPV*. NGS studies on a large number of cancers not only presented powerful information about sites of integration into the human genome but also revealed a great deal about any potential specificity for where the *HPV* genome is disrupted and/or altered.

Very recently the results obtained using an ion torrent NGS *HPV* genotyping assay with barcoded *HPV* PCR broad-spectrum general primers have been published: it was able to identify 20 different *HPV* genotypes, including the 13 high-risk genotypes, with similar performance of the Roche Linear Assay array and low per-sample cost [63].

6.2 Hepatitis C Virus (HCV)

HCV causes hepatic inflammation and fibrosis that may progress sub-clinically over decades, and accounts for approximately 15–20% cases of acute hepatitis. After acute infection, around 50–80% of *HCV* patients will develop chronic infection, the others clear the virus spontaneously. Approximately, *HCV* infects 170 million individuals worldwide. Chronic hepatitis C (CHC) patients are at high risk to develop life-threatening complications, including cirrhosis in 20% of cases and hepatocellular carcinoma (HCC) at an incidence of 4–5% per year in cirrhotic patients [64]. In the Western world, chronic *HCV* infection is the leading indication for liver transplant and the leading cause of end-stage liver disease, HCC, and liver-related death [65]. Individual outcomes are highly variable, with many patients experiencing minimal changes while others progress rapidly [65]. Fibrosis progression is uneven and may accelerate with longer duration of infection; comorbid conditions such as *HIV* infection accelerate disease progression [66]. A large meta-analysis reported the risk of cirrhosis at 7–18% after 20 years and 41% after 30 years of infection [67]. Cirrhotic patients are at high risk for hepatic decompensation (27.7–39.5% risk over 5 years) and hepatocellular carcinoma (2.8–7.4% in the first year, and 8–16.1% over 5 years) [68]. Factors that increase the risks of fibrosis, cirrhosis, and HCC include male sex, increasing age, alcohol use, and *HIV* co-infection. Liver-related mortality ranged from 15.3% to 67.1% [69]. In addition to hepatic manifestations of *HCV*, multiple cohort longitudinal studies have underlined the important morbidity of extrahepatic manifestations of *HCV* [69]. The spectrum of extrahepatic manifestations of *HCV* is wide, ranging from cryoglobulinemia to neurologic manifestations but among the more intriguing associations that has been unraveled in the past decades is the association with metabolic alterations and with increased cardiovascular diseases [69]. *HCV*, in particular genotype 3, induces steatosis, morphologically similar to that found in other causes of steatosis but with differing pathogenic mechanisms and prognostic implications [70]. In addition, the relationship between insulin resistance and *HCV* is equally complex with an increased risk of insulin resistance in *HCV* patients [70] However, despite seemingly a more metabolic profile in *HCV* subjects, *HCV* infection is associated with reduced cholesterol serum levels or a more protective lipid profile [70], further confusing the association between *HCV* and cardiovascular risk factors.

6.2.1 HCV Transmission

Most persons with *HCV* were exposed via a percutaneous exposure to infected blood. There are two primary methods of bloodborne transmission: injection drug use and nosocomial exposure. Shared injection equipment, such as

needles and syringes, contaminated with infected blood may result in exposure and transmission. *HCV* may be transmitted via sexual transmission, as *HCV* RNA is occasionally detected in the semen of viremic patients. However, studies of heterosexual couples with discordant serostatus have shown that such transmission is possible but extremely inefficient [71]. *HCV* seems to be transmitted at a higher rate in men who have sex with men (MSM), particularly those who practice unprotected anal intercourse and have *HIV*. The increasing number of *HIV*-positive MSM with acute *HCV* diagnosed in the early 2000s suggested that its epidemiology is changing [72]. Increasing *HCV* incidence and prevalence rates have now been reported in *HIV*-positive MSM in Europe, North America, Australia, and Asia [73]. Globally, the *HCV* prevalence rate in *HIV*-positive MSM has increased significantly over time, and affects an estimated 6.7% and 40% of non-injecting and injecting MSM, respectively. Two studies, with pooled data of approximately 13,000 and 6000 *HIV*-positive MSM worldwide, revealed a threefold (from 0.42 to 1.34/100 PY between 1990 and 2012) and 20-fold (from 0.07 to 1.8/100 PY between 1991 and 2014) rise in *HCV* incidence among *HIV*-positive MSM in the era of cART [74]. Although *HIV* infection is no prerequisite for sexually acquired *HCV*, *HIV*-negative MSM remain largely unaffected [75]. Heterosexual transmission of *HCV* among monogamous couples is also rare, with an estimated maximum incidence rate of 1 per 190,000 sexual contacts or 0.07% per year. A higher risk of 0.4–1.8% per year has been reported for heterosexuals with multiple partners, *HIV* co-infection or those at risk for STI [76]. Sexual *HCV* transmission requires exchange of *HCV*-infected body fluids across mucosal surfaces, most likely semen. Detection rates of *HCV* RNA in semen from men with chronic *HCV* infection vary between 10% and 44%, with the frequency increasing to 56.7% during longitudinal sampling [77]. *HCV* RNA levels in blood and semen are highly correlated, with seminal levels typically being 4.0–5.0 log lower than those in blood [78].

6.2.2 HCV Classification

After the discovery of *HCV*, great nucleotide diversity among isolates was reported [78]. Due to the error-prone viral RNA-dependent RNA polymerase (*HCV* NS5B protein), a closely related but diverse population of viral variants known as quasispecies is produced within *HCV*-infected patients [79]. There is 1–5% variation in *HCV* nucleotide sequence from a single infected patient. Accumulation of nucleotide substitutions in the virus has resulted in diversification into distinct subtypes and even genotypes. Therefore, the *HCV* RNA genome sequences are highly heterogeneous. At present, *HCV* is classified into eleven genotypes (designated as 1–11) differing in their nucleotide sequence by 30–50%, six of them are the major ones (genotypes 1–6) [80]. Within *HCV* genotype, several subtypes (designated as a, b, c, *etc*.) can be defined that differ in their nucleotide sequence by 15–30% [81]. The prevalence of *HCV* genotypes and subtypes is geographically different [82]. At present, genotype 1 is the most prevalent (46%) globally, followed by genotype 3, genotype 2, and genotype 4. Various genotypes have different infectivity and pathogenicity, thereby influencing the rate of progression to cirrhosis and the risk of HCC. *HCV* heterogeneity would also result in different responses to antiviral treatments, e.g., genotypes 1 and 4 are more resistant to interferon based therapies than genotypes 2 and 3 [79, 83]. Therefore, *HCV* heterogeneity poses a challenge to the development of pan-genotypic antiviral treatments. In addition, *HCV* heterogeneity hinders the development of a successful vaccine to against all *HCV* genotypes. Of course, *HCV* heterogeneity could also affect viral diagnosis. Though heterogeneous, different *HCV* genotypes preserve the similarity of life cycle in cells.

6.2.3 HCV Life Cycle and Genome

HCV is a small enveloped RNA virus belonging to the family *Flaviviridae* and genus *hepacivirus*. *HCV* genomic RNA is single-stranded with positive polarity, which is packaged by core protein

and enveloped by a lipid bilayer containing two viral glycoproteins (E1 and E2) to form the virion [84]. The *HCV* genomic RNA contains three distinct regions [85]: (1) a 5′UTR or non-coding region; (2) a long open reading frame (ORF) of more than 9000 nucleotides (nt); and (3) a short 3′UTR. The *HCV* 5′UTR contains 341 nt located upstream of the ORF translation initiation codon. The 5′UTR contains the internal ribosomal entry site which forms a stable pre-initiation complex by direct binding with the 40S ribosomal subunit for the *HCV* polyprotein translation. The long ORF encodes a polyprotein of approximately 3000 amino acids, which will be further processed by host and viral proteases into at least 10 different proteins, which are arranged in the order of NH_2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. The 3′UTR is principally involved in minus-strand priming during *HCV* replication. The nucleotide sequence variability is distributed throughout the entire viral genome. The 5′UTR is the most conserved region in the genome, while the regions encoding envelope proteins (E1, E2) are the most variable ones. Thus, the highly conserved 5′UTR region is usually the target of choice for *HCV* genome detection across different genotypes [85].

The *HCV* life cycle begins with the attachment of a virion to its specific receptors on hepatocytes [86]. Up to now, the high-density lipoprotein receptor scavenger receptor class B type I, tetraspanin CD81, tight junction protein claudin-1, and occludin are the known cellular receptors initiating the attachment step of *HCV* infection. It is proposed that the virus, after binding with its receptor complex, is internalized, and that the nucleocapsid is released into the cytoplasm. The virus is then uncoating to free its genomic RNA, and the *HCV* genomic RNA is used both for polyprotein translation and replication in the cytoplasm. *HCV* replication takes place within the "replication." The very low-density lipoprotein synthesis/secretion machinery is involved in the production of the infectious *HCV* particles. *HCV* uses this lipoprotein biosynthetic pathway to produce mature viral particles and to export them [79]. Because of high replication rate and no proofreading activity of the viral polymerase, *HCV* is highly variable and each possible single mutation and combination of mutations may arise every day in a given infected individual. Genome plasticity and drug-driven selection create the conditions for the emergence of resistant variants [87] and, as a consequence, most mutations associated with resistance are located within the drug target regions [88]. Despite the reduced fitness, such variants rapidly overgrow wild-type viruses and during this process they may accumulate additional, fitness-restoring, mutations. Single drug, drug family, and genotype/subtype often influence the emerging mutations [88].

6.2.4 HCV Treatment

Currently, pegIFN-α as mono-therapy, or in combination with ribavirin (in patients with *HIV* co-infection), is still the only treatment that has been adequately studied in patients with acute *HCV* infection and is approved for this indication. In patients with acute *HCV* mono-infection, cure rates between 71% and 94% have been reported with pegIFN-α mono-therapy [89]. As the therapeutic mechanisms for pegIFN-α and standard IFN-α are similar, starting the treatment with high-dose standard IFN-α 2a or 2b is common in resource-limited settings in which pegIFN-α is not available or more costly. The wide variety of cure rates within the *HIV*-co-infected population makes it difficult to interpret the effects of different treatments. Small, mostly underpowered, cohort studies with a variable genotype distribution have shown an average cure rate of 61% (standard deviation of 17) [90]. Although its role remains controversial, ribavirin is often added to pegIFN-α for the treatment of patients with *HIV* co-infection, resulting in higher SVR rates in general. Most physicians start with pegIFN-α and ribavirin, with tapering or discontinuation of ribavirin if adverse events occur [90]. However, mono-therapy could be suitable in a subset of co-infected patients treated within the first weeks after diagnosis of acute *HCV* infection [91]. In clinical practice, the optimal timing of onset of treatment remains contro-

versial. It is not easy to predict when the chances of spontaneous clearance outweigh the effects of pegIFN-based treatment. A model has been made to predict the efficacy of treatment in comparison with the chance of spontaneous clearance, and proposes treatment within the first 2 months or 4 months after transmission [92]. However, this model assumes a reliable transmission date, which is often not available. As a result, most physicians refrain from applying treatment during at least the first month after diagnosis, to await spontaneous clearance. This is in line with the current European AIDS treatment network (NEAT) and European Aids Clinical Society guidelines, recommending a 4-week period to observe a potential *HCV* RNA decline of at least 2-log, after which the chance of spontaneous clearance becomes substantially higher [93]. Without this 2-log decline, treatment can be initiated. However, observations from a large international study on 632 participants with acute *HCV* mono-infection do not support this approach. Among patients with spontaneous clearance, 33% became *HCV* RNA-negative later than 6 months after the estimated date of infection [94]. This supports the policy of waiting for a longer period. As a result, current European Association for the Study of the Liver and American Association for the Study of Liver Diseases (AASLD) guidelines are inconclusive about the observation period [95]. Patients with acute *HCV* infection have historically been treated with shorter-duration regimens, yielding higher sustained viral response (SVR) rates, than those used in patients with chronic infection. With new direct-acting antivirals (DAAs) being licensed, the treatment of chronic *HCV* infection has become very safe and effective, and early treatment may have no advantage with respect to SVR rates. As a result, current AASLD guidelines advocate waiting until infection becomes chronic and selecting treatment regimens without pegIFN. In several European countries (e.g., Belgium, The Netherlands, Spain, Italy, and the UK), DAAs can only be used in patients with advanced fibrosis or cirrhosis. Even when DAAs can be used regardless of fibrosis grade, DAAs are not European Medicines Agency-registered or Food and Drug Administration-registered for the treatment of acute *HCV* infection, as their efficacy and safety in patients with acute *HCV* infection have not yet been studied. Therefore, despite the known side effects, pegIFN-based treatment for acute *HCV* infection will remain the only available option in many countries for several years to come. For the treatment of acute *HCV* infection, only telaprevir, a first-generation *HCV* protease inhibitor, has been tested in a pilot study of acute *HCV* infection, in 19 co-infected patients. It was given for only 12 weeks in combination with pegIFN-α and ribavirin, and a promising 84% SVR rate was seen [96]. If these results can be confirmed by other ongoing studies, the use of first-generation protease inhibitors for the treatment of acute *HCV* infection could become an effective treatment strategy [97].

6.2.5 Diagnosis of HCV Infection

Majority of primary *HCV*-infected patients are asymptomatic, thus, symptoms could not be used as specific indicators for *HCV* infection. *HCV* viremia could still exist despite a normal serum alanine aminotransferase (ALT) level. Therefore, virological methods rather than ALT levels are used to diagnose *HCV* infection [98]. At present, it is difficult to isolate and culture *HCV* using clinical specimens. Furthermore, anti-*HCV* IgMs could be detected not only in 50–93% of patients with acute hepatitis C but also in 50–70% of Cronic Hepatitis C patients [99]. Therefore, anti-*HCV* IgM cannot be used as a reliable marker for the acute *HCV* infection, and IgM assays have not been used in clinical practice [100]. At present, diagnostic assays for anti-*HCV* total antibody, viral core antigen, and viral genomic RNA are used in clinical practice [98, 100].

6.2.6 Screening for HCV-Infected Patients

According to the WHO, up to 80% of *HCV*-positive patients do not show symptoms. Therefore, most cases of *HCV* infection are currently undiagnosed. The major way to diagnose *HCV* infection is to screen high-risk groups for

anti-*HCV* antibodies. Humans are the primary *HCV* reservoir [101]. *HCV* transmission occurs primarily through direct per cutaneous exposure to blood. Therefore, the most common risk factors for *HCV* infection are persons with history of injection of illicit drugs and with blood transfusion prior to July 1992. The populations with less common risk factors for *HCV* infection are persons with organ transplant prior to July 1992, receiving clotting factor concentrate prior to 1987, being born to an *HCV*-infected mother, and with a history of chronic hemodialysis, intranasal use of illicit drugs, acquiring a tattoo, incarceration, having sex with an *HCV*-infected partner, needlestick or other mucosal exposure, with persistently elevated levels of ALT [102]. Therefore, WHO recommends that anti-*HCV* EIA be performed on individuals who are part of a population with high *HCV* seroprevalence or who have a history of *HCV* risk exposure and/or behavior, rather than at the time of presentation with symptomatic diseases. In addition, it is suggested that NATs for the detection of *HCV* RNA be performed directly following a seropositive test result to establish a definitive diagnosis of *HCV* infection. The Center for Disease Control (CDC) has also recommended screening high-risk individuals for *HCV* since 1998. The CDC further modified the *HCV* screening guidelines in 2012 to include a one-time *HCV* test for all US residents born during 1945–1965, independent of risk factors [102].

6.2.6.1 Detection of Antibody Production

In general, serological tests for detecting anti-*HCV* antibodies include tests for screening and confirmation. Screening tests are used first to screen the antibody positive specimens, while confirmatory tests are then used to verify the positive screening specimens.

Screening Test

EIA

At present, the third generation test of EIA for the anti-*HCV* antibody detection is commonly used in the diagnostic laboratory [103]. Conserved antigens from the *HCV* core, NS3, NS4, and NS5 regions are used in these tests to detect anti-*HCV* antibodies. The sensitivity of third generation EIAs was estimated at 98.9% and the specificity was found at 100% in patients with chronic liver disease [104]. EIAs are easy to use and inexpensive. Furthermore, this assay could be fully automated and adapted to large volume testing. Therefore, EIAs to detect anti-*HCV* antibody are generally recommended for screening the *HCV* infections [103]. However, this assay should not be used in infants younger than 18 months due to the possibility of reactivity with maternal antibody [105]. Several Food and Drug Administration (FDA)-approved antibody-based assays are available [105]. However, the time between *HCV* infection and the appearance of detectable antibodies (serological window period) is generally more than 40 days using the third generation EIAs [106]. In 2008, the fourth generation EIA has become available which could detect the anti-*HCV* antibody significantly earlier than the other assays. The antigens utilized in the fourth generation anti-*HCV* assay are derived from the core (two different epitope clusters), NS3, NS4A, NS4B, as well as the NS5A regions. NS3 and NS4 antigens are derived from genotypes 1a, 1b, 2, and 3.

The Rapid, Point-of-Care Test

Point-of-care tests are used directly at the site of patient care, outside of the diagnostic laboratory. Several point-of-care tests (POCTs) have been developed to detect anti-*HCV* antibodies with a relatively high sensitivity and specificity [107]. The test currently approved by the FDA in 2010 is the OraQuick *HCV* Rapid Antibody Test (OraSure Technologies, Bethlehem, PA). It is approved for use in patients over 15 years old, for screening persons who are considered at risk for *HCV* infection. This test detects anti-*HCV* antibodies in different specimens, e.g., fingerstick and venipuncture whole blood, serum, plasma, or oral fluid. Recombinant proteins or synthetic peptides of core, NS3 and NS4 antigens are immobilized on a nitrocellulose membrane to perform an indirect lateral flow immunoassay, and the results are directly visualized using colloidal gold labeled protein A, which generates a reddish-purple line within

20–40 min in the presence of anti-*HCV* antibodies in the specimens. These rapid tests are suitable for resource-limited settings because they are cheap, simple to perform, and fast [108].

Confirmatory Tests

Recombinant Immunoblot Assays

Recombinant immunoblot assays (RIBA) can be used to confirm the presence of anti-*HCV* antibodies for individuals who have showed positive reactivity by EIAs. This assay is highly specific, as the presence of antibodies against each of the several *HCV* proteins is assessed as individual bands on a membrane strip [109]. The INNO-LIA™ *HCV* Score (Fujirebio Europe, previously Innogenetics) assay can be automated. This assay includes recombinant proteins and synthetic peptides from E2 hypervariable region, NS3 helicase, NS4A, NS4B, and NS5A regions. Due to the high sensitivity and specificity of anti-*HCV* EIAs, RIBA is no longer needed in the diagnostic laboratories for verification [103]. Furthermore, nucleic acid tests for viral RNA rather than RIBA are used as a confirmatory test for *HCV* infection [110]. Active *HCV* infection must be confirmed by the direct diagnostic methods.

6.2.6.2 *HCV* Viral Load

Monitoring of the course of infection and therapeutic response is based on *HCV* RNA measurement in plasma or serum of patients and, under anti-*HCV* treatment, is aimed at optimizing therapy duration, and prompting early discontinuation to prevent potential side effects and reduce unnecessary costs. Baseline viral load, extent and sharpness of viremia decay in the early phases of treatment (4 and 12 weeks), and undetectable *HCV* RNA at the end of treatment represent key parameters guiding IFN-treatment. The methods to measure *HCV* viral load have greatly evolved since their initial establishment. Today automated reverse transcription RT-PCR and transcription-mediated amplification (TMA) platforms from different vendors are widely used throughout western countries [110]. These systems accurately quantify *HCV* RNA within a broad linear range, with a lower limit of detection (LLOD) sometimes even below the lower limit of quantification (LLOQ).

Detection of Viral RNA

Based on the items used for amplification, nucleic acid amplification tests (NAT) are divided into target amplification, signal amplification, and probe amplification methods [110]. Target amplification methods (RT-PCR, TMA) and signal amplification methods (branched DNA (bDNA)) were commonly used to detect the presence of *HCV* RNA [105]. The presence of *HCV* RNA in the serum is a reliable marker of viremia. Universal standardization for *HCV* RNA titer is important. The World Health Organization (WHO) has established an international standard for *HCV* RNA quantification units [111], i.e., an *HCV* RNA international unit (IU), which is currently used in all of the commercial *HCV* RNA quantitative assays no matter what the techniques used [111].

Qualitative HCV RNA Detection

Qualitative detection assays are based on the principle of target amplification using either RT-PCR or TMA. Several FDA-approved qualitative assays for *HCV* RNA are available [105]. *HCV* RNA is extracted and converted into complementary DNA (cDNA) using reverse transcriptase. The cDNA is subsequently processed via cyclic enzymatic reactions leading to the generation of a large number of double-stranded DNAs in PCR-based assays or single-stranded RNAs in TMA. Detection of these amplified products is achieved by hybridizing the produced amplicons onto specific probes. In general, the highly conserved 5′UTR region is the target of choice for *HCV* genomic RNA detection across different genotypes [112].

Quantitative HCV RNA Detection

HCV RNA can be quantified by means of target amplification techniques (real-time RT-PCR or TMA) or signal amplification techniques (bDNA assay). Several FDA-approved quantitative assays to detect *HCV* RNA are also available

[105]. Real-time RT-PCR is the method of choice for the quantification of *HCV* RNA levels in clinical practice. This assay is highly sensitive with wide dynamic range of quantification and can prevent carryover contamination. Fully automated *HCV* NAT assays have been available in the USA since 2007, and guidelines regarding the requirements for *HCV* NAT assays were issued in 2010. However, it is necessary to remember that not all *HCV* genotypes are detected equally by NAT assays, most likely because of nucleotide mismatches which has occurred before [113]. *HCV* RNA in the serum is probably the earliest detectable marker of acute *HCV* infection, preceding the appearance of anti-*HCV* antibody by several weeks. CHC infection is defined as the presence of *HCV* RNA more than 6 months. *HCV* RNA levels remain relatively stable over time in CHC patients. Therefore, after a positive reaction screened by the anti-*HCV* antibody test, NATs to detect *HCV* RNA is often used as the confirmatory tool to diagnose CHC infection [114]. Detection of *HCV* RNA is also used to determine the viral load both prior to and during antiviral treatments. On the other hand, the *HCV* RNA level has no prognostic value [115]. The level of *HCV* genomic RNA, reflection of *HCV* replication, does not correlate with the severity of liver disease, not with the risk of liver disease progression to cirrhosis or HCC.

6.2.6.3 Detection of Viral Core Antigen

Compared to other diagnostic methods like EIA, the advantages of NATs are having higher specificity and sensitivity. However, the disadvantages of these assays are time-consuming and require sophisticated technical equipment, trained technicians, dedicated laboratory space, and expensive reagents. In patients with *HCV* infection, it has been demonstrated that the *HCV* core antigen level strongly correlates with the *HCV* RNA level for various genotypes [116]. Thus, due to cheap and easy-to-perform, the *HCV* core antigen quantification assay can be used as an alternative method to NATs to detect *HCV* RNA [117]. Currently, core antigen detection by means of a chemiluminescent microparticle immunoassay can be fully automated in the Architect *HCV* Core antigen test (Abbott Laboratories) [118]. The Architect *HCV* Ag assay had a specificity of 100%, with a lower limit of detection of 3 fmol/L corresponds to approximately 1000 IU/mL of *HCV* RNA [117]. Whereas, current *HCV* RNA assays have a lower level of detection between 5 and 15 IU/mL [117]. In general, about 90% of *HCV* RNA positive samples are positive with a viral load above 10,000 IU/mL [118], well in the sensitivity range of the *HCV* core antigen assay [117]. Therefore, *HCV* antigen detection might be the next step following a positive antibody screening test. Several combination assays for detection of both anti-*HCV* antibodies and *HCV* core antigen have been developed [119]. At present, EIA to detect *HCV* core antigen is too insensitive to replace the NATs to detect *HCV* RNA in the blood bank setting and in the treatment monitoring according to the current clinical practice guidelines. However, it could be used as a supplemental test in resource-limited settings [120]. The Architect *HCV* Ag assay has been suggested as a better monitoring tool in the era of new all-oral, interferon-free antiviral treatments that do not require high analytical sensitivity [116].

6.2.6.4 Interpretations of Diagnostic Results

The presence of *HCV* RNA in the absence of anti-*HCV* antibodies is strongly indicative of acute hepatitis C (AHC), which can be confirmed by seroconversion (i.e., the appearance of anti-*HCV* antibodies) a few days or weeks later. However, there are still other possibilities for the presence of *HCV* RNA in the absence of anti-*HCV* antibodies, e.g., cronic hepatitis C infection in the immunodepressed patients, hemodialysis patients or agammaglobulinemic subjects. The presence of both anti-*HCV* and *HCV* RNA does not allow one to distinguish acute form from an acute exacerbation of cronic hepatitis C. However, the anti-*HCV* IgG avidity index within the first 8 days following the onset of clinical symptoms may be useful in identifying actual acute hepatitis C [120]. If the antibody test is positive and the *HCV* RNA test is negative, this result indicates a

resolution of *HCV* infection or acute hepatitis C during a period of low-level viremia. If the *HCV* RNA assay is negative and remains negative for more than 6 months, then the individuals are recovered from a past *HCV* infection. Cronic Hepatitis C is defined as the persistence of *HCV* RNA for more than 6 months. In patients with clinical signs of chronic liver disease, cronic hepatitis C is certain when both anti-*HCV* antibodies and *HCV* RNA are present.

6.2.6.5 Genotyping

Different *HCV* genotypes would result in different responses to antiviral treatments [116]. Thus, genotyping is important to predict the likelihood of response and determine the optimal duration of therapy [105].

Serological Method

The *HCV* genotype can be determined by detection of antibodies against *HCV* genotype-specific epitopes using a competitive EIA [121]. The currently available assay (Murex *HCV* serotyping 1–6 HC02, Abbott Laboratories, North Chicago, IL) could identify the six *HCV* genotypes (1–6) but not subtypes, and provide interpretable results in approximately 90% of chronically infected immunocompetent patients [122].

Molecular Techniques

The reference method for *HCV* genotyping is genome sequencing of the core/E1 or the NS5B regions and subsequent phylogenetic analysis [122]. However, this in-house method is restricted to reference centers. *HCV* genotyping assays approved for in vitro diagnostic use are also commercially available [112]. The Linear Array *HCV* Genotyping Test (Roche Molecular Systems) targets the 5′UTR [123]. This assay is based on conventional PCR amplification followed by reverse hybridization onto membrane strips containing specific probes. The obtained band pattern can be either visually interpreted or read by a scanner. Assays targeting other regions in addition to the 5′UTR have been recently developed to better discriminate between subtypes 1a and 1b. The Versant *HCV* genotype 2.0 assay (Siemens) is also based on reverse hybridization and targets the 5′UTR and core regions [124]. On the other hand, the Abbott RealTime *HCV* Genotype II (Abbott Molecular) targets the 5′UTR and NS5B regions. This assay is based on a single-step real-time RT-PCR with labeled genotype-/subtype-specific probes that minimize contamination with amplified products [125].

6.2.6.6 Subtyping

HCV subtyping is important for epidemiological studies, especially in the case of outbreaks, but it is not considered to be clinically relevant for the treatment of interferon-α and ribavirin. However, subtyping may be clinically relevant in the era of DAAs. For example, the phase 3 studies of telaprevir, boceprevir, faldaprevir, and simeprevir showed lower SVR rates for *HCV*-subtype 1a than those for subtype 1b [126]. In addition, BILB 1941, a non-nucleoside inhibitor of *HCV* NS5B, has been shown to have better antiviral efficacy in patients with subtype 1b than in those with subtype 1a [127]. Therefore, methods to determine the *HCV* subtypes should be important in the era of DAAs. The second-generation line probe assay, a reverse hybridization assay that uses probes targeting both the 5′UTR and core-coding region, correctly identified *HCV* subtypes 1a and 1b in more than 99% of cases. Thus, this assay could be used to differentiate *HCV* subtypes 1a and 1b in clinical trials and practice [125].

6.2.6.7 Next-Generation Sequencing and *HCV* Infection

Currently, the assessment of viral genotype commonly uses probe-based assays that target the highly conserved untranslated region (5 = UTR), while the detection of resistant strains currently relies upon the targeted analysis of genomic regions that rely on PCR Sanger sequencing; the application of this method is limited by problems with primer design for highly divergent *HCV* genotypes, genome coverage, and a restricted and inconsistent ability to detect both minor populations of resistant strains as well as mixed-genotype/subtype [geno(sub)type] infections that may be relevant for treatment response. To fulfil this gap, NGS technologies for the generation of

full-length *HCV* sequences, with the potential to accurately define *HCV* geno(sub)type while also simultaneously identifying both resistant strains and minor variant populations across the entire genome have been developed. NGS detects and quantitates variants present at frequencies as low as 0.5% and, therefore, permits early detection of resistance mutations, definition of their kinetics, and progressive disappearance after treatment suspension [128, 129]. Although there are no commercial kits, many laboratories analyze NS3–4A, NS5A, and NS5B regions with NGS but its use in clinical practice is still limited and requires expert guidance for interpretation

6.3 Human Immunodeficiency Virus (HIV)

HIV is part of the Lentivirus genus, a family of Retroviridae [130]. Many species are infected with Lentivirus, which are typically responsible for long-lasting illnesses with a long incubation period [130]. It characterized by chronic infections that are poorly responsive to the immune response and evolve slowly but progressively and which, if untreated, may have a fatal outcome. The average survival time after *HIV* infection is considerably longer in patients following therapy, in fact, it can be said that aging of the *HIV*-infected population. Without therapy, the average survival time after contracting *HIV* is estimated from 9 to 11 years, depending on the *HIV* subtype. *HIV* infection occurs with the transfer of blood, semen, vaginal fluid, pre-ejaculation, or breast milk. Within these body fluids *HIV* is present in both free particles and within infected immune cells [131].

HIV is transmitted as single-stranded RNA viruses, in a positive sense. At the entry into the target cell, viral RNA is converted (reverse transcript) into double filament DNA by means of transverse transcriptase transported along with the virus genome into the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cell DNA through a virus-encoded integrator and host co-factors. Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection from the immune system. Alternatively, the virus can be transcribed, producing new RNA genomes and viral proteins that are packaged and released by the cell as new virus particles and have the ability to start a new replication cycle [132]. Based on current knowledge, *HIV* is divided into two strains: *HIV*-1 and *HIV*-2. *HIV*-1 is the virus that was initially discovered and defined both LAV and HTLV-III: it is more virulent, more infectious, and is the cause of most *HIV* infections worldwide [133]. The first of the two is predominantly located in Europe, Central America, and Africa; *HIV*-2, however, is mostly found in West Africa and Asia and causes a clinically more moderate syndrome than the previous strain [134].

Since the beginning of the epidemic, more than 70 million people have been infected with the *HIV* virus and about 35 million people have died of *HIV*. Globally, 36.7 million [30.8–42.9 million] people were living with *HIV* at the end of 2016. An estimated 0.8% [0.7–0.9%] of adults aged 15–49 years worldwide are living with *HIV*, although the burden of the epidemic continues to vary considerably between countries and regions. Sub-Saharan Africa remains most severely affected, with nearly 1 in every 25 adults (4.2%) living with *HIV* and accounting for nearly two-thirds of the people living with *HIV* worldwide [134].

6.3.1 HIV Transmission

The most important factor that increases the risk of sexual transmission of *HIV*-1 is the number of copies per mL of plasma *HIV*-1 RNA (viral load), with a 24 times increased risk of sexual transmission for every 1 log 10 increase [135]. Acute *HIV* infection, which causes very high plasma viral loads in the first few months, is an important driver of *HIV* epidemics [136]. A reduction in plasma viral load of 0.7 log 10 is estimated to reduce *HIV*-1 transmission by 50% [136]. Seminal and endocervical viral load independently predict risk of *HIV*-1 sexual transmission, after adjustment for plasma viral load [137].

Other factors associated with increased risk of sexual transmission of *HIV* include sexually transmitted infections (notably genital ulcers of any cause [138], herpes simplex [139] type-2 infection, and bacterial vaginosis [140], pregnancy [141], and receptive anal intercourse [142]. Male circu*MC*ision is associated with a reduced risk of sexual transmission of *HIV* [143]. Behavioral factors that increase *HIV*-1 sexual transmission include many sexual partners, and concurrent partnerships [144]. The concentration of virus particles is highest at the time of primary infection and in the stage of advanced immunodeficiency (10^6–10^7 copies/mL in the blood). The higher the viral burden, the higher the risk of transmission by sexual contact; *HIV* transmission is unlikely if the *HIV*-positive individual has a consistently low viral count (less than 50 copies/mL) and no other concomitant sexually transmitted diseases [137]. Among all sexual practices, being the receptive partner in unprotected anal intercourse confers the highest risk of contracting *HIV*—up to 1.4%, depending on the viral count of the *HIV*-positive person [138]. The risk of *HIV* transmission is elevated by a factor of 3–10 by the concomitant presence of a florid sexually transmitted infection [138]. Such infections are common among persons with *HIV* (13–16%) [137]. Sexually transmitted diseases take a more complicated course in *HIV*-positive persons than in *HIV*-negative persons; they also induce a rise in viral counts and progression of the *HIV* disease.

6.3.2 HIV Classification

Many distinct strains of *HIV*-1 have been isolated, and their variability in biological, serological, and molecular features indicates the virus is highly heterologous. These differences, become apparent in the level of virus entry, replication kinetics, production the modulation of CD4 expression, cytopathicity, latency and inducibility, genetic evolution, viral tropism, and coreceptor usage.

R5 M-tropic strains are dominant in the early stages of infection, and more importantly they persist to advanced stages of disease. In a progressive study we have shown the importance of macrophages, the persistence and role of R5 M-tropic *HIV*-1 strains in disease progression [145]. These R5 strains from the advanced stage of disease are biologically *fit* and more infectious than those derived from the early time of infection. They are able to infect productively monocytes and macrophages. On the other hand, the R5 variants appear to be restricted in replication, particularly in undifferentiated monocytes at the level of viral entry [145]. Thus, R5 from patients with AIDS seem to have an enhanced cytopathic activity and higher affinity for binding to CD4 and/or CCR5. We have shown that X4 variants are absent in approximately 60% of *HIV*-1 infected patients at the very advanced stage of disease. It has been shown that *HIV*-1 infects macrophages and microglia in the central nervous system and causes *HIV*-associated dementia in 10% of patients with AIDS [146]. This raises the possibility of interactions and/or contributions of multiple factors exerted in maintaining R5 persistence, such as the effect of viral evolution, specific host genetic factors, and immune system pressure. In addition we have also shown that infection of monocyte and macrophage by *HIV*-1 is regulated by various factors [147]. These factors, in brief, include cell maturation and differentiation, sensitivity to beta-chemokines, and cytokines [147].

Years after chronic infection is established, CXCR4 utilizing strains emerge in approximately 40% of infected individuals. The importance of the emergence of X4 strains in late stages of infection has always been directed towards their role in immunodeficiency and rapid progression to AIDS. This is due to the seemingly obvious association between their enhanced cytopathicity and replicative ability linked to CD4+ T-cell depletion in late stages of infection. However, the mechanism by which X4 viruses are associated with accelerated disease progression has never been properly elucidated. The acceleration of *HIV*-1 disease progression has been attributed to the expanded spectrum of CXCR4+ precursor cells susceptible to infection

by X4 strains. It has also been postulated that the decline of the host immune system associated with clinical AIDS may allow X4 viruses to evolve and replicate freely in late-stage infection [148]. Furthermore, a critical question must be asked: how are X4 strains maintained at such high levels during late stages of infection when one of the main cell targets, CD4+T cells, is significantly depleted? At the same time macrophages as the other major target cells and 60% were infected by X4 strains and only 10% were shown to be resistant. This is still a major paradox in *HIV* pathogenesis and raises a few questions [149].

6.3.3 HIV Life Cycle and Genome

HIV is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell [150]. It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease, and integrase [151].

The genome of human immunodeficiency virus (*HIV*) encodes 16 viral proteins playing essential roles during the *HIV* life cycle. Three major genes, gag, pol, and env, code for structural proteins (matrix, capsid, nucleocapsid, and p6), viral enzymes (protease, reverse transcriptase [RT], and integrase), and envelope proteins (GP120 and GP41) [151]. The remaining genes code for regulatory proteins (Tat and Rev) and accessory proteins (Vif, Vpu/Vpx, Vpr, and Nef) [152]. Vpu is found exclusively in *HIV* type 1 (*HIV*-1), whereas Vpx is carried by *HIV*-2. Although *HIV* genomes code for only 16 viral proteins, a great number of physical interactions between pairs of *HIV* proteins, so-called *HIV* pairwise protein interactions, provide essential mechanisms for *HIV* to achieve efficient viral replication at different stages of the *HIV* life cycle [153].

A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle. This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the *HIV* Envelope protein, which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules which anchor the structure into the viral envelope. The Envelope protein, encoded by the *HIV* env gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell membrane releasing the viral contents into the cell and initiating the infectious cycle [151].

The main target of *HIV* is activated CD4 T lymphocytes; entry is via interactions with CD4 and the chemokine coreceptors, CCR5 or CXCR4. Other cells bearing CD4 and chemokine receptors are also infected, including resting CD4 T cells, monocytes and macrophages, and dendritic cells. CD4-independent *HIV* infection of cells can happen, notably in astrocytes [154] and renal epithelial cells, and subsequent *HIV* gene expression has an important role in the pathogenesis of *HIV*-associated neurocognitive disorder (related to astrocytes) and nephropathy (related to epithelial cells). A range of host proteins interact with *HIV* proteins or *HIV* DNA to either restrict or promote virus replication in specific cell types. Transmission of *HIV* across mucosal membranes is usually established by one founder virus, which has unique phenotypic properties including usage of CCR5 rather than CXR4 for entry, enhanced interaction with dendritic cells, and resistance to interferon-α [155].

Transmission of the founder virus is followed by a rapid increase in *HIV* replication and then a striking induction of inflammatory cytokines and chemokines, which is in stark contrast to the minimum initial response to other chronic viral infections such as hepatitis B or hepatitis C [156]. Viral load then decreases to a so-called setpoint, the level of which is established largely by innate and adaptive immune *HIV*-specific CD8 killing

of productively infected cells mediated by T cells happens soon after infection, and the potent adaptive immune response to *HIV* selects for the emergence of mutations in key epitopes, often leading to immune escape [157]. In nearly all individuals, progressive exhaustion of *HIV*-specific T cells happens, characterized by high expression of programmed death 1 (PD-1) on both total and *HIV*-specific T cells and a loss of effector function [158]. Neutralizing antibodies arise roughly 3 months after transmission and select for viral escape mutants [159]. Broadly neutralizing antibodies, which can neutralize many *HIV*-1 subtypes, are produced by about 20% of patients [160]. These antibodies are characterized by a high frequency of somatic mutations that often take years to develop [161]. Broadly neutralizing antibodies do not usually provide benefit to the patient because of the development of viral escape mutants [162]. The production of broadly neutralizing antibodies by the use of new immunogen design strategies is a major focus of vaccine research. The innate immune response to *HIV* is largely mediated by natural killer cells, and is also crucial for virus control. Viral escape mutants also emerge and restrict the antiviral effects of natural killer cells [163].

Shortly after the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule. The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the antisense cDNA [164]. Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called integrase. This integrated viral DNA may then lie dormant, in the latent stage of *HIV* infection [165]. To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T cells become activated [166]. This means that those cells most likely to be killed by *HIV* are those currently fighting infection. During viral replication, the integrated DNA provirus is transcribed into RNA, some of which then undergo RNA splicing to produce mature mRNAs. These mRNAs are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to full-length, unspliced copies of virus RNAs and allows them to leave the nucleus [164]. Some of these full-length RNAs function as new copies of the virus genome, while others function as mRNAs that are translated to produce the structural proteins Gag and Env. Gag proteins bind to copies of the virus RNA genome to package them into new virus particles. *HIV*-1 and *HIV*-2 appear to package their RNA differently. *HIV*-1 will bind to any appropriate RNA. *HIV*-2 will preferentially bind to the mRNA that was used to create the Gag protein itself.

6.3.4 HIV Pathogenesis

Infection with *HIV* starts without symptoms or ill-feeling and is accompanied by slight changes in the immune system. This stage spans up to 3 months after infection until seroconversion, where *HIV*-specific antibodies can be detected in individuals following recent exposure. The outcome of infection and duration for disease progression with clinical symptoms may vary greatly between individuals, but often it progresses fairly slowly [166]. It takes several years from primary infection to the development of symptoms of advanced *HIV* diseases and immunosuppression. During primary infection, although individuals may look healthy, the virus is actively replicating in the lymph nodes and blood stream of infected individuals. As a result, the immune system may get slowly damaged by the burst of viral load in

their bodies [167]. Symptomatic stage of disease indicates the late phase of *HIV* disease (AIDS) where individuals may be susceptible to other opportunistic infections (OIs) [167], such as infections with *Mycobacterium avium*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, CMV, toxoplasmosis, and candidiasis. It is agreed that infected individuals develop an AIDS status when their plasma *HIV* load is high and the CD4+ T count is less than 200 mm [168]. The availability of the highly active antiretroviral therapy (HAART) may question the dilemma as to whether everyone who seroconverts to *HIV* will develop AIDS. One mechanism *HIV* weakens the immune system is by infecting and destroying CD4+ T cells, which in turn leads to immunodeficiency at later stage of disease [167].

6.3.5 HIV Treatment

Combination antiretroviral therapy regimens that were able to suppress viral replication were developed in the late 1990s and transformed *HIV* from a progressive illness with a fatal outcome into a chronic manageable disease. More than 25 licensed drugs that block *HIV* replication at many steps in the virus life cycle are available. Recommended antiretroviral therapy regimens are less toxic, more effective, have a lower pill burden, and are dosed less frequently than the initial protease inhibitor-based regimens. Standard anti-retroviral therapy regimens combine two nucleoside reverse transcriptase inhibitors (emtricitabine or lamivudine together with one of abacavir, tenofovir, or zidovudine) with a non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or integrase inhibitor. Several effective nucleoside reverse transcriptase inhibitor-sparing regimens can be used if intolerance or resistance to nucleoside reverse transcriptase inhibitors develops. After initiation of antiretroviral therapy, the plasma viral load decreases to concentrations below the lower limit of detection of available commercial assays in most people, usually within 3 months. By contrast, the recovery of CD4 T cells in individuals on antiretroviral therapy is variable.

Guidelines in high-income countries allow clinicians to choose a starting regimen of dual nucleoside reverse transcriptase inhibitors combined with either a non-nucleoside reverse transcriptase inhibitor, a ritonavir-boosted protease inhibitor, or an integrase inhibitor, because these three regimens have similar efficacy and tolerability.

Subsequent antiretroviral therapy regimen switches for virological failure are guided by the results of resistance testing. For low-income and middle-income countries, WHO recommends a public health approach to use antiretroviral therapy with standardized first-line (non-nucleoside reverse transcriptase inhibitor plus dual nucleoside reverse transcriptase inhibitors) and second-line (ritonavir-boosted protease inhibitor plus dual nucleoside reverse transcriptase inhibitors) regimens, and restricted monitoring for both efficacy and toxic effects.

6.3.6 Diagnosis of HIV Infection

6.3.6.1 Serology

HIV-1 testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *HIV*-1 [168]. Specimens with a nonreactive result from the initial ELISA are considered *HIV*-negative unless new exposure to an infected partner or partner of unknown *HIV* status has occurred. Specimens with a reactive ELISA result are retested in duplicate. If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., Western blot or, less commonly, an immunofluorescence assay (IFA)) [169]. Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered *HIV*-positive and indicative of *HIV* infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate Western blot result, which may be either an incomplete antibody response to *HIV* in an infected person or nonspecific reactions in an uninfected person. Although IFA can be used to confirm infection in these ambiguous

cases, this assay is not widely used. In general, a second specimen should be collected more than a month later and retested for persons with indeterminate Western blot results. Specimens with a reactive antigen/antibody combination immunoassay result (or repeatedly reactive, if repeat testing is recommended by the manufacturer or required by regulatory authorities) should be tested with an FDA-approved antibody immunoassay that differentiates *HIV*-1 antibodies from *HIV*-2 antibodies. Reactive results on the initial antigen/antibody combination immunoassay and the *HIV*-1/*HIV*-2 antibody differentiation immunoassay should be interpreted as positive for *HIV*-1 antibodies, *HIV*-2 antibodies, or *HIV* antibodies, undifferentiated. Specimens that are reactive on the initial antigen/antibody combination immunoassay and non-reactive or indeterminate on the *HIV*1/*HIV*2 antibody differentiation immunoassay should be tested with an FDA-approved *HIV*1 nucleic acid test (NAT). A reactive *HIV* 1 NAT result and nonreactive *HIV*1/*HIV*2 antibody differentiation immunoassay result indicates laboratory evidence for acute *HIV*1 infection; a reactive *HIV* 1 NAT result and indeterminate *HIV*1/*HIV* 2 antibody differentiation immunoassay result indicates the presence of *HIV* 1 infection confirmed by *HIV*1 NAT; a negative *HIV*1 NAT result and nonreactive or indeterminate *HIV*1/*HIV*2 antibody differentiation immunoassay result indicates a false positive result on the initial immunoassay. In addition, a few tested specimens might provide inconclusive results because of a low quantity specimen. In these situations, a second specimen is collected and tested for *HIV* infection. *HIV*-1 virus has high mutation rate since they replicate their copies using RNA polymerase, which could have high errors in the gene copying [168]. This high mutation is very critical for the virus survival and evolution. The mutation rate is responsible for conferring the drug resistance during viral infection in the human host. Because of its high mutation and recombination rates, *HIV*-1 always exists as a diverse population in hosts. Therefore, many drug resistance mutations exist as quasispecies at a frequency of less than 20% of the mix [170]. Also, it will lead to the variance of viral strains. Next-generation sequencing technology is a currently developed DNA/RNA sequencing tool in gene identification [170].

6.3.6.2 Sequencing and Next-Generation Sequencing

For many years, virus population sequencing using Sanger's technique has been the gold standard for *HIV*-1 drug resistance testing, both for research and clinical routine. Sanger sequencing is feasible for most laboratories with basic molecular biology equipment, is straightforward to perform and generally affordable, particularly with home-brew methods. It is easily scalable to a few dozen tests per week using a single PCR instrument and a single technician with part-time dedication, which fits many small or mid-scale *HIV*-1 laboratories. Sanger-based genotyping has been extensively validated in clinical trials and is supported by equally validated and often publicly available laboratory protocols and interpretation algorithms and rules that can be retrieved automatically [171]. This allows standardized reporting of resistance testing results to clinicians, researchers, and public health officials, which has been instrumental in the past to ensure its acceptability among *HIV*-1 caregivers and policymakers. However, due to its intrinsic sequencing chemistry, Sanger sequencing can only provide a consensus sequence of the whole quasispecies in each *HIV*-1-infected individual, being able to detect only those nucleotides present in at least 10–20% of the virus population. There is solid evidence that, at least in some cases, low-frequency genotypic information missed by Sanger sequencing might impact ART efficacy and could be important to improve *HIV*-1 resistance surveillance [172]. The advent and rapid technical evolution of NGS platforms, coupled with rapid reductions in costs, simplification of laboratory procedures, improvements in turnaround time to results and testing scalability, as well as the development of automated bioinformatic pipelines are gradually increasing NGS use in *HIV*-1 diagnostics. Advances in next-generation sequencing have played an important role in shaping the direction of *HIV* clinical research. In 2006, the Solexa Corporation (later acquired by Illumina,

Inc.) released their first short read sequencer, the Solexa Genome Analyzer. Using reversibly-terminated, fluorescently-labeled nucleotides, Illumina instruments detect base incorporation into template strands bound to the surface of an optically transparent glass slide called a "flowcell" [173]. The Illumina sequencing-by-synthesis involves the cyclic addition of polymerase and reversibly-terminated, fluorescently-labeled dNTPs. A single nucleotide complementary to the template is incorporated in each cycle and its identity is determined optically following laser excitation of the fluorophore; four digital images per flow cell "tile," each using a different bandpass filter, are captured per sequencing reaction cycle. The fluorescent dye terminators are subsequently removed and the next cycle of base incorporation and imaging proceeds [173]. Depending on the instrument and reagents chosen, up to 300 sequencing cycles are performed—double this number if "paired-end" sequencing is used. Next-generation sequencing technologies are well known to suffer from substantial sequencing error rates in comparison to conventional capillary-based sequencing. In published comparisons of different platforms, the Illumina MiSeq platform has historically exhibited a relatively lower overall error rate [174] and greater throughput while producing shorter read lengths. This overall difference in error rates is largely due to the excessive insertion and deletion (indel) errors in homopolymeric regions that have plagued other platforms, such as the Ion Torrent Personal Genome Machine (PGM) or the Roche 454 systems. By incorporating a single reversibly-terminated nucleotide per cycle, the Illumina sequencing-by-synthesis process avoids the elevated error rate associated with homopolymeric regions. This elevated indel error rate was associated with the tendency of those platforms to incorrectly call the number of repeated bases within homopolymers [175]. Instead, the Illumina error profile is dominated by nucleotide substitutions. On average, an approximate error rate of 0.1% per base is observed [176]; however, this rate is not uniform across the length of a read. Error rates of 1% or more are typically seen towards the end of a 250-bp read, and random cycles with error rates exceeding 10% are occasionally observed [177]. Furthermore, a "strand bias" has been described for Illumina platforms [178], where there is a significant statistical association between read direction and the proportion of reads with a mismatch error. Finally, higher mismatch error rates have been reported in associations with A's and C's than G's and T's [179] sequence motifs [178], such as the GGC motif that was reported for the earlier Illumina Genome Analyzer platform. All NGS platforms available to date require reverse transcription and PCR amplification before *HIV*-1 sequencing. This limits the lower sensitivity threshold to the intrinsic error rate of the reverse transcriptase, i.e., 10^{-4} or 1 error per every 10^4 nucleotides copied. Therefore, even in the presence of high *HIV*-1 RNA levels, it is unrealistic to expect any reliable detection of *HIV*-1 variants below 0.5–1% in the virus population. Also, true assay sensitivity of any ultrasensitive genotyping method depends on the number of RNA molecules in the original sample. The RNA copy in the assay depends on the plasma *HIV*-1 RNA concentration, the volume of plasma used and the efficiency of the RNA extraction process. The efficiency of the reverse transcriptase step also determines the starting copy number, since NGS platforms sequence DNA and not RNA molecules. As a rule of thumb, reliable detection of variants at 1% frequency will require *HIV*-1 RNA levels of at least 1000 copies/mL. Although mutant detection above 1% frequency is generally robust and reliable, linear quantification of mutants in the 1–100% range is often affected by biases during library preparation due to the presence of resistance mutations or polymorphisms in primer binding sites, PCR-founding effects or random resampling of input DNA molecules. Primer ID partially avoids PCR resampling bias by including a random sequence tag in the first primer so that every template receives a unique ID [180]. Sequences obtained with this strategy can then be identified, the initial copy number can be quantified and the error and bias can be corrected to a great extent. However, this approach requires an amplicon-based strategy and high coverage to obtain enough reads with identical primer IDs,

what makes it an expensive procedure. As NGS is approaching the clinic, a number of challenges must be overcome before it becomes generally available for routine diagnostics. Accessibility to NGS testing is improving for *HIV*-1 clinicians, with companies like Monogram Biosciences (San Francisco, California) already using NGS for proviral DNA *HIV*-1 genotyping (GenoSure Arc*HIV*e Assay), and Quest Diagnostics (Marlton, NJ) which has a tropism test with reflex to deep sequencing. Hands on time and platform costs for laboratories processing NGS samples are decreasing. The cost per megabase of raw data of DNA sequence decreased 370-fold from US $5.200 in 2001 to US $0.014 in 2015. Equipment and maintenance costs, however, remain unaffordable for many low-income countries. The most important limitation, however, is the lack of automated, validated, and robust but simplified bioinformatic analyses coupled with *HIV*-1 resistance interpretations to enable NGS use and interpretation by laboratory technicians, but even this is improving rapidly.

6.4 Herpes Simplex Virus (HSV)

Genital herpes is one of the most common, persistent, and highly infectious sexually transmitted viral infections mostly caused by herpes simplex virus-2 (*HSV*-2) and in many emerging first time cases, by *HSV*-1 [181]. Primary and recurrent genital herpes infections most commonly result in lesions and inflammation around the genital area. In women, the sites of infection are mainly the vulva and the vagina, with some cases involving the regions of cervix and perianal. In heterosexual men infection is typically on the glans or the shaft of the penis, whereas anal infection is also reported with homosexual men. More than 500 million people are infected worldwide and most cases reported are among the age groups between 16 and 40 years that coincides with increased sexual activity among this age group [182]. While these numbers are an estimate, the actual numbers may be underestimated as many people are either asymptomatic or are unaware of the infection [183]. Herpesviruses are among the most ubiquitous of human infections. After infection with *HSV*, it is thought that the virus and the immune response to the virus persist through the life of the host. *HSV* infections are measured by testing various populations for the presence of antibodies specific to the virus. An estimated 90% of all people worldwide have one or both viruses [184]. *HSV*-1 is the more prevalent virus with 65% of persons in the USA having antibodies to *HSV*-1 [185], while *HSV*-2 infections are markedly less frequent, with 15–80% of people in various populations infected [186]. *HSV*-1 and *HSV*-2 infection rates widely vary between countries. The increase in genital *HSV*-1 is mainly attributed to an increase in oral sex among youngsters and adults which is viewed safer than intercourse [187]. Due to this, in the USA, Canada, and other European countries, at least half of the first episodes for genital herpes have been caused by *HSV*-1 in the past decade [188]. In a study performed by the CDC it is estimated that about one in six Americans aged 14–49 are infected with *HSV*-2 and the prevalence in women was 20.9%, twice as high as among men [189]. While a surge of *HSV*-2 seroprevalence from 16.4% to 21.8% was observed from 1976 to 1994 [190], this trend has reversed, dropping to 17.2% in 2004 [191]. In Africa and other developing countries, there is a high burden of *HSV*-2 infections with >50% prevalence in the population [192]. Around 82% of women and 53% of men in the sub-Saharan Africa are seropositive for *HSV*-2 [193]. *HSV*-2 infection rates also depend on the rates of sexual activity and are more prevalent in heavily exposed populations, such as commercial sex workers, who are nearly 100% positive, suggesting an urgent need for education and new measures for prevention [194].

6.4.1 HSV Transmission

Genital herpes is predominantly transmitted through sexual contact. Viral transmission by oro-genital contact is mostly *HSV*-1 and, therefore, the number of genital *HSV*-1 cases is on the rise [195]. Virus shedding is more predominant in

sites like mouth and mucosal surfaces such as the vagina. Contact with any one of these increases the risk of being infected with *HSV*. An episode or outbreak is termed as the phase in which individuals experience symptoms and the severity of these episodes depends on previous immunity to *HSV*. Notably, almost 25% of people presenting with a first clinical episode of genital herpes have serological evidence of past *HSV*-2 infection at the time of presentation, suggesting initial infection was asymptomatic [196]. In many other instances of primary infections where the patient encounters *HSV* for the first time the first episode may occur anywhere between 2 days and 2 weeks after primary infection. Due to its low environmental stability *HSV* can only remain infectious for a period of days on moist surfaces [183]. It can, therefore, be assumed with a high level of certainty that when normal hygiene (including bodily hygiene) is maintained, modes of transmission other than sexual intercourse do not play a significant role. Intrauterine and perinatal viral transmission are the exceptions. Both primary and recurrent *HSV* infection in pregnant women can result in intrauterine viral transmission and congenital *HSV* infection, although the incidence is low at just 5% of all *HSV* infections in newborns [197]. The clinical consequences of fetal infection described include abortion, stillbirth, or other congenital manifestations usually including skin and eye lesions and/or neurological symptoms [198]. The highest risk of fetal infection is during the first 20 weeks of pregnancy, and with primary maternal *HSV*-2 infection. Viral transmission to the child via the mother's genital tract during labor is regarded as the most common cause of neonatal *HSV* infection; of these infections 70–85% are caused by *HSV*-2 [199]. The incidence in the USA is quoted at 5–31 per 100,000 live births with a worse prognosis for *HSV*-2 infection compared to *HSV*-1 [197]. The highest risk is with perinatal maternal primary *HSV* infection; however, most neonatal infections occur around the birth in the presence of asymptomatic genital tract viral shedding [197]. Disease manifests as localized infection of the skin, eyes, and mucous membranes, central nervous system infection or disseminated systemic infection [200].

6.4.2 HSV Life Cycle and Genome

HSV are linear, double-stranded DNA viruses capable of establishing latency in humans. They belong to the family of Herpesviridae and more specifically to the sub-family of Alphaherpesvirinae. There are two subtypes: *HSV*-1 and *HSV*-2 that are closely related but differ slightly in tissue tropism and antigenic properties. The viral DNA is present in the core that is enclosed in a protein shell called the capsid. The icosahedral shaped capsid is ~125 nm in diameter, which is connected to and surrounded by a glycoprotein expressing lipid bilayer membrane envelope via a protein coat called the tegument. The viral envelope contains at least 12 glycoproteins many of which play major roles in the entry and egress of the virus. The life cycle of *HSV* has been mostly studied and characterized using *HSV*-1 infections [201]. However, *HSV*-2 infections are considered similar to *HSV*-1 infections. Different stages in the *HSV* life cycle can be broadly classified into:

6.4.2.1 Attachment

Initiation of infection begins with the attachment of viral glycoproteins to the cell surface. Heparan sulfate proteoglycans (HSPGs) on the cells serve as attachment sites for *HSV* [201]. Glycoproteins B and C (gB and gC) on the *HSV* envelop bind to the HSPGs and are essential to initiate attachment [202].

6.4.2.2 Entry

After the initial attachment to the cell surface, virus entry is the next step in the life cycle. Various modes of viral entry have been established. The virus is taken into the cells by either direct fusion with the plasma membrane, which is independent of pH change, or through endocytosis mediated by specific cellular receptors. The glycoprotein D (gD) on *HSV* plays an important role in both of the aforementioned uptake processes and glycoproteins H and L (gH and gL) act in concert to complete the fusion machinery. To date the following receptors have been identified for gD: herpes virus entry mediator (HVEM), nectin-1 and -2 and 3- O sulfated heparan sulfate

(3-OS HS). HVEM was the first identified *HSV* receptor that belongs to the tumor necrosis factor (TNF) superfamily. The next set of receptors identified is represented by nectin-1 and -2. They belong to the immunoglobulin superfamily. The last receptor is a rare modification of the large sugar molecule HS mediated by the 3-O-sulfotransferase 3 (3-OST-3). 3-OST-3 belongs to the family of 3-O sulfotransferases (3-OSTs) that place sulfate groups at the 3-OH position on the glucosamine in HS [203]. Even though gD is needed for receptor-mediated endocytosis and also for the direct fusion of viral envelop to the plasma membrane, there seems to be no clear consensus on how and which mode of entry the viruses use in human hosts or animal models [203].

6.4.2.3 Capsid Transport and Replication

Upon successful entry into cells, the viral capsid and tegument proteins are released into the cytoplasm. The virion host shutoff protein (vhs) is a viral tegument protein that is released into the cytoplasm after entry and degrades host mRNAs that regulate stress response. The capsid then translocates to the nucleus along microtubules via the dynein and dynactin motor proteins and releases the viral DNA into the nucleoplasm [203].

6.4.2.4 Replication and Assembly

Once inside the nucleus, several viral genes are expressed in an ordered fashion. The proteins of the α genes or intermediate early (IE) genes are the first to be transcribed. The products of these genes are termed as infected cell protein (ICP) and there are five ICPs: 0, 4, 22, 27, and 47. The virus encodes a tegument protein: VP16 that aids in the transcription of the α genes. The expression of ICP4 is then thought to drive the expression of the β genes or the early genes. The β genes encode for various proteins that promote viral DNA replication, including the enzyme thymidine kinase (TK) [204].

The virus utilizes TK for replication leading to the expression of the γ or late genes. The proteins of the γ genes encode for several components of the viral structure including capsid and envelop proteins. Various viral components are formed which then assemble and the viral DNA is repackaged into a new capsid. Fully assembled capsid exits from the nucleus by acquiring a glycoprotein-containing envelop at the inner nuclear membrane and losing it at the outer membrane when the naked capsid is released in the cytoplasm for re-envelopment using a Golgi-derived membrane [205].

6.4.2.5 Latency and Reactivation

One of the key traits of this family of viruses is to go latent for the life of the host after primary infection. How and why the virus goes latent is only partially understood and is one of the hot topics in herpes research. After a lytic infection the virus has the ability to evade and mask itself from the host defense. Latency is established when the virus migrates to the sensory ganglia via a retrograde fashion and invades the nucleus of the neurons. In the nucleus the *HSV* genome is maintained in a circular form and remains in a silent state. During this state, a region of the genome that encodes for the latency associated transcripts (LATs) remains active [206]. LATs help in reducing the expression of the viral genome thereby maintaining them in a latent state protected from the immune system and they protect infected neurons from apoptosis, thus increasing the amount of latent transcripts that would eventually increase the viral load upon reactivation [207]. Some evidence also suggests the role of neuronal function in maintaining latency [208]. Furthermore, during latent infection, the ability of some parts of the *HSV* genome to remain transcriptionally active and inactive suggested the presence of epigenetic control.

Reactivation of the latent virus occurs when an external stimuli or "stress" is applied to the neuron. Various factors such as environmental conditions, fever, exposure to sunlight, and other unknown conditions have been attributed to cause reactivation but their exact targets at the molecular level remain unknown. When the virus reactivates it travels from the sensory ganglia via anti-retrograde fashion to the primary infection site or sites of high neuron innervations where

active virus replication and shedding occur and symptoms like pain, inflammation, and lesions develop [209].

6.4.3 HSV Pathogenesis

Primary infections are clinically most severe and most likely symptomatic [210]. Symptoms like fever, itching, and muscle pains usually in the lower part of the body are most common in primary infection; 40% of men and 70% of women also report fever, headache, malaise, and myalgias [211]. Papule formulation followed by a wide distribution of blisters or lesions appear around the genital areas that eventually break to form ulcers. Over a period of time the ulcers crust and heal. In women common sites for lesion are the cervix, vagina, labia majora and minora, and perianal region through infected vaginal fluid and in men it is mostly on the shaft or the glans of the penis. Anal lesions are also reported in homosexual men. Primary infections either by *HSV*-1 or by *HSV*-2 cannot be differentiated just by clinical symptoms; additional laboratory testing is needed to differentiate between the two viruses.

At the tissue and molecular level, *HSV*-2 infects the epithelial cells on the genital mucosa leading to an increase in inflammatory response and cell death at the site of infection. Multinucleated cells and syncytia formation are the most common observation in cells infected with *HSV*. The recruitment of macrophages, natural killer cells, B-cell and T-cell mediated immunity [212], and the release of cytokines have been reported to play a role in innate and adaptive immunity to *HSV* infections. This contributes to a chronic inflammatory state in genital skin and mucosa. During the course of primary infection, the virus spreads via a retrograde fashion along the microtubules lining the axons to the dorsal root ganglia (DRG), where the neuronal cells act as reservoirs for the virus to remain latent [213]. Upon reactivation due to factors such as stress and other unknown conditions, the virus spreads from the DRG to the epithelial cells via an anterograde fashion where a lytic replication of the virus follows, resulting in virus shedding. This is the cause of recurrent infections and these infections are usually asymptomatic or may be associated with a classic genital ulcer. Following the primary eruption the virus establishes lifelong latency in sensory neural ganglions [214]; in the case of primary genital infection the sacral ganglions are mainly involved. From here the virus can reactivate, causing recurrent infection. Viral reactivation is common in the presence of immunogenetic predisposition, though reactivations decrease with increasing age. Numerous physiological and environmental factors such as fever, UV light, menstruation, stress, or trauma can function as triggers [215]. Endogenous viral reactivations may manifest as recurrent herpes genitalis. Recurrences occur in almost every person suffering symptomatic primary herpes genitalis due to *HSV*-2, in a third of patients frequently (at least 6 times a year) [216]. Recurrent genital *HSV*-1 infections occur over five times less commonly [217]. Recurrences almost always initially present with prodromal symptoms such as neuralgic symptoms, dysaesthesia, or lumbosacral dermatome pain 1–2 days before skin and mucosal lesions erupt [218]. Compared to primary infection, symptoms of recurrence are much less severe and the clinical course shorter [219]. In the majority of cases endogenous viral reactivation is characterized by asymptomatic genital viral shedding. Most commonly *HSV*-2 is shed by *HSV*-2 seropositive patients, and this is the case for almost anyone who is anti-*HSV*-2 IgG positive [202]. In contrast, *HSV*-1 shedding is uncommon. These data allow the assumption, with a high level of certainty, that *HSV*-2 seropositive people should always be regarded as potential virus excretors.

6.4.4 HSV Treatment

Standard first-line drugs include acyclovir, valacyclovir, and fa*MC*iclovir. The specific antiviral action of these acyclic nucleoside analogues [220] is based on their phosphorylation to monophosphate form by thymidine kinase (TK), the key enzyme of *HSV*-1 and *HSV*-2, with subsequent phosphorylation via di- to triphosphate form by cellular enzymes. The triphosphate

nucleoside analogues inhibit and fixate the viral DNA polymerase by being incorporated into the growing DNA chain as "false" enzyme substrates. In the case of acyclovir/valacyclovir this leads to chain termination, since hydroxyl groups in the 3′ position, which are essential for further linkage, are missing. Fa*MC*iclovir may be incorporated into the growing DNA chain. Acyclovir is the first choice therapeutic agent for *HSV* infections, including herpes genitalis. However, bioavailability is only 15–30% with oral administration. Infections of the skin and mucous membranes including herpes genitalis are treated orally in immune competent people. Severe *HSV* infections, particularly in immunodeficient patients, should be treated with intravenous (i.v.) acyclovir. Acyclovir dosage for the treatment of herpes genitalis is dependant on infection status, immune competence and whether or not the patient is pregnant. If recurrences occur at a rate of over four to six episodes annually [221, 222], long-term treatment to suppress the virus (prophylaxis) should be considered. The benefits of prophylaxis have been proven particularly during pregnancy [223]. Topical acyclovir is only recommended for herpes labialis, herpes keratoconjunctivitis, and mildly symptomatic herpes genitalis. Officially acyclovir is not licensed for use in pregnancy, though administration should be avoided particularly before the 15th week of gestation. Valacyclovir is a prodrug (an L-valyl ester) of acyclovir suitable for oral administration. After ingestion it is converted to acyclovir by the hepatic enzyme valacyclovir hydrolase. Oral valacyclovir has a bioavailability of 54%, achieving active ingredient concentrations three to four times higher than oral acyclovir. This allows increased dose intervals and is associated with better compliance. Valacyclovir is also a standard treatment for herpes genitalis in immunocompetent patients and studies have shown its efficacy for viral suppression and prevention of recurrent herpes genitalis [223]. Valacyclovir is not licensed for antiviral treatment in children and adolescents since its efficacy and safety profiles have not yet been adequately studied in this population. This applies to pregnancy too as there is also little data on its safety in this context [224]. Possible side effects are similar to those of acyclovir. Fa*MC*iclovir is the inactive diacetyl ester prodrug of the only topically effective acyclic nucleotide analogue penciclovir, which arises after cleavage of two ester groups in the small bowel and liver. The bioavailability of fa*MC*iclovir is 77% after oral application. Fa*MC*iclovir is also regarded as one of the standard therapeutic agents for herpes genitalis, along with acyclovir and valacyclovir, and is also not licensed for use in children and adolescents, immunosuppressed patients under the age of 25 years or in pregnancy. It should, therefore, not be used as the treatment of choice in pregnancy [225]. In rare cases fa*MC*iclovir can cause headaches, nausea, and confusion. Long-term prophylaxis and treatment with ACV, VACV, or FCV can result in the development of resistance, especially in immunocompromised patients. The prevalence of ACV- or PCV-resistant *HSV* isolates differs greatly for immunocompetent and immunocompromised patients. This difference is most likely due to two reasons [226]. First, virus replication may be prolonged in immunocompromised patients, having a tendency toward true persistence. Second, and most important, the host responses are impaired and less pathogenic viruses that would not survive in the face of normal host responses may continue to replicate. Thus, even if some resistant viruses are less pathogenic, they are capable of producing overt diseases in these patients.

6.4.5 Diagnosis of HSV Infection

6.4.5.1 Virus Detection

The laboratory diagnosis of acute genital *HSV* infection or asymptomatic virus shedding is made via direct viral detection. The method of choice is demonstration of viral genomes in skin or mucous membrane swabs using the polymerase chain reaction (PCR) [227]. The content of vesicles provides the best swab material. The sample should be sent in physiological saline solution or viral transport medium. In the presence of complications involving other organs the examination of liquor, tissue samples, bronchoalveolar lavage,

amniotic fluid, intraocular fluid, serum, or EDTA blood should be considered. The PCR test should be able to differentiate between *HSV*-1 and *HSV*-2 [227] and various in-house assays and commercially available kits are used [228]. The sensitivity of PCR is quoted at ≥98% independent of the qualitative or quantitative method used, the specificity at almost 100% [228]. Considering these data and the reduced stability of viral DNA when samples are stored for a few days at temperatures over 20 °C, a negative PCR by no means entirely excludes *HSV* infection. Current guidelines thus recommend starting antiviral treatment when typical herpes genitalis symptoms are present regardless of laboratory results [229]. Alternatively, acute genital *HSV* infection or asymptomatic viral shedding can be diagnosed by growing the virus in tissue cultures, whereby typing of viral isolates is performed by immunofluorescence using appropriate fluorescein labeled *HSV* serotype-specific monoclonal antibodies. Virus isolation is a sensitive method of detecting *HSV* since both *HSV*-1 and *HSV*-2 grow well in various cell types, such as diploid human embryonic fibroblasts or permanent Vero cells and HEp-2 cells. However, in view of its higher sensitivity [230], PCR is rightly regarded as the gold standard of diagnosis in many laboratories. Virus isolation continues to be recommended as an alternative method for diagnosis of genital herpes [228]. Direct *HSV* antigen detection using commercially available immunofluorescence tests is a commonly used and economic method of diagnosis that provides results within hours; sensitivity and specificity are, however, limited [231]. It must be remembered that direct viral detection does not differentiate between primary and recurrent infection or asymptomatic virus shedding.

6.4.5.2 Antibody Detection

The detection of virus-specific antibodies for confirming *HSV* infection is widely used in clinical practice. One should, however, be aware of the limited value of serology results. *HSV* serology is mainly useful for confirming seroconversion following primary infection, through demonstration of IgG. This can be of particular value in the diagnosis of *HSV*-2 infections in the context of antenatal care. Confirming seroconversion is also possible by demonstrating type-specific IgG antibodies, and since *HSV*-1 and *HSV*-2 are so closely related this is only possible using ELISA/immunoblot on the basis of *HSV*-1 gG-1 or gC-1, and *HSV*-2 gG-2 [232]. When interpreting results it is important to consider that partial cross-immunity exists between *HSV*-1 and *HSV*-2. The importance of *HSV* type-specific IgG is mostly that it allows rapid, reliable, and economical identification of *HSV*-2 carriers and potential virus shedders [233]. Thus a patient in whom anti-*HSV*-2 IgG is detected can be considered a potential virus shedder and transmitter who may also suffer from anogenital *HSV* infection. If an initial serum sample is available from the early stage of a herpes genitalis infection, primary and recurrent infections can be differentiated from one another through the detection of virus type-specific DNA by PCR in combination with virus type-specific IgG [234]. As an example, this means that when *HSV*-2 is detected on genital swab in a pregnant woman, primary genital herpes can be differentiated from a recurrence up to a few weeks before delivery using *HSV* type-specific IgG. This differentiation is of great significance, since the risk of severe neonatal *HSV* infection is many times higher following primary infection than with recurrent infection. Avidity testing can also assist in differentiating between primary and recurrent infections although to date experience with this method is limited [235]. Negative anti-*HSV* IgG excludes recurrent *HSV* infection. The detection of anti-*HSV* IgM is of limited significance for early confirmation of acute *HSV* infection. False positive IgM results are possible due to cross-reactivity with other herpes viruses, e.g. the varicella-zoster virus. Confirmation of acute *HSV* infection is only possible using non type-specific *HSV* IgM tests that have high sensitivity and specificity [236]. It must, however, be noted that the positive predictive value of anti-*HSV* IgM is low, and that it does not allow differentiation between primary and recurrent infection. Although IgM is usually positive following primary infection, it can also be positive in the context of recurrence, independent of clinical symptoms. The rather unreliable

measurement of *HSV* type-specific IgM antibodies should be avoided in clinical practice [236].

6.4.5.3 Antiviral Drug Resistance Detection

The persistence of lesions for more than 1 week after the beginning of therapy without appreciable decrease in size, an atypical appearance of the lesions, or the emergence of new satellite lesions despite antiviral administration is suggestive of treatment failure. After healing of an infection caused by a Timidine Kinase-negative drug-resistant *HSV* strain and stopping antiviral therapy, recurrences are most often associated with a drug-sensitive strain, although some cases of spontaneous recurrences of ACV-resistant viruses have been reported [237]. Laboratory diagnosis of ACV resistance is required to guide clinicians toward different treatment options in cases of therapy failure. ACV resistance can be diagnosed by testing a virus against antiviral agents (phenotypic assays) or by the identification of a specific mutation conferring resistance to antiviral drugs (genotypic assays).

Phenotypic Methods

The common basis of phenotypic assays is the measurement of virus growth inhibition in the presence of the antiviral drug. After appropriate periods of incubation with the antiviral, viral replication is measured based on the reduction of virus-induced cytopathic effect or plaque formation, which is evaluated either colorimetrically or microscopically [238].

Genotypic Methods

The objective of genotypic assays is the search for specific mutations in the viral genes encoding the activating/phosphorylating TK enzyme (*UL23*) and the target DNA pol enzyme (*UL30*). The gene of interest is amplified by PCR, and the PCR products are then sequenced. The mutations detected in the gene of interest are then interpreted by comparison with the whole panels of mutations described in the literature. For the mutations that have not been characterized previously, some predictions can be made from the knowledge of the conserved regions implicated in the structural integrity of the enzyme or in its catalytic functions. However, in order for genotypic methods to be helpful in clinical practice, it is essential to be able to discriminate between random variations (polymorphism) and true drug resistance mutations. The generation of recombinant mutant viruses allows the formal assessment of the role of nonconfirmed mutations in antiviral drug resistance.

6.4.5.4 HSV Next Sequencing Generation

The next-generation sequencing technology was applied to study the mode of emergence of acyclovir ACV-resistant (ACVr) *HSV*-1 in patients with hematopoietic stem cell transplantation (HSCT) by quantitatively detecting mutations in the viral thymidine kinase gene in the *HSV*-1 isolates recovered from HSCT patients. It was showed that mutations detected with the Sanger sequencing method in the viral Thymidine Kinase genes of *HSV*-1 isolates were also detected with the NGS assay. Furthermore, different mutations, which conferred ACV resistance and not identified with the Sanger sequencing method, were also detected in a quantitative manner by using the NGS assay. The NGS assay makes it possible to make a diagnosis of vTK gene mutation-associated ACVr *HSV*-1 infections at the early stage, which the ratio of ACVr *HSV*-1 is much lower than that of ACV-sensitive *HSV*-1 [239]

6.5 Molluscum Contagiosum (MC)

Molluscus Contagiosum (MC) is a common cutaneous viral infection caused by the *Molluscipox* virus that affects both children and adults. Clinically, *MC* is characterized by small, waxy, dome-shaped umbilicated papules [240]. Secondary bacterial infection can occur, particularly if patients are scratching their lesions. Inflammatory reaction to *MC*, molluscum dermatitis, inflamed *MC* lesions, and Gianotti-Crosti syndrome-like reactions are common [241]. Plantar localization of *MC* is uncommon and can cause pain on walking [242]. *MC* involving the intraoral mucosa has been documented but is rare

[243]. Molluscum contagiosum (*MC*) is a common skin condition, caused by a member of the poxvirus family [244] that causes considerable parental anxiety and results in primary and secondary care consultations. It is common in children and generally presents with asymptomatic lesions; however, it can present with pruritus, erythema and, on some occasions, bacterial superinfections with inflammation and pain [245]. Dermatological conditions can impact upon quality of life; in severe cases, they can have similar impacts to that of chronic conditions [244]. The reported incidence and prevalence of *MC* varies widely; therefore, it is difficult to estimate the true number affected by *MC*. Evidence of factors increasing the risk of transmission is mixed.

Whereas mollusca contagiosa are rather frequent in 1- to 5-year-old children and can be localized almost anywhere on the body, their appearance in adults characteristically involves the genital area and is mostly regarded as a sexually transmitted infection case. In these cases, the pubic area is typically involved. Shaving represents a risk factor for a high lesion number but not the extension beyond the pubis. Sexually acquired molluscum is rare in younger children but becomes quite common during adolescence and young adulthood. The entity of congenital molluscum has been debated in the literature, but it is accepted that molluscum infections in neonates are likely vertically transmitted [246].

6.5.1 MC Classification

Analysis of DNA restriction fragment patterns shows four genetic types of molluscum contagiosum virus (I, II, III, and IV), and variants based on type-specific restriction endonuclease recognition sites (DNA fingerprinting) [247]. These types are closely related at the level of the nucleotide sequence, induce indistinguishable lesions, and can be used for molecular epidemiology surveys. Types I and IV are the most common types detected in patients, followed by type II, which is more common in patients with *HIV* [248]. Types I and II can be distinguished by nucleotide sequencing or restriction analysis [249]. Several surveys of the molecular epidemiology of molluscum contagiosum virus infection have shown geographical variations in the distribution of the virus.

6.5.2 MC Life Cycle and Genome

Molluscum contagiosum is the only member of the Molluscipoxvirus genus in the family Poxviridae [250]. It is phylogenetically distinct from other poxviruses and has several unique biological characteristics. Replication is limited to the human epidermis; the virus enhances cell mitosis and disrupts epidermal cell differentiation. Although molluscum contagiosum does not encode an epidermal growth factor homologue, expression of epidermal growth factor receptor is upregulated in infected epidermis [251]. Like all poxviruses, molluscum contagiosum virus is a cytoplasmically replicating virus. Viral cores are first found in the basal layer of the epidermis, where keratinocytes display markers of cell activation, over-express epidermal growth factor receptor, and are hyperplastic [251]. Henderson-Paterson bodies, the viral inclusion bodies, are found about three to four layers above the basal cell layer, and grow bigger as they progress towards the granular cell layer; they represent the typical cytoplasmic site where the virus is assembled. As the assembly sites grow, the cellular organelles, including the nucleus, are pushed to the side. Maturation of molluscum contagiosum virus takes 5 days. The stratum corneum ultimately disintegrates as the inclusions enlarge, releasing high numbers of infectious virions. Rupture and discharge of molluscum contagiosum virus through a central dimple-like ostium in the skin lesion is similar to holocrine secretion, an egress process that differs from that of other poxviruses [246].

The genome of molluscum contagiosum virus is a linear, double-stranded DNA molecule with a high guanine–cytosine content (63% compared with 30% in vaccinia virus), featuring about 4.2 kbp of terminally inverted repeats, typical of poxviral genomes. The complete genome of

molluscum contagiosum virus type I (1/80) has been cloned and sequenced, and shown to comprise 190–289 bp (GenBank accession U60315: molluscum contagiosum virus type 1/80) [252]. The genome was at first reported to encode 164 open reading frames, probably encoding hypothetical proteins: genes *MC001R* to *MC164L*, starting in terminally inverted repeats [252]. However, a more detailed analysis subsequently revealed 182 genes, with 154 that were highly likely to be coding genes [253]. One hundred and five hypothetical proteins have homologues to smallpox virus and other poxviruses. The remaining open reading frames are unique to molluscum contagiosum virus and might be involved in the suppression of the host response to infection, nucleotide biosynthesis, and cell proliferation [253]. Most of the genes conserved in other poxviruses are in the central part of the genome. Unique molluscum contagiosum virus genes with cellular homologues or no identified homologues are located on the flanks of the genome and dispersed between conserved genes. Few genes have been analyzed for function and these were expressed only in isolation in bacterial or viral expression systems [254].

6.5.3 MC Treatment

Spontaneous clearance occurs in immunocompetent individuals but often over a prolonged period of months to a few years. Most patients prefer treatment, if lesions persist more than a month or two. There is no etiologically directed treatment of mollusca contagiosa, and the majority of treatment options are mechanical, causing a certain degree of discomfort. Traditional treatment includes curettage as the most efficient [255] and cryotherapy. Both methods are painful but can be ameliorated with the use of topical anesthetics. Some physicians use cantharidin 0.7% or 0.9% liquid for treatment of mollusca contagiosa. It must be applied with care and washed off 2–6 h later. Use on the face or genital areas is not recommended [256]. Other topical therapeutic modalities include retinoid cream [257], imiquimod cream [258], salicylic acid, cidofovir [259], silver nitrate paste and tape stripping [260], 10% potassium hydroxide solution [261], and topical application of essential oil of *Melaleuca alternifolia* and organically bound iodine [262]. Ultrapulsed dye laser is also a treatment [263]. Oral cimetidine is questionably helpful [264]. Because molluscum contagiosum is considered a self-limiting disease, debate continues about whether lesions associated with this disease (*MC*V lesions) should be treated or allowed to resolve spontaneously. Many clinicians recommend treatment of genital *MC*V lesions to reduce the risk of sexual transmission and to prevent autoinoculation. Although *MC*V lesions will generally resolve if left untreated, the resolution rate is extremely variable [265] and immunocompromised patients are at greater risk for secondary inflammation and bacterial infection. In addition to the risk for transmission to sexual partners, some patients may experience pain and discomfort that adversely affect their quality of life. Emotional and psychological discomfort, particularly embarrassment, may also be associated with genital *MC*V lesions. Therefore, it would be advantageous in most cases for physicians to treat *MC*V lesions, provided the treatment was safe, effective, painless, and convenient to administer. Several treatment options, both physician-administered and patient-administered, for genital *MC*V lesions are available.

6.5.4 Diagnosis of MC Infection

Although easily diagnosed, *MC* may present as a single lesion or as several small, inflamed lesions of difficult diagnosis. If so, dermatoscopy performed on *MC* may be superior to dermatologic examination. The presence of orifices, vessels, and specific vascular patterns aids in the diagnosis [266]. Recently, a fluorescence resonance energy transfer-based real-time PCR has been developed that provides a very sensitive and specific detection of the *MC* virus [267]. Diagnosis of genital molluscum contagiosum is generally made by clinical examination of lesions. Lesions caused by *MC*V typically appear as white, pink, or flesh-colored, umbilicated, raised papules

(1–5 mm in diameter) or nodules (6–10 mm in diameter). Molluscum contagiosum lesions may occur as single or multiple lesions (usually <30 papules). Differential diagnosis includes condylomata acuminata and vulvar syringoma for multiple small *MC*V lesions and squamous or basal cell carcinoma for large, solitary lesions. Analysis of biopsy specimens of lesions with hematoxylin and eosin staining to identify epidermal changes may facilitate a more definitive diagnosis. If genital molluscum contagiosum is identified, patients should also be tested for other STDs as a precautionary measure.

Molecular laboratory diagnosis of molluscum contagiosum virus is important as lesions can be confused with those caused by Cryptococcus neoformans, herpes simplex virus, *Human papillomavirus*, and varicella-zoster virus. A rapid method for identifying patients infected with *MC*V via swab sampling is suggested by the use of a two dual-labeled probe real-time PCR assays, with high specificity and concordance of 99.9%, one homologous to the p43K gene and one to the *MC*080R gene. The p43K PCR was designed to be used in conjunction with pyrosequencing for confirmation of PCR products of the p43K PCR product that was capable of providing enough nucleotide sequence to definitively differentiate *MC*V1 and *MC*V2 [268].

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7 The Laboratory Diagnosis of Bacterial Sexually Transmitted Diseases

Vittorio Sambri

7.1 Introduction

Sexually transmitted diseases (STDs) include a wide number of infections that are transmissible via sexual intercourse. The etiological agents of these diseases encompass different microbes, including bacteria, viruses, and protozoa. Mankind coexists and develops with the STDs since many centuries: in the medieval age syphilis and gonorrhea were already present and affecting several thousands of patients, but their etiology was totally unknown. One not completely convincing theory postulated that syphilis was entering in Europe brought by the crew of Cristoforo Colombo returning from the newly discovered Americas: today this hypothesis has been debated since there was evidence that *T. pallidum* was likely already circulating outside the Americas before Colombo's voyages. The identification of the causative pathogens was possible only several centuries later, in the golden age of Microbiology when many infections were finally linked to the respective etiologic agents: the gonococcus was identified by Albert Ludwig Sigesmund Neisser in 1879, *T. pallidum* was discovered in syphilis lesions and named *Spirochaeta pallida* by Schaudinn and Hoffmann in 1905, and 2 years later (1907) Stanislaus von Prowazek identified *C. trachomatis*. Since those achievements, the identification of pathogen causing STDs has moved forward and today this body of knowledge is at the basis of the possibility to currently perform the laboratory diagnosis of STDs. Several different reasons are sustaining the requirement to achieve a precise and possibly fast diagnosis of STDs:

1. the knowledge of the pathogen(s) involved in each case of STD is a powerful tool to appropriately manage and control each patient and the community involved in order to stop the spread to healthy persons and to prevent the long-term consequences of these infections;
2. the availability of etiologic data is at the bottom line of any surveillance activity for the epidemiological control of STDs: in particular the prevalence data obtained with the screening diagnostic approach applied to asymptomatic individuals, such as for *C. trachomatis* infections in young women of childbearing age, is of outmost importance to control this frequently unapparent but extremely relevant infection;
3. the recently applied "syndromic approach" to the STDs, that enables the simultaneous detection of different pathogens possibly underlying a similar clinical picture, represents a major improvement since this technique allows a

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faster and more accurate therapy, the so-called pathogen driven treatment;
4. considering the increasing evidence of antibiotic resistance phenomena among bacterial STDs related germs (gonococcus and *M. genitalium* in the front line) the availability of in vitro data for the antimicrobial susceptibility testing (AST) of each pathogen is extremely relevant to set up the most appropriate treatment: the classical techniques for the evaluation of the AST are based on culture methods, and since the implementation of nucleic acids amplification tests (NAATs) for the diagnosis of STD many data about the AST are also determined by using the molecular tests. This on one side is a great improvement in respect of the standard culture based techniques since the AST data are available also for non-viable of difficult to grow microbes, but on the other side the large use of NAATs for the determination of AST opens the big question about the clinical meaning of identifying resistance gene rather than antimicrobial resistance phenotypes.
5. the large panel of different tests offered on the market opens two principal questions: (1) which is the test most appropriate and effective in each laboratory setting for individual STDs; (2) which are the standards of quality that each laboratory must ask for and how to survey the quality performance of the adopted methods.

This chapter will discuss the most recent techniques presently available to perform the laboratory diagnosis of some of the major STD of bacterial and protozoan origin, in detail:

1. Syphilis (caused by the spirochete *Treponema pallidum*).
2. *Chlamydia trachomatis* infection.
3. Gonorrhea (caused by the gram negative bacterium *Neisseria gonorrhoeae*).
4. Trichomoniasis (caused by the protozoan *Trichomonas vaginalis*).
5. Infection by genital mycoplasma (mainly by *Mycoplasma genitalium* and *M. hominis*).
6. Infection by *Haemophilus ducrey* (chancroid).
7. Donovanosis (infection by *Calymmatobacterium granulomatis*).

At the end of this chapter a brief discussion about the most recent developments in the field of laboratory diagnosis of bacterial vaginosis (BV) is provided, even if BV is not considered an infective STD but a dysbiotic condition that can facilitate the acquisition of STDs.

Each section includes two distinct parts: one dedicated to the so-called direct diagnosis (i.e., all the techniques that bring to the identification of the microbes or some germ components), while the second is devoted to the serological methods (indirect diagnosis).

7.1.1 Syphilis

Syphilis is a multistage STD that holds relevant importance worldwide with an estimated number of total disease burden ranging from 18 to 56 patients. This chronic and progressive infection is caused by *T. pallidum,* a spirochete that is known since 1905 and that until 2018 has not been efficiently cultivated in vitro [1]. This extreme difficulty in achieving the in vitro growth of *T. pallidum* has prompted to the wide use of diagnostic methods other than the culture based techniques. The choice of technique for diagnosing syphilis is largely dependent on the stage of the infection, since the direct diagnostic techniques can only be applied in those patients presenting symptoms related to early infections (such as syphilitic ulcers, condiloma lata or lesions of congenital infection) since in later stages of disease *T. pallidum* invades tissues and organs so that it cannot be directly identified.

Direct Diagnosis *T. pallidum* cannot be grown efficiently for diagnostic purposes in vitro and consequently the laboratory diagnosis of syphilis can be obtained essentially by methods that are based on microscopy, immune-histochemistry, or nucleic acid amplification (PCR). Microscopy has been used for direct detection since one century

now and this method is nowadays infrequently applied. It is of note that the dimension of *T. pallidum* cells is below the limit of detection of direct light microscopy (0.2 μm): this fact makes necessary the use of different microscopy techniques such as dark field (DF) or direct immunofluorescence (DFA-TP) to observe the spirochetes in biological samples from fresh cutaneous or mucosal lesions. The most recent European guidelines are strongly against the use of DFA-TP in the diagnosis of syphilis and the reagent is no more available [2]. PCR based methods are becoming more widely used since the last decade even if no test have received an international approval to be used for the diagnosis of syphilis and species-specific or subspecies specific *T. pallidum* PCR tests remain primary in research laboratories [3]. The most relevant limiting issue about the use of nucleic acid amplification technologies for *T. pallidum* identification still remains the uncertainty about the level of target gene conservation among the wild strain of this spirochete and the limited verification of the analytical parameters (sensitivity and specificity) of these methods. Table 7.1 summarizes the most relevant characteristics of the direct diagnostic methods for syphilis.

Table 7.1 This tables summarizes the most relevant features of some direct techniques for the identification of *T. pallidum* in clinical specimens

Technique	Biological sample	Major advantages	Major disadvantages
PCR (nucleic acid amplification techniques—NAAT)	Fluid or tissue biopsy from skin and/or mucosal lesions (not verified on blood or cerebrospinal fluid)	1. Possible use for oral or rectal ulcers	1. No international standardization 2. Negative result does not rule out the diagnosis 3. Laboratory preparedness and equipment/expertise
Immunohistochemistry	Paraffin embedded or formalin fixed biopsies	1. Samples can be shipped away or stored for long time 2. Pregnancy derived tissues 3. Retrospective evaluation of initially not syphilis suspected tissue	1. Negative results do not rule out syphilis 2. Pathology specialized laboratory settings required 3. Subjective interpretation/ high level of training for the correct evaluation of the slides
DFA-TP	Fluids from chancres or erosive cutaneous lesions from primary, secondary of congenital syphilis	1. Can be used for oral mucosa lesions 2. Quite high specificity (depending on the fluorescent anti-*T. pallidum* antibodies) 3. Samples can be shipped away or stored for long time	1. Negative results do not rule out syphilis 2. Anti-*T. pallidum* fluorescence conjugated antibodies no more commercially available. 3. Subjective interpretation/ high level of training for the correct evaluation of the slides
DF microscopy	Freshly (<20 min) collected fluids from chancres or erosive cutaneous lesions from primary, secondary of congenital syphilis	1. On-site (medical office) diagnostic if the specific spiral shaped motile bacteria are seen	1. About 30% false negative (low sensitivity) 2. Not to be used on oral or rectal specimens (presence or not pathogenic spirochetes) 3. Specific expertise needed 4. Subjective interpretation/ high level of training for the correct evaluation of the slides

The preferred type of samples and the most relevant advantages and disadvantages are shown in the columns

Serological Diagnosis Given the above summarized limitation of the method to direct identify *T. pallidum* in syphilis patients, the most relevant role in the laboratory diagnosis of this STD is played by serological testing. This approach can also be used to diagnose asymptomatic subjects and these tests are usually categorized into two main groups: non-treponemal test (NTTs) and treponemal test (TTs).

NTTs These methods identify the immune response (IgM and IgG) that raises during syphilis in response to lipoidal substances (mainly cardiolipin) released from the spirochete and dying host cells. The most used techniques include "rapid plasma regains—RPR" (Fig. 7.1) "toluidine red unheated serum test—TRUST," and "Venereal Disease Research Laboratory—VDRL": all of these tests are based on flocculation that demonstrates the serum antibodies against a suspension of lecithin (phosphatidylcholine and phosphatidylethanolamine), cholesterol, and cardiolipin. The appearance of these antibodies is generally after 2 weeks from the onset of the primary lesion(s) so that about 25–30% of the primary syphilis are initially tested negative [4]. A common feature of the NTTs is simplicity coupled with a low cost: unfortunately all of these methods are mainly manually performed and their interpretation is largely subjective. Recently [5] a review of their use in the diagnosis of syphilis stated that these methods are not fulfilling the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust and deliverable to those who need them) criteria for the methods that can be used at a point of care (POC). In the absence of specific therapy for *T. pallidum*, the anti-lipoidal antibody usually peaks at 2 week post onset and remains positive for long time even in the late disease. When patients are treated, a different antibodies behavior is generally present: the titers decline until negativity within 6 months, even if about 20% of infected individual show a persistence of this response [6]. This condition is referred as "serofast status" and is more commonly observed in patients that receive therapy for late rather than early infection. Biological false positive ratio of NTTs is ranging from 2 to 5% and it is mainly caused by acute intercurrent factors such as acute febrile status, immunization, or even pregnancy or more chronic conditions (autoimmune diseases, hepatitis C, and leprosy) [7]. On the other hand, also false negative results are expectable, mainly due to prozone effect.

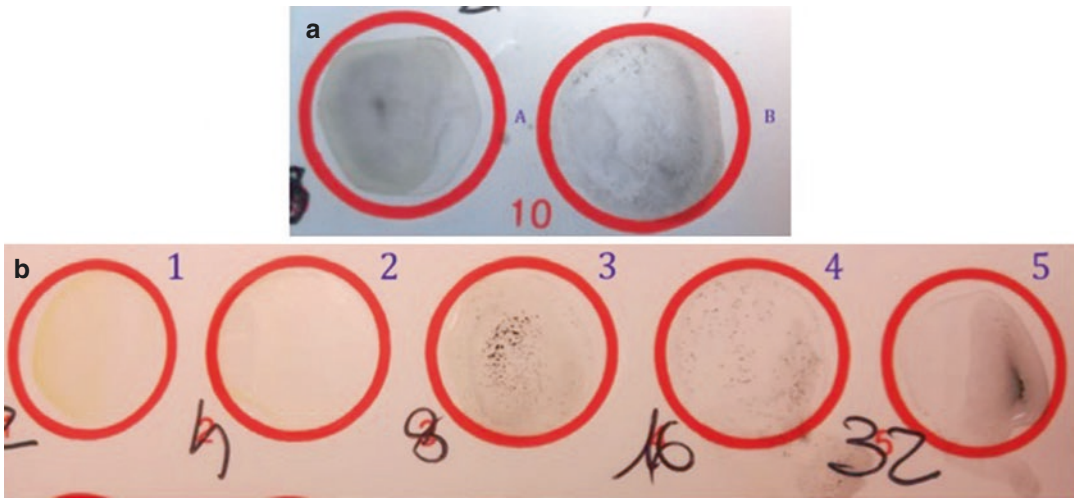


Fig. 7.1 RPR test for the detection of non-treponemal antibodies for the laboratory diagnosis of syphilis. Panel (**a**): well A (right) contains a negative control serum, well (**b**) (left) contains a negative serum specimen. The absence of flocculation is evident in both wells. Panel B: the wells from 1 to 5 contain a serial (from 1:2 to 1:32) dilution of a RPR positive serum. Wells 1 and 2 do not contain the RPR reactive, while the presence of flocculation is evident in wells 3 and 4, being absent in well 5. The interpretation of this result is: RPR titer of 1:16

TTs These methods are used to detect anti-*T. pallidum* antibodies and are more specific than NTTs. An important feature that distinguishes TTs from NTTs is also the life-long persistency of the antibody response detected by TTs that consequently cannot be used to take apart active and previously treated infections. In addition TTs are not expected to be useful to determine the efficacy of antibiotic therapy for syphilis. This category of test usually turns to positive results after 6–14 days after the primary lesions and thus TTs could be used to define the diagnosis in those patients that gave a negative NTTs result in early syphilis. In general, TTs are also used as confirmatory test for the NTTs results, thanks to their higher specificity. The use of TTs out of the laboratory setting is quite uncommon since these methods require specific laboratory equipment. The list of TTs includes the fluorescence based "treponemal antibody absorbed-FTA-ABS" that could be used either for IgM and IgG detection and the series of technical variants of the microhemagglutination assay (MHA-TP, TPHA, TTPA) (Fig. 7.2). In the last decade many TTs based on the use of specific *T. pallidum* recombinant antigens have been proposed: the main three technical variants of these newer methods are enzyme immune assay (EIA), immunoblotting (IB) (Fig. 7.3), and chemiluminescence (CLIA) [8, 9]. All of these techniques are largely automatable and not susceptible to subjective interpretation since most are performed on automatic instrumentations [10]. In the Western countries a large proportion of large laboratories have adapted the newer TTs as screening methods for syphilis: this approach has been defined as the "reverse algorithm" since the results obtained with EIA and/or CLIA are further evaluated and confirmed by NTTs. See Fig. 7.4 for details about the two (traditional and reverse) algorithms. These two ways to approach the issue of laboratory diagnosis of syphilis are theoretically similar and should provide overall identical results. On the practical stand point, the reverse algorithm is likely to identify those patients with early stage disease (TT-positive and NTT-negative) that could be missed by using the traditional way.

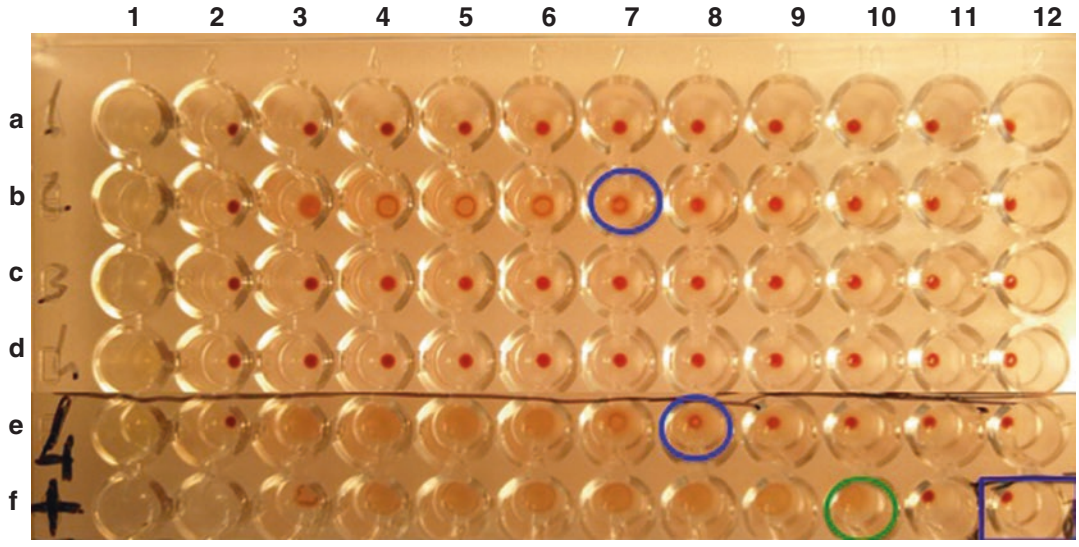


Fig. 7.2 TPHA test for the detection of treponemal antibodies for the laboratory diagnosis of syphilis. Rows (horizontally) from A to F contain: A-E serum samples, F positive control. Lanes (vertically) from 1 to 12 contain: 1 and 2 chicken red blood cell suspension (1 coated with *T. pallidum* antigens and 2 not coated) only; 3–12 contain chicken red blood cells coated with *T. pallidum* antigens and serum specimens diluted from 1:40 (lane 3) up to 1:20480 (lane 12). Samples in rows A, C, and D are negative, sample in row B has 1:640 titer (blue circle), sample in row E has 1:1280 titer (blue circle). The titer of positive control (row F) is 1:5120 (green circle). The black rectangle (row F, lane 12) shows the negative control. The evaluation of the test is performed by checking the absence of a solid red blood cell button at the bottom of each well

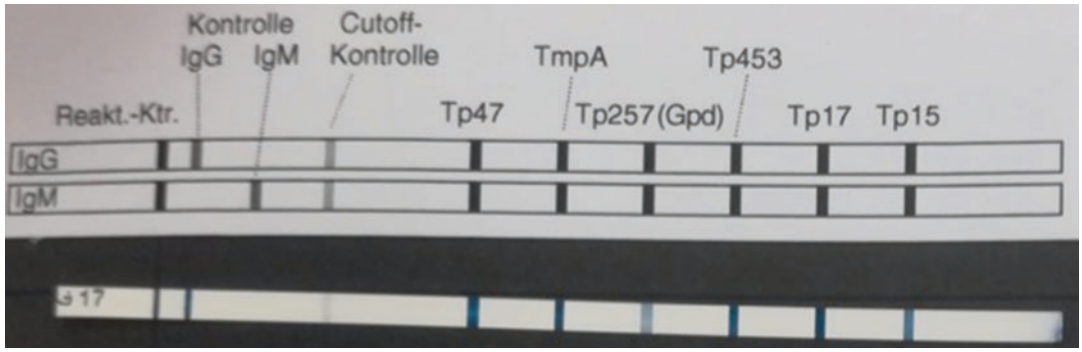


Fig. 7.3 Immunoblotting test for the detection of IgG antobodies against *T. pallidum*. The white background panel contains the position of the relevant antigenic lines for IgG (upper) and IgM (lower) immune responses. The black background panel shows the IgG reactive lanes identified by a positive serum sample. Note that the interpretation of the test is based on a score system that considers the positivity and the intensity of each single recombinant antigens identified

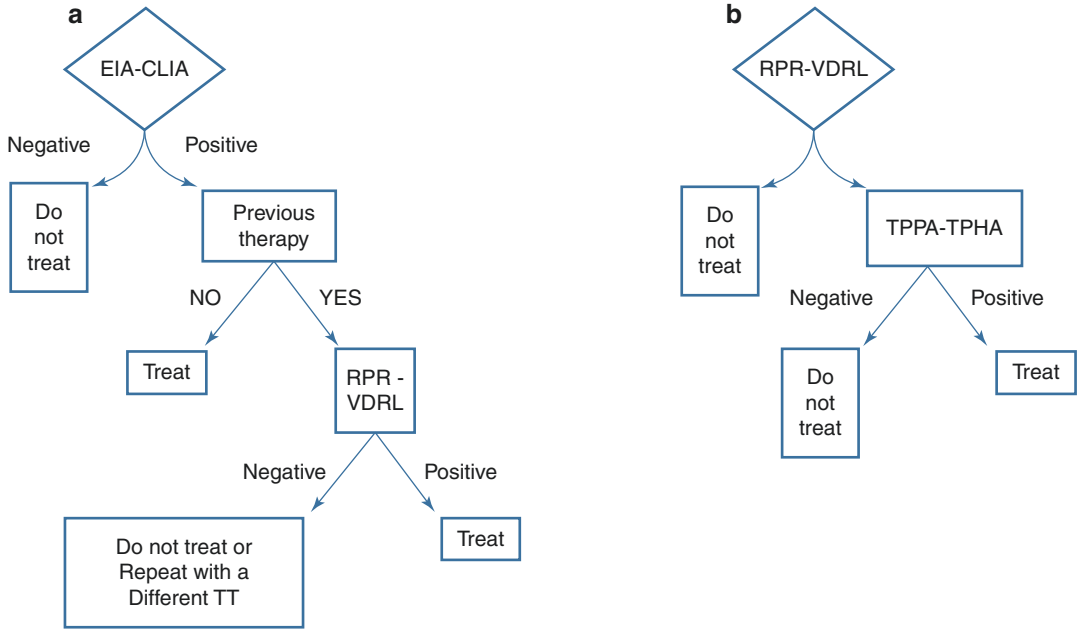


Fig. 7.4 Parts (**a**) and (**b**) show the "Reverse" and the "Traditional" algorithms for the diagnosis of syphilis, respectively

POCTs In addition to the above described TTs and NTTs, in the last years a series of simplified, rapid, and inexpensive methods have been developed to perform the diagnosis of syphilis in setting with limited laboratory capacity: the so-called point of care TTs (POC-TTs). These tests are mainly using the immune-chromatographic technology and require a small amount of whole blood (finger-prick) to be performed. Additional features that makes these tests widely usable in the low resources setting are: low price, room temperature storage, minimal training requirement, and turnaround time (TAT) faster than 30 min (that means the patient should site and

waiting to be properly and immediately treated). The POC-TTs of course share with the "normal TTs" the characteristics of being unable to distinguish between recent and previously treated infections so that the risk of overtreatment should be considered but, given the low-income setting in which these POC-TTs are widely used, this risk is indeed quite low. The very fast TAT of these methods is particularly relevant for those patients that need to get treated as fast as possible such as the pregnant women. In the case of non-pregnant population with POC-TTs positive result the general recommendation is to treat the subject with no prior history of anti-*T. pallidum* therapy and to have a NTT performed in those previously treated. More recently POC-TTs have evolved with the possibility to combine in only one diagnostic device the detection of non-treponemal and treponemal antibodies and to test also for HIV immune response: these combined tests hold a large expectation for the eradication program for STD worldwide [10]. The main features of NTTs, TTs, and POC-TTs are summarized in Table 7.2.

Table 7.2 This table contains the main features of non-treponemal tests (NTTs), treponemal tests (TTs), and point of care treponemal tests (POC-TTs) currently used for the indirect diagnosis of syphilis

Technique	Biological sample	Major advantages	Major disadvantages
NTTs			
VDRL	Serum, plasma, CSF	• Possible use as test to monitor the efficacy of therapy • Only test for neurosyphilis on CSF • TAT <15 min • Sensitivity 71–100% • Specificity 98%	• False positive for acute and chronic conditions • Manual • Interpretation subjective • Microscope needed • Necessary to make fresh antigen suspension • Cannot be used on whole blood
RPR or TRUST	Serum or plasma	• Possible use as test to monitor the efficacy of therapy • Less expensive than VDRL • No microscope needed • Antigen commercially available • TAT <15 min • Sensitivity 73–100% (depending on infection stage) • Specificity 98%	• False positive for acute and chronic conditions or dusty environment • False negative (early syphilis or prozone effect) • Manual • Interpretation subjective • Microscope needed • Cards only disposable • Cannot be used on whole blood
TTs			
FTA-ABS	Serum, plasma, CSF	• It has been the diagnostic reference in middle- and low-income countries • Sensitivity 96–100% • Specificity 99%	• Not recommended for resolution of discrepancies between TTs and NTTs results • Time consuming and high training need • Microscope needed • Manual • Possible biological false positive results when underlying autoimmune diseases
TPPA	Serum, plasma	• Inexpensive, large availability • Sensitivity 82–100% • Specificity 99%	• Manual • Subjective interpretation
TPHA and MHA-TP	Serum, plasma	• Inexpensive, less available than TPPA • Sensitivity 82–100% • Specificity 99%	• Manual • Subjective interpretation

(continued)

Table 7.2 (continued)

Technique	Biological sample	Major advantages	Major disadvantages
EIA	Serum	• Can be automated • High throughput • Screening of asymptomatic and blood donors populations • Sensitivity 82–100% • Specificity 99%	• More expensive than NTTs and MHA-TP • Instrumentation needed • IgM detection with low sensitivity in active disease • Not useful for staging or monitoring therapy
CLIA	Serum	• Can be automated • High throughput • Screening of asymptomatic and blood donors populations • Sensitivity 82–100% • Specificity 99%	• Same as EIA but more expensive
POC-TTs			
TT	Whole blood, plasma, serum	• Can be used for a rapid diagnosis "while you wait" • TAT < 20 min • Quite inexpensive • Sensitivity 86% (84% serum, 80% whole blood)	• Cannot distinguish new and treated syphilis
Dual TT and NTT	Whole blood, plasma, serum	• Can be used for a rapid diagnosis "while you wait" • TAT < 20 min • Can distinguish new and treated syphilis • Overall diagnostic performance ranging from 87% to 89%	• More expensive that traditional TTs
Dual syphilis and HIV	Whole blood, plasma, serum	• Can detect HIV and syphilis simultaneously • HIV results 98% in agreement with results of laboratory based tests • Syphilis results 85% in agreement with results of laboratory based tests	• Lower diagnostic performance for syphilis than HIV

CSF cerebrospinal fluid
The preferred type of samples and the most relevant advantages and disadvantages are shown in the columns

7.2 Special Testing Conditions

Neurosyphilis The diagnosis of neurosyphilis requires large laboratory experience and still remains a challenging procedure. The reference test is currently VDRL performed on CSF. Many other TTs, NTTs, and NATT tests have been proposed so far but there is still the need for additional study in order to state the clinical value of their results.

Congenital Syphilis The diagnostic methods for congenital syphilis are not completely fitting into the clinical needs: one major point that must be taken into consideration when facing with the neonatal diagnosis of *T. pallidum* is the transfer of maternal Igg to the fetus during the last weeks of pregnancy. This fact hampers the use of serological tests in newborns until about 6 months. A possible solution is to search for *T. pallidum* specific IgM response by using IB but the data so far available are still partially conclusive and the IgM detection is not recommended by all the guidelines.

Screening (Antenatal and Blood Donations) The availability of highly automated TTs made, since the last decade, possible to screen for

T. pallidum antibodies large populations, including pregnant women and blood donors. Of course the main purpose of screening is different in these two groups: in pregnant women the main goal is the earliest possible identification of pregnancy developing in not treated syphilitic women (to avoid the severe consequences of congenital syphilis), while blood donors must be screened to ensure the biological safety of the donations. Most of the screening strategies applied in Europe are based on the use of EIA or CLIA tests.

7.2.1 *Chlamydia trachomatis* Infection

C. trachomatis is a unique intracellular microorganism that has a biological cycle sustained by two different forms: the reticulate (RB) and the elementary (EB) bodies (Fig. 7.5). EB are the infecting form of *C. trachomatis* that enter the host cells and develop into the RBs. The RBs are capable to multiply inside cytoplasmic inclusions that at the end of the cycle provoke the rupture of the host cell with the release of newly generated infecting EBs that could start a new infection cycle [11]. *C. trachomatis* most frequently infects the lower urogenital tract in male and female thus generating different clinical syndromes, such as urethritis, cervicitis, and pelvic inflammatory disease (PID) in women or rectal and pharyngeal infection particularly in men who have sex with men (MSM) The most recent issued guidelines for the management of *C. trachomatis* infections have been issued in 2014 by the CDC of USA [12] and in Europe in 2015 [13].

Direct Diagnosis In the late 1970s, after many years during which the only way to grow Chlamydiae was the inoculation in embryonated hen eggs, several technical variants of in vitro cells cultures have been developed to isolate *C. trachomatis* strains from genital secretions (most from urethral in males and cervical secretion in females). Of course those techniques were highly labor intensive, required the constant availability of fresh in vitro cells culture (McCoy, HeLa229, or BGMK clones) and were quite long (due to the above described development cycle of Chlamydiae the TAT was at least 72 h after inoculum). A major improvement was achieved at the beginning of the 1980s when fluorescent conjugated *C. trachomatis* specific monoclonal antibodies against the major outer membrane protein—(MOMP and the LPS) become available and the direct fluorescent assay on genital smears was set as the new gold standard for the diagnosis of genital chlamydia infections. These monoclonal antibodies have

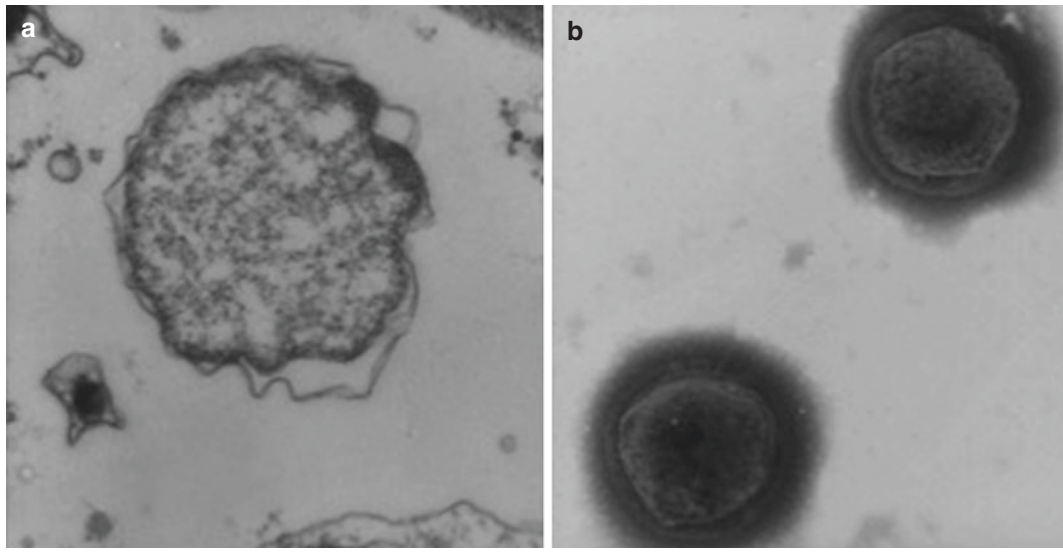


Fig. 7.5 Electron microscopy image of *Chlamydia trachomatis* reticulate body (**a**) and elementary body (**b**)

also been used to stain the intracytoplasmatic inclusion that develops after in vitro inoculation: so far the use of Giemsa or iodine stains is no more acceptable due to low specificity and sensitivity [12]. Since the last 15 years many different NAAT tests have been developed and made commercially available: all of these techniques identify *C. trachomatis* specific RNA or DNA in different clinical specimens and are nowadays recommended for routine diagnostics due to their superior sensitivity and specificity and the fast (less than 3 h) TAT [14]. Since the start of the routine use of NAATs for detection of Chlamydiae about 10–30% more samples gave positive results in comparison to culture. In general there is no proven time frame for the minimum interval between the beginning of *C. trachomatis* infection and the NAAT detectability but it is generally hypothesized that this interval could be 1–3 days since acquiring the infection. The precise following of the manufacturers' indication is crucial to obtain the best diagnostic performance from NAATs: this includes collection, transportation, and storage of samples, using of ICQ at the prescribed time interval, and participation to EQA schemes. Since the validated NAATs are highly specific and the risk of losing low positive results in repeated testing, the confirmatory testing of positive specimens is not recommended [15]. It is also of note that many commercially available tests can simultaneously detect *C. trachomatis* and *N. gonorrhoeae.* The most widely used biological sample for NAAT testing are urine in male and vulvo-vaginal swabs in females instead of the more invasively collected urethral or cervical secretions. In both genders, anorectal swabs are acceptable. The overall diagnostic performance of "home brewed" differs from that of commercial NAATs and consequently the use of test approved by regulatory authorities (FDA in the USA and CE-IVD marked in the EU). As far as the sensitivity of NAATs is concerned, it must be considered that many different substances could interfere with the NAATs performance: as a consequence adequate extraction, amplification, and internal controls must be used for each individual run according to the instruction of the manufacturer. Since a new variant of *C. trachomatis* (with 377 bp deletion in the cryptic plasmid that was used a NAAT target) has been reported in Sweden in 2006 only tests that are certified capable to detect this variant can be accepted for the routine diagnosis of *C. trachomatis*. Because anorectal infection by the LGV (lymphogranuloma venereum) serovars requires a different clinical management in comparison to non-LGV infections, it is of outmost relevance to specifically test for LGV in all the MSM who report receptive anal sex in the 6 months preceding the anorectal infection. In detail, LGV is a condition caused by three invasive serovars of *C. trachomatis*, named L1, L2, and L3. These strains are capable to generate an invasive infection after the appearance of genital ulcers and papules with femoral lymphadenopathy and inguinal tender [16].

Diagnosis of LGV The laboratory diagnosis of LGV is relevant for two main reasons: first to prevent long-term complications and second to stop the transmission to sex partners. In addition the exact definition of LGV related infection has relevant implication for the therapy that in these patients must be prolonged in comparison with non-LGV chlamydia infections [17]. In the past, the use of microimmunofluorescence or complement fixation tests have been proposed for the diagnosis of LGV, with cutoff titers >1:256 or >1:64, respectively. Nowadays these techniques are totally obsolete since their lack of standardization and clinical interpretation criteria. The use of NAAT is highly advised also for the diagnosis of LGV: most of these techniques are not capable to distinguish the LGV related serovars from the other strains of *C. trachomatis*, so that once positive results have been obtained by standard NAAT, additional specific testing is necessary to rule out the hypothesis of a LGV related disease. In other words, when a positive NAAT result is achieved by testing a lymph node biopsy from a suspected LGV patient, it is necessary to further evaluate the samples for the identification of LGV. This typing procedure is performed by genotype specific PCRs followed by restriction fragments length polymorphism (RFLP) or sequence analysis (target on the *omp-1* gene). An alternative target for LGV identification is the

pmpH gene: this sequence shows a characteristic deletion of 30 bp in all the LGV isolates. This techniques is not capable to specifically identify LGV individual genotypes but showed a high sensitivity, with about 25% of the not LGV serovars missed [16, 17].

The recommended first-choice specimens for *C. trachomatis* genital infection identification are first-void urine in male and (self-collected) vulvo-vaginal swabs for females [13]. First-void urine (up to 20 mL collected >1 h after previous micturition) is well accepted by the patients due to the easy of sampling. In female patients the sensitivity of first-void urine is lower than in males and consequently the use of vulvo-vaginal swabs is strongly advised: these samples could be collected by health-care providers or self-collected) with equal overall diagnostic performances. In the case of clinical evaluation, the collection of a cervical swab is also advised but the more recent data stated that these two types of swabs have equal sensitivity. Presently no commercially available NAATs method has been certified for extra-genital urinary specimens: in particular for rectal specimens the sensitivity and specificity of NAATs are lower when compared to urogenital specimens. NAATs can also detect *C. trachomatis* in semen with a good agreement with the results obtained in first-void urines (that is indeed easier to be collected): so testing semen is strongly discouraged.

Serological Diagnosis Serology has been proven not useful in the laboratory diagnosis of uncomplicated STD caused by *C. trachomatis*. In selected patients, only invasive infection by *C. trachomatis* could raise detectable level of specific immune response with a persistency of many years. Serology has no value in diagnosing uncomplicated lower genital tract infections and has a very limited value for ascending infections and for infertility work up—Neonatal pneumonias are the only *C. trachomatis* related infection that must be diagnosed by detecting the IgM specific immune response.

POCTs A recent review paper reported on the possibility to use POCT and the so-called near POCT for the diagnosis of *C. trachomatis* genital infections [18]. The overall concept about this diagnostic strategy is to achieve a rapid diagnosis ("while you wait") in the doctor office (or in low level of cure settings) with cheap device that demonstrates high sensitivity and specificity. In practice, the POCTs should fit in the above reported ASSURED criteria in order to be used in the diagnosis of *C. trachomatis* genital diseases. Most of the POCTs have been compared with NAATs as reference standard and the level of agreement shown is quite satisfactory: antigen detection rapid POCTs had a specificity ranging from 97 to 100% with a pooled sensitivity of 37% for vaginal swabs, 53% for cervical secretion, and 63% for urines. Among the best performer it is the aQcare Chlamydia TRF kit that uses a fluorescent nanoparticle based lateral flow technique. A "near POCT" test is the GeneXpert CT/NG from Cepheid: the simple technology (including very easy to use instrumentation) of the test allows its use on a near POCT setting. It is anyway necessary to underline that all the GeneXpert tests should be performed in a laboratory setting in order to ensure the best diagnostic performance. This method showed comparable sensitivity with that of NAATs for self-collected vaginal specimens, as well as for cervical secretion and urines. The specificity ranged from 99.4% to 99.9%. As a conclusion it must be underlined that most of the tests enlisted in the below Table 7.3 have been evaluated in small studies with a limited population and consequently the affordability of these techniques must be additionally verified [19]. Table 7.3 summarizes some features of the most recent POCT and near POCT tests for *C. trachomatis*.

7.2.2 Gonorrhea

The last estimated number of cases of gonorrhea in the world is about 78 million, with the highest prevalence in the Western Pacific Area: globally the largest diffusion is among countries with mid to low income [19]. If this infection remains untreated, severe consequences can arise for the patients, including PID, ectopic pregnancy, infer-

Table 7.3 Some features of the most recent point of care tests (POCT) and near POCT tests for the diagnosis of *C. trachomatis* genital infections are summarized, including: manufacturer and test identification, principle of technology used, turnaround time (TAT), and storage conditions

Technique	Manufacturer	Test technology	TAT (in minutes)	Storage temperature
POCTs for antigen detection				
ACON Chlamydia and NG	ACON (China)	LF	<30	RT
aQcare Chlamydia TRF	Medisensor (RK)	LF with fluorescent nanoparticles	15	RT
BioRapid	Biokit (E)	LF	20	RT
BioStar Chlamydia	Inverness Medical (USA)	Optical immunoassay	<30	RT
Chlamydia RT	DRW (UK)	LF	25	RT
ClearView	Alere (USA	LF	30	2–8 °C
Chlamydia test card	Ultimed (G)	LF	10	RT
HandiLab-C	HandiLab (USA)	Enzyme detection	<15	RT
QuickView	Quidel (USA)	LF	15	RT
Near POCT				
GeneXpert CT/NG	Cepheid (USA)	RT PCR	<90	RT

LF later flow immunochromatography, *RT* room temperature

tility, increased risk to acquire HIV. In addition, since the last years the increased number of multi-drug resistant gonococci poses additional threats to the public health [20], raising the need not only for a fast and accurate laboratory diagnosis of gonorrhea, but also imposing the requirement to identify all the *N. gonorrhoeae* strains with reduced sensitivity to antibiotics.

Direct Diagnosis The basic step for the direct diagnosis of gonorrhea is the microscopic evaluation of a gram stained smear obtained from the male urethra during acute stage of *N. gonorrhoeae* genital infection (Fig. 7.6). The large presence of polymorphonuclear leukocytes with intracellular gram negative diplococcic is strongly indicative of gonorrhea. Of course this approach cannot be used to rule out the diagnosis when the smear is negative. Gram stain is also not sufficient to make a diagnostic statement of gonorrhea in cervical female secretion and in rectal or pharyngeal specimens. In the case of multiple testing from one single anatomical site sample, the first that must be performed is under any circumstance the culture for gonococcus. It is also important to underline that genital secretions must not be collected by using wood shafts swans and cotton tips that could be inhibiting for the growth of the germs. The sampling procedure for male urethra must be done with the insertion of the swab tip for at least 2–3 cm in the lumen; in the case of cervical sampling, the insertion into the endocervical canal should be for 1–2 cm followed by a series of 2–3 rotations. Due to the fastidious nature of gonococcus, the specimens must be inserted immediately upon collection in non-nutritive transport media that ensure the vitality of the microbes. Culture transport media

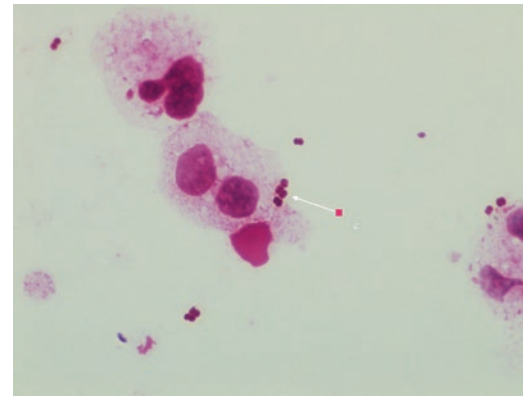


Fig. 7.6 Gram staining of a smear prepared from the urethral secretion of a man suffering from acute gonorrhea. The presence of several gram negative diplococci outside and inside (as pointed by the white arrow) the polymorphonuclear cells is shown

are largely preferable since the higher capability to keep the optimal metabolic condition for bacterial growth since the very beginning: it is mandatory to place the inoculated media under CO_2-enriched condition for transportation and culture. The strict adhesion to the above rules is the only way to achieve an optimal recovery rate for gonococcus: in particular the highest rate of positivity is reached when specimens are directly inoculated in medium incubated under increased atmosphere of CO_2 within the shortest possible time. For the growth of gonococci, samples are streaked on selective medium (like the Thayer-Martin) when obtained from non-sterile anatomical sites or on non-selective plates (chocolate agar) when collected from sterile sites. All the growth media for *N. gonorrhoeae* must be "chocolatized" (heated equine or bovine blood) (Fig. 7.7). To make the growth media selective many different antibiotics could be added, including vancomycin, colistin, and nystatin). The incubation periods should be 24 and 48 h at 36 °C under 5% CO_2. The presumptive identification of gonococcus isolates is made in the presence of oxidase positive gram negative diplococci. The definitive identification is mandatory since the presence of non-pathogenic bacteria could mime the presence of gonococci: this could be obtained by biochemical and serological testing and/or by MALDI-ToF or specific PCR. In general the culture for gonococcus is moderately expensive and could provide extremely relevant data about the antimicrobial susceptibility of individual isolates. Since cephalosporins are the unique class of antimicrobial recommended for the treatment of gonorrhea, the local capability to evaluate the susceptibility of individual *N. gonorrhoeae* isolates to this class of drug is of outmost relevance in the case of treatment failure.

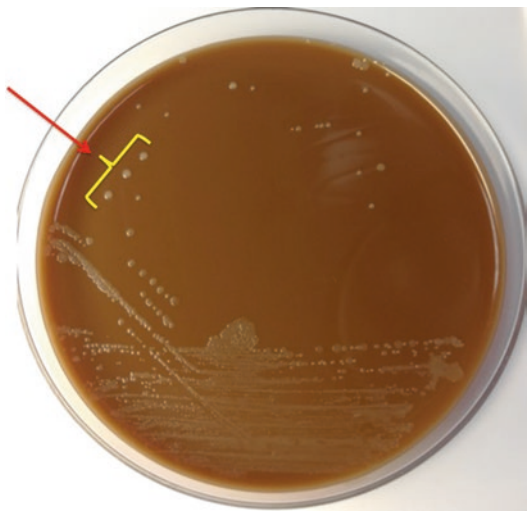


Fig. 7.7 *Neisseria gonorrhoeae* grown on Thayer-Martin agar. The red arrow indicates a series of three isolated colonies showing the typical round "creamy" white translucent morphology

Since the last 5 years many NAATs are available on the market for identification of gonococcus: the large majority of these methods are capable to contemporarily identify also *C. trachomatis*. The tests can be used for the diagnosis of both symptomatic and asymptomatic males and females. A (of course not complete) list of the most widely used FDA cleared and/or CE-IVD marked test is the following: Abbott RT m2000 CT/NG, BD ProbeTec ET and QX, GeneXpert CT/NG Cepheid, Aptima CT/NG Hologic, Amplicor and Cobas CT/NG Roche, Anyplex II STI-7 Seegene. Of course NAATs are capable to generate inaccurate results and laboratories must take all the required measures to minimize this inconvenient, including the use of appropriate controls and participation to EQA schemes. In any way, the diagnostic performance of NAATs is well above to that of cultures due to the superior and expanded sensitivity (that in part derives from the lack of necessity of metabolically active bacteria to achieve positive results): overall NAATs can detect *N. gonorrhoeae* with a sensitivity above 90% and a specificity of >99%. The specimen categories acceptable for NAATs detection of *N. gonorrhoeae* are similar to those enlisted above for *C. trachomatis*. It must be clearly underlined that in general no test of cure is required after a therapy administered following a positive NAAT result for *N. gonorrhoeae* (and *C. trachomatis*), since the residual NAs from germs weakened or killed by the antimicrobials could generate a strong positive NAAT result without any clinical relevance in term of efficacy of the treatment. All of the above methods differ one from another for technical details (type of

nucleic acid amplification and detection of the amplified sequences) and for the targets genes: for a detailed description of each technology and for the list of validated specimens (that is out of the scope of this chapter) please refer to Papp et al. [12]. An additional issue is the use of molecular test for the detection of antimicrobial resistance in *N. gonorrhoeae*. As above reported, the number of gonococcus isolates with reduced sensitivity to antibiotic is increasing worldwide and since the large and constantly increased use of NAATs for the diagnosis of *gonorrhoeae* it is becoming more and more uncommon to perform the AST on isolated strains. In addition, the detection of specific resistance gene is not a common feature for the NAATs. In principle, an ideal NAAT for the detection of antibiotic resistance in gonococcus should be in complete agreement with the MICs of the diverse molecules tested in vitro. Unfortunately, the phenotypic resistance gonococcus is generated by multiple genes with a wide possibility of single nucleotide (SNPs) mutations that make difficult to standardize the NAAT. In other words, different SNPs can generate the same phenotypic resistance pattern but with difference in the genetic sequences. For a detailed list of the genes involved in antimicrobial resistance of *N. gonorrhoeae* and for the most relevant requirement of the NAAT to detect the sequences, please refer to Low and Unemo [21].

Serological Diagnosis Serology has been proven not useful in the laboratory diagnosis of gonorrhea.

POCTs As above reported for *C. trachomatis*, also in the case of gonorrhea the diagnosis can be achieved by using POCTs and "near POCTs". The use of POCTs is particular relevant in mid- to low-income settings where laboratory services are less frequently available for large population. Also in the case of gonococcus, the POCTs must fit in the set of ASSURED criteria in order to provide reliable results. Traditionally the POCTs for the detection of gonococcus.

Specific antigens suffer from low sensitivity and alternative techniques are currently under development. At present the evaluation of POCTs for gonorrhea is generally deriving from a limited number of small studies. The only "near POCT" method that has been quite extensively evaluated is the GeneXpert CT/NG from Cepheid: this assay has unfortunately the requirement for a specific (even if small and very simple to be used) instrumentation and has a TAT of about 90 minutes. For detailed description of the currently existing POCTs for *N. gonorrhoeae* please refer to Guy et al. [22].

7.3 Trichomoniasis

Trichomonas vaginalis is a protozoan parasite that causes trichomoniasis, the most diffuse non-viral STD worldwide with an estimated number of cases of about 180 million [23]. In men the infection by *T. vaginalis* is frequently asymptomatic with some patient suffering from dysuria and urethral discharge. In women the disease is more clinically evident (50% asymptomatic) with polymorphic manifestations and potentially severe sequelae: purulent vaginal discharge, with pain and local inflammatory signs, infertility, premature rupture of the membranes in pregnancy, low birth weight infants, and neonatal death [24]. Recently, trichomoniasis has been identified as a risk factor for HIV infection [25].

Direct Diagnosis Since the first identification in 1836 *T. vaginalis* has been mainly seen under the microscope in genital fluids. The so-called wet mount test" (a technique of microscopic visualization of freshly—few minutes—harvested vaginal secretions diluted in saline) has been for a long time the reference diagnostic method even with quite a low sensitivity (from 38% to 82% in symptomatic females) that is largely dependent on the interval between the sampling and the observation. If pear shaped motile trichomonads are observed the diagnosis could be placed with 100% specificity. In order to achieve a sufficient sensitivity at least 10^4 protozoa/mL must be present in the sample. Trichomoniasis can also be diagnosed during a Pap test, but in this case sensitivity and specificity are lower. As an alternative

strategy, *T. vaginalis* can be cultured since at least two major media formulation can sustain the propagation of this protozoan. The Diamond's modified medium and the InPuoch device are both used even if the second one holds the advantage to allow the direct microscopic observation to ensure the growth of the parasites. Unfortunately the incubation time is long (at least 4 and up to 7 days) and a daily check is necessary to evaluate the growth thus making the culture a labor intensive and time requiring process. The long incubation at 37 °C is also generating with elevated frequency the overgrowth of bacteria from the vaginal flora, since the addition of antibiotic is seldom enough to block superimposed bacterial growth. Vaginal swabs for culture could be either self-collected or by heath care provider, showing no diagnostic differences. An alternative to growth *T. vaginalis* in synthetic media is the inoculation in cells monolayers that is more sensitive (limit of detection about 3 protozoa/mL): regrettably this is a very complicated, labor intensive, and expensive approach that is not used in routine laboratories. Nowadays, the most widely and reliably used diagnostic method for trichomoniasis are NAATs: all the commercially available tests have high sensitivity (above 98%) and specificity (90%). Most of the NAATs used for *T. vaginalis* are multiplex test that can also detect other STD pathogens such as *C. trachomatis*, *N. gonorrhoeae*, and genital mycoplasmas. The TAT of these methods ranges from 60 to 120 min and the target differs from one to another: the first test FDA cleared and CE-IVD marked for the diagnosis of trichomoniasis in women was the APTIMA TV (Hologic) that is based on TMA technology with a sensitivity ranging from 74% to 98% and specificity between 87% and 98% (these data are depending on the comparative method used and on the specimens tested). Of course, as for all of the NAATs, the presence of viable protozoa is not necessary and consequently the handling of the samples is much simpler than for microscopy and culture. The APTIMA test requires a complex and quite expensive instrumentation and has a TAT of about 3 h. Nowadays many other newer tests are available: all of these latest methods are validated for use in both symptomatic and asymptomatic females with a large panel of usable specimens including urines, urethral and vaginal swabs, and endocervical samples. A recent study performed on more than 3000 women and 2500 men [26] showed an increased detection rate for NAAT in comparison to wet mount of 1.3, thus suggesting that NAATs must be selected in order to ameliorate the clinical and epidemiological management of trichomoniasis. By the way the only NAAT that has been cleared for use with men specimens is the Cepheid GeneXpert. Other laboratory based NAATs include Solana TV assay (Quidel) that is based on isothermal-helicase dependent amplification (HAD), GeneXpert (Cepheid) that for its simplicity is also included in the "near POCT" list, and the AmpliVue (Quidel) that uses the same HAD technology as the Solana with a different platform.

Serological Diagnosis Serology has been proven not useful in the laboratory diagnosis of trichomoniasis.

POCTs The number of POCTs for the detection of *T. vaginalis* has increased in the last years and nowadays many of these techniques are used in routine laboratories. As discussed for the other infections, the POCTs must meet the ASSURED criteria: the first POCT for *T. vaginalis* that filled this condition has been the OSOM *Trichomonas* test (Sekisui Diagnostics) with a sensitivity ranging from 83.3% to 90% and a specificity between 98.8% and 100%. The TAT is only 10 min and the basic technology is the immunochromatography capillary flow immune assay for the membrane proteins of the protozoan. The OSOM test has also been tested for self-performance at home by women after ordering a home *T. vaginalis* kit over the internet.

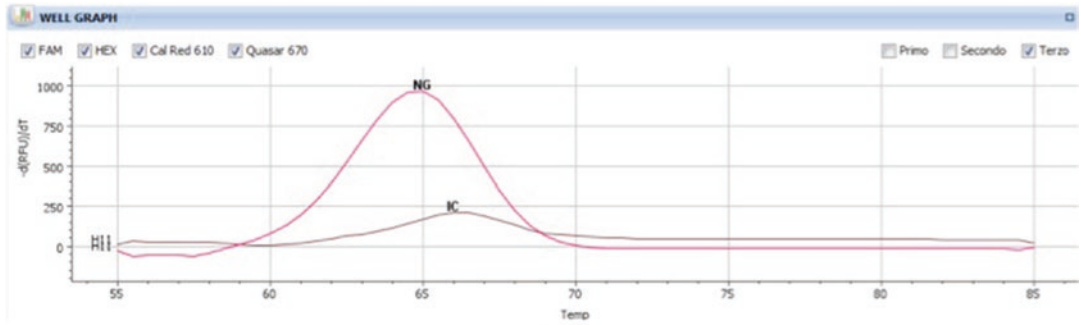
Infection by Genital Mycoplasma Bacteria of the genus *Mycoplasma* and *Ureaplasma* are widely diffuse and some of the species of these genuses have been since long time involved in STD: *M. hominis*, *U. parvum*, and *U. urealyticum* all have a potential to cause STD infections but their precise pathogenic role is under debate: *U. urealyticum* and *M. hominis* look like to play a

role in adverse pregnancy outcome since they can colonize the human genital tract and in the presence of host factors that may additionally lead to pregnancy adverse outcome [27]. The last identified (cultured after 50 days incubation from urethral exudates of two men suffering from non-gonococcal urethritis in 1981 as reported by [28]) member of this group of pathogens is *M. genitalium*. This section will discuss most of the diagnostic issues related to this emerging pathogen, not taking into detail the other above reported species.

Direct Diagnosis The role of *M. genitalium* in STDs is now well known: this *Mollicutes* is a fastidious intracellular obligate germ with a tiny genome of 580 kb that makes this bacterium the smallest prokaryote capable of autonomous replication [29]. Since 1991 the presence of papers describing molecular assays for the detection of *M. genitalium* has increased on a yearly bases, thus witnessing the interest for this pathogen and its diagnostic.

Given the extreme difficulty to grow *M. genitalium* in vitro, culture is not an option for the diagnosis. NAATs are indeed expected to play the major role for the laboratory identification of *M. genitalium*: two main groups are presently commercially available, targeted at DNA and RNA, respectively. Specific primers for *M. genitalium* are part of many different PCR based techniques for STDs detection, including the Anyplex II STI-7 Seegene (Fig. 7.8), the STDetect chip

a



b

Tipo	FAM		HEX		Cal Red 610		Quasa...	Quasa...	Interpretazione automat...
	UU	UP	MG	MH	NG	CT	TV	IC	
SAMPLE	-	-	-	-	-	-	-	++	-
SAMPLE	-	+++	-	-	-	-	-	++	UP(+++)
SAMPLE	-	-	-	-	-	-	-	++	-
SAMPLE	-	-	-	-	-	-	-	++	-
SAMPLE	-	-	-	-	+	-	-	++	NG(+)
SAMPLE	-	-	-	-	++	-	-	++	NG(++)
SAMPLE	-	-	-	-	-	-	-	++	-

Fig. 7.8 Panel (**a**) shows the amplification curve obtained with the Seegene Anyplex II STI-7 method for *Neisseria gonorrhoeae* (NG, upper red curve) and the Internal Control (IC, gray lower curve). The peak in the red curve indicates a positive result for gonococcus. Panel (**b**) shows the interpretation plot of the test: the cell in the row marked "UP" with +++ indicates a positive results for *Ureaplasma parvum* and the two cells in the row marked "NG" with + and ++, respectively, indicate two distinct samples positive for gonococcus. The row marked "IC" shows ++ in all the cells and this means that the amplification reaction was performed correctly. The last row "Interpretazione automat" summarizes the result obtained for each one of the 7 microbes for each sample tested: the sample in the second horizontal line is positive for *U. parvum* and the samples in the fifth and in the sixth line are positive for gonococcus, all the remaining samples are negative

LabGenomics (that uses a PCR microarray technology to identify 13 different STD pathogens), and the InvaderPlus LSI Medience Co. that detects *M. genitalium* (MG), *M. homins* (MH), *U. urealyticum* (UU), and *U. parvum* (UP). A simpler approach is the use of "closed devices" that include in one pouch all the required reagents and whose are automatically interpreted by a dedicated analysis software, such as the FilmArray STI BioFire. On the other side the *M. genitalium* kit from TiB MolBiol is just providing specific primers and probes and requires the NA extraction and amplification to be performed on an open platform. BioRad released a CE-IVD labeled test (BioRad Dx CT/NG/MG) that includes an internal control and must be performed manually for the NA extraction: this test can be used for testing vaginal, cervical, and urethral secretions and first-void urines from males. The same technology is available in the AmpliSens NG/CT/TV/MG from the Russian company InterLabScience. The Hyplex STD Mycoplasma assay from Amplex is also CE-IVD marked and detects NMG, MH, UU, and UP.

Two relevant features of ResistancePlus MG kit (a qPCR based method) are the possibility to quantitate the MG bacterial load and the identification of mutation in to the 23 S rRNA gene that encodes for macrolides resistance [30]. This characteristic is of particular importance since nowadays *M. gentalium* is likely the second bacterial STD worldwide after *C. trachomatis* and because the antimicrobial resistance of *M. genitalium* is arising year after year. In 1997, about 10% of the *M. genitalium* infection was susceptible to 1 g of azithromycin, while in 2009 the percentage of resistance was grown up to above 30% with some data that suggest that the resistance mutation would be selected in one over ten patients treated with this antibiotic regimen [31]. As the resistance to macrolides increased, a second-line therapy with moxifloxacin (10 days) was proposed, but in 2006 the first case of *M. genitalium* infection resistant to moxifloxacin was reported. Since nowadays *M. genitalium* is largely diagnosed in high-income setting due to the availability of highly efficient NAATs methods, a demand for the capability to identify those cases caused by multi-resistant strains is indeed raising. As far as the amplification of RNA is concerned, there is one test that is based on transcription-mediated amplification (TMA) for *M. genitalium*: the APTIMA kit from Hologic. This test is based on two different target genes (16S rRNA and MgPa) and has sensitivity of 88.8% and specificity of 100% as determined on male urine specimens. When applied to vaginal and cervical swabs these values decreased to 84% and 6%, respectively.

Serologic Diagnosis Many attempts to use serology for the diagnosis of genital infection by *M. genitalium* have been confounded by the antigenic variation and cross reactions with other (even non) pathogenic members of the genus (that could colonize human beings) such has *M. pneumoniae*. Presently no serological tests are used for the diagnosis of this infection.

Infection by *Haemophilus ducrey* (Chancroid) *H. ducrey* is a gram negative germ that causes a STD named chancroid. This infection is characterized by genital ulcerations accompanied by pain and that are persisting for several weeks if not appropriately treated with antimicrobials [32].

Recently *H. ducrey* has also been associated with skin ulcers of the legs and limbs in patients that have been living in Africa or in the Pacific region. The genital infection is acquired after a traumatic interruption of the epithelial layer and it is generally accepted that the minimal infecting load could be as low as 1 CFU. Since the late 1990s, when STD treatment was mainly switched to a syndromic approach, chancroid was the most diffuse type of genital ulceration in most tropical areas. Nowadays, *H. ducrey* related STD is substantially declined, but the laboratory diagnosis is still a challenging procedure [33].

Direct diagnosis. The most relevant biological sample for the laboratory diagnosis of chancroid is the material collected from the bottom of the ulcer obtained, when necessary, after the removal of the purulent exudate that covers the surface of the lesion. The time lapse between the collection and the inoculation of agar plates is a fundamental factor for the optimal isolation of

the pathogen, since *H. ducrey* can only survive for a few hours in the swabs. So the direct inoculation "at the bed side" is the preferred approach; as an alternative the use of Amies' medium with fast shipment to the laboratory can guarantee an acceptable performance. An alternative specimen, with lower sensitivity for culture based techniques, is the pus from bubo lesions (that sometimes represents the only possible sample when ulceration is absent). As per the normal culture of purulent material the sensitivity is largely sub optimal. At the microscopic examination *H. ducrey* frequently shows a picture resembling a "railroad track" but this feature is totally insufficient to make the diagnosis. The gold standard has been for many years the culture: the most widely used media include as the basic formulation both gonococcal and Mueller Hinton. The addition of Isovitalex (a nutrition supplement) and 1% hemoglobin plus 5% fetal calf serum or 5% chocolatized blood is required in order to maximize the capacity to sustain the growth. To prevent the over growth of gram positive germs the addition of vancomycin at 3 mg/L is advised. Plates must be incubated at 33–35 °C under microaerophilic or anaerobic atmosphere. Temperature should not exceed 35 °C in order to ensure the viability of *H. ducrey*. The identification of the suspected colonies is made by using the so-called clumping (these colonies can be pushed across the plate without modification of their shape) phenomenon and gram staining. The positivity of the oxidase test accompanied by a negative catalase reaction also allows a presumptive diagnosis. The use of commercial identification systems based on biochemical testing is hampered by the very fastidious growth characteristics of *H. ducrey*. The use of NAATs is nowadays becoming widely common for the diagnosis of chancroid: many PCR based techniques have been proposed with different sensitivity and specificity values. The most promising is a method that can be used to contemporary distinguish different bacterial causes of genital ulcers: *H. ducrey*, *T. pallidum*, and *Calymmatobacterium granulomatis* [34]. Some of these multiplex PCR techniques have been made commercially available (See gene STI Master Panel 5 tests) but the performance of these methods still need to be independently evaluated in highly endemic areas for chancroid.

Serologic Diagnosis The specific antibodies against *H. ducrey* usually rise only when the ulcerative lesion is evident and the identification of immune response against selected components (such as the LPS) or the whole bacterium did not prove to be effective to identify the cause of chancroid.

Donovanosis (Infection by *Calymmatobacterium granulomatis*) Donovanosis or "granuloma inguinale" is another type of genital ulcerative lesion that has the characteristic to bleed readily upon touch. The causative agent is *Calymmatobacterium granulomatis*, a gram negative pleiomorphic bacterium that has been recently propose to be re-classified in the genus *Klebsiella* due to the wide (99%) homology with *K. pneumoniae* and *K. rhinoscleromatis*. A later study reported lower level of homology with *Klebsiella* so the latest information proposed to leave the classification in a "fluid status" by now [35]. The infection is geographically limited to a selected area in the Latin America and the Caribbean, India, New Guinea, and southern Africa (KwaZulu-Natal).

Direct Diagnosis A typical feature of donovanosis is the observation inside histiocytes of typical vacuoles containing the causative bacteria: these cells are named "Donovan bodies" and their observation is pathognomonic of granuloma inguinale [36]. The isolation of *C. granulomatis* in synthetic media has not been obtained so far and the only way to grow the germ in vitro is by using a cell culture system: the most used are monocytes and the Hep-2 line. The sample to be used for the detection of Donovan bodies is principally the exudate from the bottom of the ulceration collected after a swab soft cleaning to remove the purulent material generated by over infecting germs. Alternatively a punch biopsy from the same area could be used after being crushed and spread to make a smear on a glass slide: the crushed biopsy has higher sensitivity

than the exudate smear. A simple Giemsa is the technique used to stain the smear: as an alternative the Leishman technique could be applied. The possible alternative to the identification of the Donovan bodies is represented by NAATs: The main target used for the NA amplification of *C. granulomatis* is the *phoE* gene and this technique has also refined by applying a colorimetric detection systems [37].

Serologic Diagnosis The detection of specific immune response against *C. granulomatis* has been performed by immunofluorescence using as an antigen tissue sections from infected individual suffering from well-developed granuloma inguinale lesions. Unfortunately the diagnostic accuracy of this test is so low that it cannot be used for diagnostic purposes [36].

Laboratory Diagnosis of Bacterial Vaginosis Bacterial vaginosis (BV) is among the most frequent microbiological syndrome in the population of women in their childbearing phase [38]. The basic condition that is found among women suffering from BV is a substantial change in their vaginal microbiota: the commonly predominant *Lactobacillus* spp. leave the place to a more complex and less characterized flora composed by several different bacterial species, including *Gardnerella* spp. (Fig. 7.9), *Prevotella* spp., *Atopobium* spp., *Peptostreptococcus* spp., *Mobiluncus* spp., *Sneathia* spp., *Leptotrichia* spp., and *Mycoplasma* spp. [39]. Nowadays, BV is generally considered as a form of dysbiosis and it is linked, even if BV is mostly an asymptomatic condition, to increased risk of adverse pregnancy outcome, pelvic inflammatory disease (PID), and to an amplified possibility to acquire other STDs such as *C. trachomatis*, herpes virus, *T. vaginalis*, and *N. gonorrhoeae*. BV is usually clinically diagnosed based on the identification of three out of the four Amsel criteria [40]: (1) non-inflammatory and homogeneous vaginal discharge; (2) microscopic identification of clue cells; (3) pH over 4.5 in the vaginal fluid, and (4) a positive "whiff test" (fish odor after the addition of 10% potassium hydroxide to the vaginal secretions). The application of another score system based on the microscopic

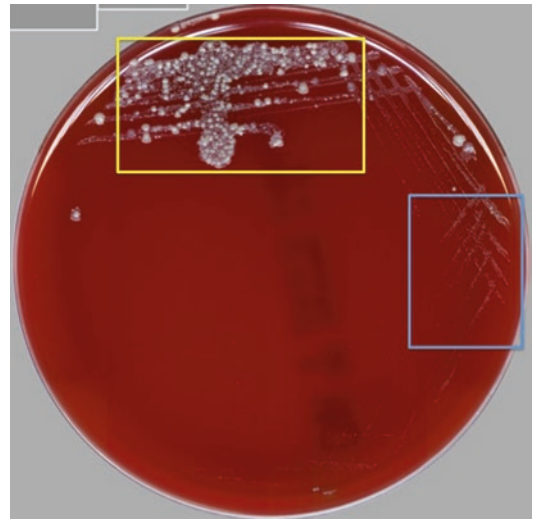


Fig. 7.9 *Gardnerella vaginalis* grown in sheep blood CNS agar. The yellow rectangle indicates an area with growth of mixed vaginal microbial flora. The blue rectangle highlights the area in which only *G. vaginalis* tiny punctiform, almost colorless, non-hemolytic, colonies are present

semi-quantitation of the presence of *Lactobacillus* in a gram stained vaginal smear, the so-called Nugent criteria [41], has been proven more problematic under the diagnostic point of view, since a large proportion of women that showed a positive Nugent score are totally asymptomatic, with large differences among diverse ethnic groups. For many years the diagnosis of BV was also related to the identification of *G. vaginalis*: this germ holds the capacity to build up a biofilm matrix that serves as substratum for the other species that predominate in the dysbiotic status of BV.

Direct Diagnosis The microscopic evaluation of a vaginal smear stain with the Gram techniques has been (and mainly it still is) the gold standard for the laboratory diagnosis of BV, since the mid-1960s. The above reported Nugent criteria are based on a standardized scoring system of the microscopic observation of the vaginal smears (wet mount): the amount of gram positive rods and lactobacilli (normal vaginal flora) and gram negative or gram variable bacteria (BV flora) is evaluated. With a score from 0 to 3 the diagnosis of BV is excluded, when the score ranges from 4 to 6 the flora is mixed, and with a score from 7 to 10 the

BV is diagnosed. The diagnostic performance of the Nugent score system is more accurate than that of the Amsel criteria: from about 40% to 50% of women with mixed flora (score 4 to 6) were diagnosed as suffering from BV with the Amsel panel of criteria [42].

NAATs and NA Probe Tests Considering the above reported limitation of the clinical and microscopic approaches for the diagnosis of BV, quite recently the use of molecular techniques have been proposed. Two main techniques are currently available: the direct probe assays (DPA) and the amplification of NA (mainly based on multiplex real-time PCRs).

The DPA uses synthetic oligonucleotide probes to directly test the presence of different germs into the vaginal fluid and the following are the tests nowadays available: Affirm VP (Becton Dickinson), and the Bacterial Vaginosis/Vaginitis panel from Quest Diagnostics (whose use is limited to symptomatic patients). The Affirm VP is rapid (30 min) and detect *G. vaginalis*: the detection of this microbe at a concentration above 5×10^5 CFU per ml of vaginal fluid is diagnostic of BV (the principle is basically similar to the detection of clue cells in wet mount microscopy). When compared to the wet mount this test has a sensitivity of 90% and a specificity of 97%; the comparison with the Nugent criteria showed 81% and 94% specificity and sensitivity, respectively. The method from Quest Diagnostics is considered negative for load below 2×10^5/mL. The commercial NAATs techniques for the diagnosis of BV are enlisted following: NuSwab (Laboratory Corporation of America), SureSwabBV (Quest Diagnostics), BD Max vaginal panel (Becton Dickinson), BV panel (Medical Diagnostic Labs), and the HP Vaginitis/Vaginosis (AusDiagnostics). Basically all of these tests are capable to quantitatively detect the NA of different germs (among which *G. vaginalis*, *Atopobium vaginae*, different species of *Candida* spp., and *Lactobacillus* spp.) and the diagnosis of BV is based on the comparative analysis of the combination of germs detected. Additional studies are necessary to investigate the possibility to use newer molecular techniques for the diagnosis of BV, such as the Next Generation Sequencing (NGS) that holds the potential to investigate the presence of different germs in complex biological samples such as the vaginal fluid [43].

POCTs Rapid tests, mainly with the format of dipsticks and based on chromogenic reactions that detected the presence of sialidases (OSOM BV Blue Genzyme) and FemExam (Cooper Surgical) that measures the vaginal pH and the presence of trimethylamine are also available. Both the tests have good practical application, mainly due to good sensitivity and specificity compared to the classical diagnosis and the "easy to use" that allow the use as POCTs.

7.4 Conclusions

The laboratory diagnosis of the major STDs caused by bacteria and protozoa has evolved in the last decades. With the only exception of syphilis, whose laboratory diagnosis still relies mainly of detection of non-treponemal and treponemal antibodies, the large majority of the diagnostic tests are nowadays based on nucleic acid amplification techniques (NAATs). This evolution has several advantages with respect to the classical approach based on microscopy and culture based tests. NAATs have shorted TAT, increased sensitivity and specificity and in a simplified version could be used as POCTs. The availability of POCT for the diagnosis of STDs represents a major diagnostic development that has relevant improvements in the overall management of STD patients: reduction of the cost and shorter TAT (results could be available "while you wait") among the most prominent. Future development of the laboratory diagnosis of these STD will be necessary in order to make available technologies for the routine detection of the antimicrobial resistance, in particular in the case of gonococcus and *M. genitalium*. In addition, the more accurate diagnosis of BV that is currently becoming possible with the use of NAATs will improve the possibility to treat BV, that is recognized as a factor that facilitates the acquisition and transmission of other STDs [43].

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Part IV

The Clinical Spectrum of STI

Marco Cusini

The fourth part of the handbook deals with the clinical spectrum of selected STI, which recently raised the interest of clinicians and researchers due to changes in epidemiology or in clinical picture, or due to the development of new treatments. Different clinicians were called to present the wide clinical spectrum of selected infections. The chapter by Marco Cusini focuses on syphilis which still has a high morbidity in women and infants in developing countries. Furthermore, in the last two decades, upon from the HIV-1 epidemic there has also been a serious raise in incidence rates of syphilis among men who have sex with men. Regarding syphilis, not only have a number of cases increased, but peculiar clinical presentations have also been registered. Gonorrhoea is becoming an untreatable disease and Antonio Cristaudo and collaborators stress this concern in their chapter and explain how the development of antimicrobial resistance of the N. gonorrhoeae has narrowed the range of effective antibiotics. Chlamydia trachomatis is of great epidemiological relevance among young people and is considered to be among the major causes of female infertility and its early identification remains a challenge for the venereologists and the gynaecologists. The team of Francesco De Seta have edited a comprehensive chapter on the C. trachomatis infections from the point of view of a modern clinical approach. Recently, there have been many new developments in the field of bacterial vaginitis and vaginosis and these clinical identities themselves represent quite a new entity and the development of research regarding microbioma is bringing more knowledge. This is made clear in the chapter of Secondo Guaschino and collaborators. Antonio Volpi and Lauren Stamberry remind us that the old concept that Herpes Simplex type 1 is always over and Herpes simplex type 2 is always under the belt is no longer valid and new clinical aspects have emerged and new research is now focusing on genomic and gene recombination. Anogenital warts are by far the most frequent viral STI and their treatment is still a matter of discussion. Alessandra Latini will take us on a clinical journey through the different aspects of the HPV infections, also of those that have crossed the boundaries of genital areas. Modern venereology has encountered the liver as a targeted internal organ of new STI pathogens, such as the hepatotropic viruses. The sexual transmission of viral hepatitis has been demonstrated and

the control of this modality of spreading must be taken into consideration. These aspects are well covered by Massimo Puoti and collaborator in their chapter.

When HIV infection made its appearance as the most worrying diseases of the twentieth century, nobody could have imagined the wide range of aetiopathogenetic, clinical and treatment aspects that would dramatically opened in the biomedical sciences. Today HIV infection has been transformed from an acute fatal illness to a chronic disease and the implications of this revolutionary shift are investigated in depth in the chapter of Massimo Andreoni. Lastly, tropical STIs and endemic treponematosis maintain a high level of interest in developing countries but also Western societies must not neglect a possible re-emergence of these infections as demonstrated by the Lymphogranuloma venereum in the early 2000s. These issues will be discussed in two separate chapters edited by Aldo Morrone and Marty van Valls.

The Syphilis After the Year 2000

8

Marco Cusini and Stefano Ramoni

8.1 Introduction

Syphilis is a bacterial sexually transmitted disease, caused by *Treponema pallidum subspecies pallidum* (TP). TP belongs to the order of *Spirochetales*, and the family of *Spirochaetaceae*. There are almost three more pathogenic members of the family: *T. pertenue*, *T. carateum*, and *T. pallidum subspecies endemicum*, causing, respectively, yaws, pinta, and endemic syphilis, that are not sexually transmitted. The four bacteria shares many common features and they are morphologically, immunologically, and serologically indistinguishable. The importance of syphilis is related to serious adverse pregnancy outcomes, such as stillbirth and congenital syphilis, and to their biological and epidemiological interrelationships with HIV-1 infection. In the last years, this infection as a strong biological marker of a recent at-risk sexual behaviour was utilized as outcome in many epidemiological studies aimed to evaluate the effect size of preventive interventions.

8.2 The Beginning of Syphilis

From the very beginning, syphilis has been a stigmatized, disgraceful disease; each country whose population was affected by the infection blamed the neighbouring and often enemy countries for the outbreak [1]. As a matter of fact syphilis became the "French Disease" for the inhabitants of today's Italy, Germany, and United Kingdom; in France it was "the Neapolitan disease", the Russians called syphilis "Polish disease", and the Polish "the German disease". The Danish, the Portuguese, and the inhabitants of Northern Africa named it "the Spanish/Castilian disease" and the Turks coined the term "Christian disease". Moreover, in Northern India, the Muslims blamed the Hindu for the outbreak of the affliction. However, the Hindu blamed the Muslims and finally all Asiatic people accused the Europeans [2–4].

Moreover, syphilis was known in different geographic areas with more than 50 names of saints (Saint Job, Sainte Reine, Saint Evagrius, and so on). This reflects the hope that these saints can help in healing the disease and also the fact that people tried to take the responsibility for this new disease away from their own [5].

The disease was first mentioned by Grünpeck in 1496 in *De pestilentiali sorra* and was attributed to astrological origins. In 1503, Grünpeck called it "mentulagra" (sickness of the "mentula", the male genitals) in his book

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Latin Hieronymus Fracastorius, (born c. 1478, Verona, Republic of Venice [now in Italy]—died Aug. 8, 1553, Caffi [now Affi], near Verona), Italian physician, poet, astronomer, and geologist, who proposed a scientific germ theory of disease more than 300 years before its empirical formulation by Louis Pasteur and Robert Koch. At the University of Padua Hieronymus Fracastoro was a colleague of the astronomer Copernicus. As a physician, he maintained a private practice in Verona.

Fig. 8.1 Portrait of Girolamo Fracastoro (1476–1553) painted by unknown artist

De mentulagra and he considered it to be a "filthy" contagion ("sordid contagio").

In the beginning of sixteenth century, Jean Fernelius, a Parisian teacher whose work and interests were devoted to the mercury treatment of the condition, coined the term "lues venerea" ("venereal pest") in his treaty dedicated to the affliction [6].

The term Syphilis was introduced by Girolamo Fracastoro (1478–1553), a poet, geographer, and medical personality in Verona [7–9] (Fig. 8.1). In 1530 he published a Latin poem titled "Syphilis sive morbus gallicus" in three books. In book I, he described the evolution of the disease: incubation (I, 319,329), first lesions (I, 330,331), secondary dissemination (I, 344,346), other forms and variants (I, 347,364). He described the disease as being a source of defilement and disgrace ("lues" cited seven times). He stated that this "vulgar" disease was born in the west of the Atlantic seas, over those unhappy recently discovered edges (Box 8.1) [10].

In books II and III, Fracastoro discusses the main treatments of syphilis in his era: mercury and Guaiacum, the "sacred wood of the American Indians" [6].

In book III, written at the insistence of Cardinal Bembo (to whom the work was dedicated), Fracastoro told the story of a Greek shepherd, Syphilus, who led a revolt against the god of the Sun and suffered later from this disease [6]. It was thought that through the character of Syphilus, Fracastoro was referring to Syphilus, one of the 11 sons of Niobe, who was cursed by Apollo, god of the Sun. Syphilus suffered this terrible fate, because his mother (Niobe) claimed that her children were more beautiful than Apollo [11].

We do not know more about the etymology of the words "syphilis" and "syphilus." The majority of Renaissance authors used the term "syphilis" after Fracastoro had mentioned Syphilus's myth in his book ("syphilidemque ab eo labem dixere coloni").

Andre du Laurens and Fallopio justified the etymology of "syphilis" as meaning lover of

Box 8.1 Latin Quotation on the Origin of Syphilis from G. Fracastoro (1478–1553)

Oceano tamen in magno sub sole cadente,
Qua misera inventum nuper gens accolit orbem,
Passim oritur, nullisque locis non cognito vulgo est.

swine (from the Homeric Greek or Latin "sus" and Greek "philos").

Fracastoro did not mention the transmission of this contagious disease. Only 16 years later, he discussed sexual transmission of syphilis in *De Contagione* (1546) [12].

The origin of Syphilis was debated for many years and two main theories were proposed: the pre-Columbian theory and the Columbian theory.

According to the Columbian theory, the sailors on Colombo ships brought the disease to Europe, returning from the new world [13]. The history of syphilis in Europe began officially in the spring of 1493, with the return of Colombo's ships from the Americas. According to Fernandez de Oviedo and Ruy Diaz de Isla, two Spanish physicians, who attended the return of Colombo from the Americas, several members of the original crew, and some of the indigenes that were brought to Europe, had manifestations of a new unknown disease [12].

The supporters of the pre-Columbian theory state that syphilis and the other treponematosis were present in Europe and Africa before 1493, and many of the cases were considered as leprosy [14].

There is a third unitarian hypothesis that considers syphilis and the other treponematosis as a variant of the same disease, that geographic and climate variations and the degree of cultural development of populations within disparate areas were the cause of the different clinical pictures.

Venereal syphilis is thus the results of the evolution of the infection in those areas where inhabitants exhibited a civilized society and paid more attention to personal hygiene.

The existence of syphilis or of treponemal infections before 1492 has been postulated in several studies that examined skeletal samples from the Median Eve and found bones abnormalities suggesting treponematosis [15].

As a matter of fact, syphilis spread all over Europe starting from 1493 with great mortality and morbidity and remained a major health problem until the twentieth century.

After the discovery of penicillin syphilis became a curable disease, but still present and still representing an important public health issue.

8.3 Epidemiology

Despite the high effectiveness of penicillin treatment, syphilis is still an important public health problem worldwide. The World Health Organization (WHO) estimated 5,6 new cases among adults in 2012 all over the world, with a major diffusion in sub-Saharan Africa and south Asia [16]. In Africa the prevalence of infection in pregnant women ranges from 4% to 15% and primary syphilis is responsible for 25% of intrauterine death and 14% on neonatal death (http://www.epicentro.iss.it/problemi/sifilide/epid.asp). According to CDC, the US rate of primary and secondary syphilis has increased every year since 2000–2001 and this epidemiological trend has been mainly attributable to the increased cases among who have sex with men (MSM). The incidence rate increased from 2.1 cases per 100,000 population in 2000 to 9.5 cases per 100,000 population in 2017 (Fig. 8.2). In 2017 a total of 30,644 new diagnosis of primary and secondary syphilis were reported in the US; this represents an increase of 10.5% compared with 2016, and an increase of 72.7% compared with 2013.

As observed in previous years, in 2017 the incidence rate of primary and secondary syphilis in male patients (16.9 cases per 100,000 males) was significantly higher than in female ones (2.3 cases per 100,000 females) with the MSMs still represent the majority of early syphilis cases (Fig. 8.3). While in men the rate increased every year since 2000, in women the rate remained stable until 2013 when it started to increase; during 2013–2017 the incidence rate in women increased to 155.6% (Fig. 8.4).

In the US syphilis was diagnosed mainly in young patients, with the highest rate among persons aged 25–29 years as shown in Fig. 8.5 (https://www.cdc.gov/std/stats17/syphilis.htm).

A similar situation is registered in Europe; according to ECDC data, in 2016 the European incidence rate was 6.1 cases per 100.000 population with the highest rate in the UK (9.9 cases per 100,000 population), followed by Malta, Iceland, and Germany (Table 8.1).

As in the US, also in Europe syphilis is more common in males (10.8 cases per 100,000 population) than in females (1.3 cases per 100,000 population), with a male-to-female ratio of 7.9:1 (Fig. 8.6).

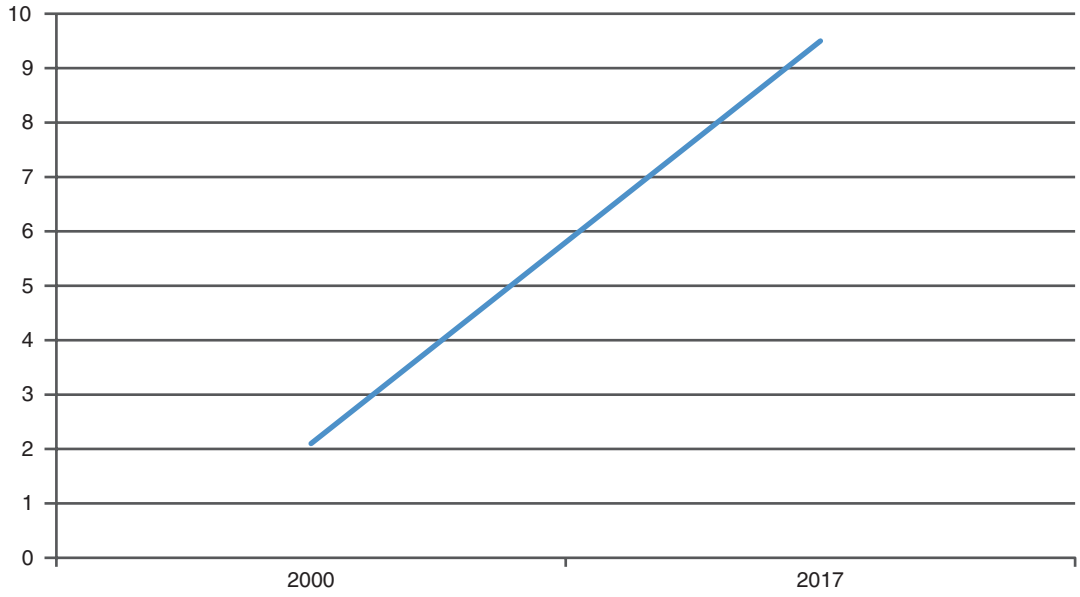

Fig. 8.2 Incidence rates × 100,000 of early syphilis (primary, secondary, and early latent) from 2000 to 2017 (modified from CDC, Sexually Transmitted Diseases Surveillance 2017)

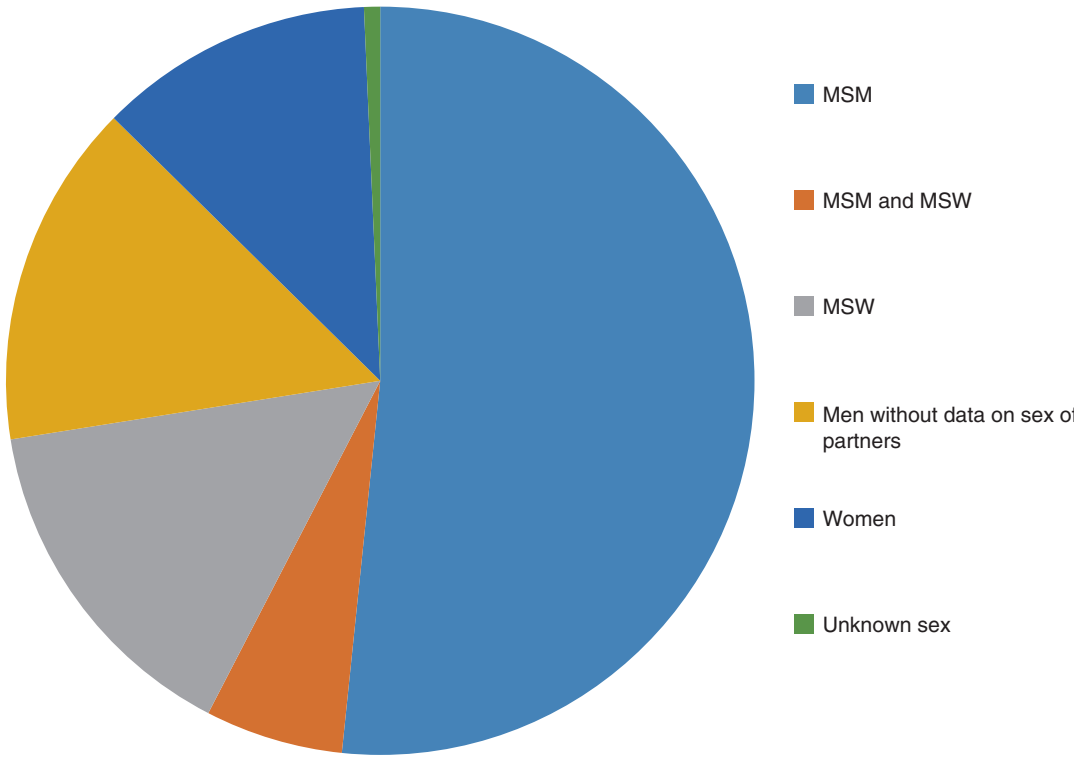

Fig. 8.3 Distribution of primary and secondary syphilis cases by sex and male sexual orientation, USA 2017 (*modified from CDC, Sexually Transmitted Diseases Surveillance 2017*). Legend: *MSM* men who have sex with men, *MSW* men who have sex with women

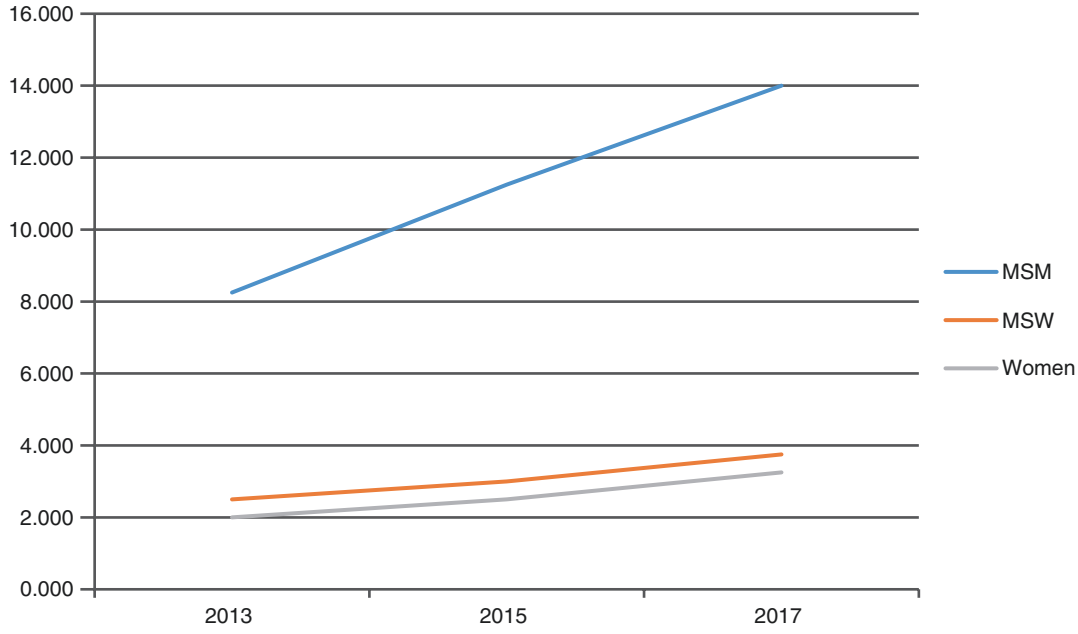

Fig. 8.4 Cases of primary and secondary syphilis by sex and sexual behaviour, US 2013–2017 (*modified from CDC, Sexually Transmitted Diseases Surveillance 2017*)

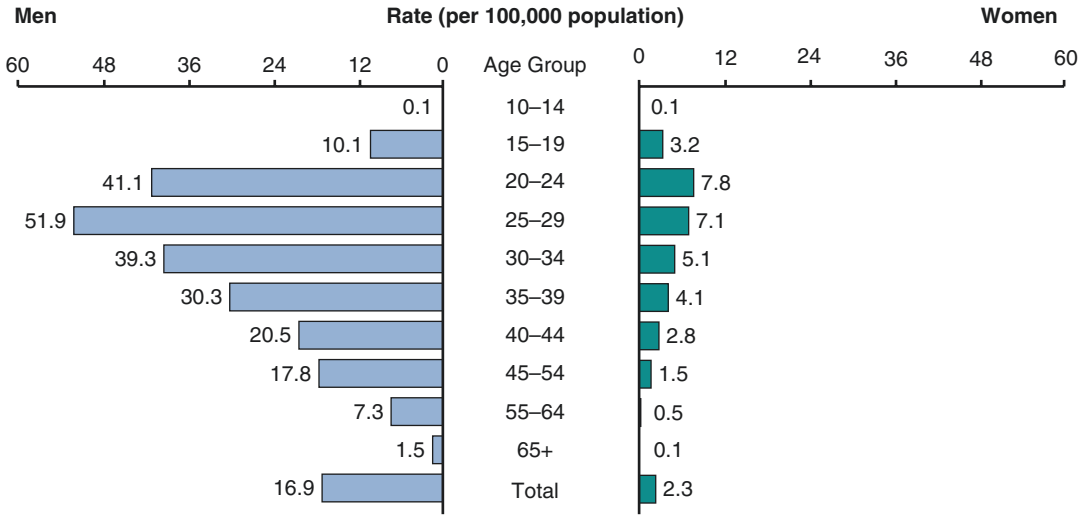

Fig. 8.5 Incidence rates of primary and secondary syphilis by age and sex in 2017 (*modified from CDC, Sexually Transmitted Diseases Surveillance 2017*)

The majority of diagnosis were made in two age groups: above 45 years (31% of diagnosis) and 25–34 years (30% of diagnosis). Data between 2010 and 2016 showed an increase of reported case in MSM; cases among heterosexuals seem to be stable (Fig. 8.7) [17].

After 2000

In the US and Europe syphilis became a rare disease between 1970s and 1990s and this is in part related to the HIV epidemic and the consequent public health campaign. The introduction of combined antiretroviral therapy (cART)

progressively changed the appreciation of HIV infection and nowadays HIV is not yet considered a fatal condition; so risky behaviours restarted, thus leading to a recurrence of all STIs, mainly in MSM. Since 2000–2001 outbreaks of syphilis were registered in many countries. Between 2000 and 2002 the number of primary and secondary syphilis in Milan increased by 400%, the majority of cases (85%) were diagnosed in MSM [18].

After the failure of the Soviet Union in the 1990s, with the collapse of the public health, a large epidemic of syphilis was registered in western Europe [18], but the most dramatic situation was observed in China where syphilis has made a significant resurgence in the last 20 years: during the cultural revolution, all STIs were very uncommon but, as Chinese economy expanded, all STIs reappeared, and now syphilis represents one of the top 5 reported communicable diseases in China and one of the most important problems of public health, with more syphilis cases in the province of Guangdong in 2008 than in Europe during the same year [19].

Table 8.1 Cases of syphilis reported in Europe in 2016 (*modified from ECDC Annual epidemiological report 2016*)

	Year 2016	
Geographical area	No. of cases	Incidence rate
Europe	29,635	6.1
United Kingdom	6470	9.9
Malta	40	9.2
Iceland	30	9.0
Germany	7157	8.7

8.4 Clinical Features

In about 30–40% of cases syphilis remains asymptomatic and it is called latent syphilis; the diagnosis is possible only using specific serological tests. In symptomatic cases, the clinical course is conventionally divided into main 3 stages: primary syphilis (PS), secondary syphilis (SS), and tertiary syphilis (TS) [20].

Primary Syphilis After an incubation period of 2–4 weeks (it can range from 10 to 90 days) a chancre (primary syphiloma) appears in the site of TP inoculation, mainly the genitalia. It presents as a single dark red nodule, one-centimeter in diameter, hard in consistence, superficially eroded, and secreting a clear serosity (Fig. 8.8).

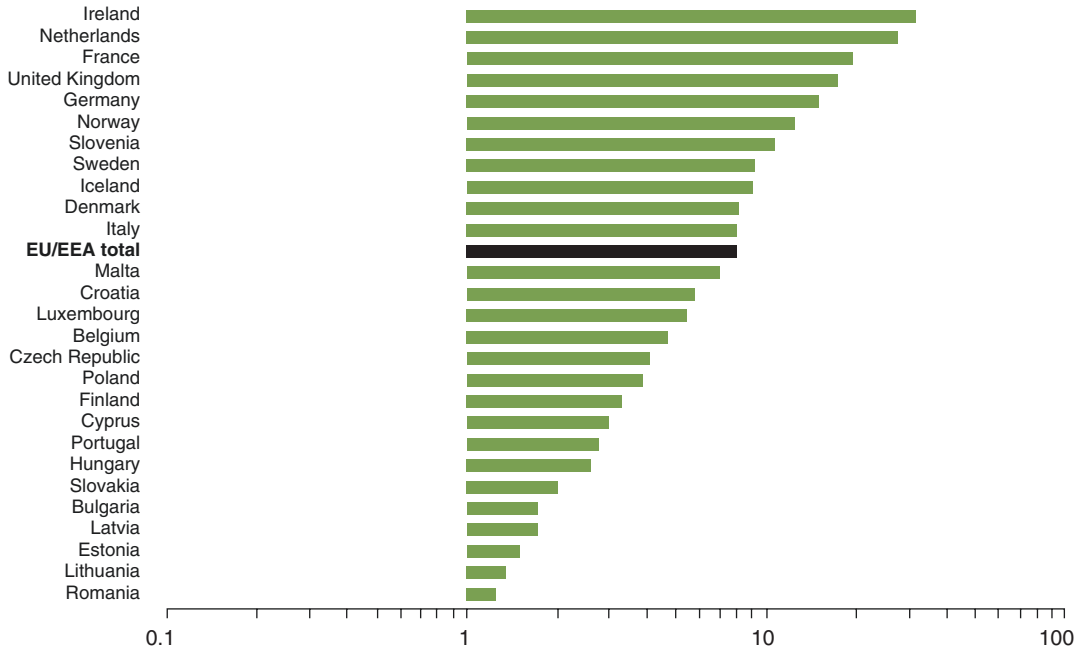

Fig. 8.6 Male-to-female ratio for syphilis in Europe 2016. (*From ECDC Annual epidemiological report 2016*)

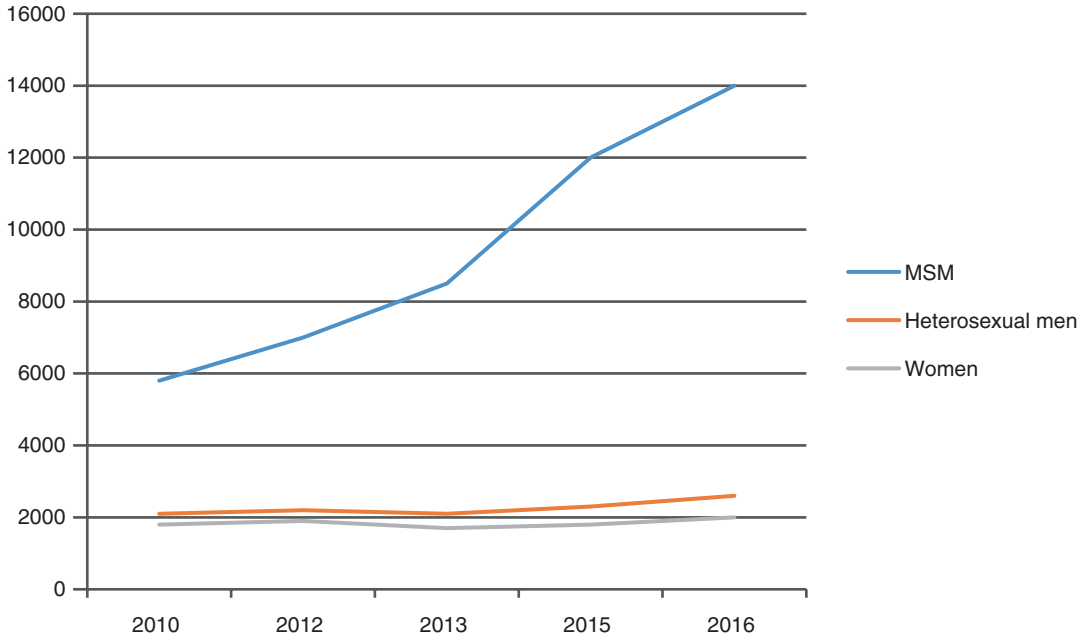

Fig. 8.7 Cases of syphilis by sex and sexual behaviour in Europe 2010–2016 (*modified from ECDC Annual epidemiological report 2016*)

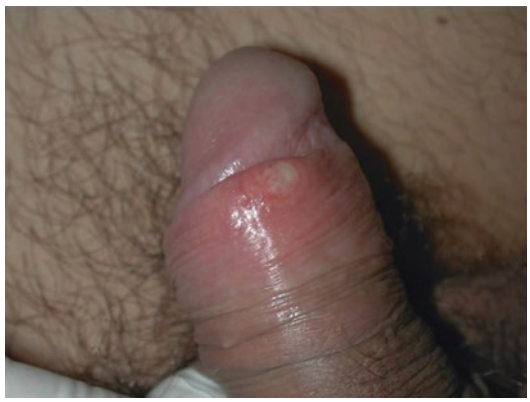

Fig. 8.8 Typical syphilitic chancre

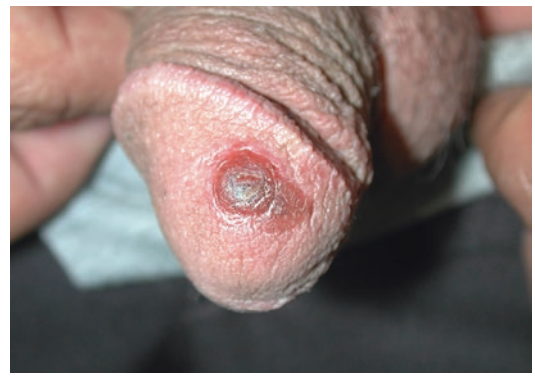

Fig. 8.9 Papular syphilitic chancre

Syphilitic chancre is generally painless, so the patient may not notice it; only sometimes it is painful due to irritative phenomenon [21]. The classic feature of the chancre, as described above, is observed only in 50–60% of cases [22]; in 40–50% of cases the chancre is atypical for:

Clinical Feature Possible clinical variants of syphilitic chancre are the *papular chancre,* characterized by the presence of a papular lesion lacking of superficial erosion—(Fig. 8.9), the *ulcerated chancre*, characterized by a loss of substance reaching the dermis (Fig. 8.10), the *gangrenous chancre,* characterized by an extensive loss of substance caused by pyogenic superinfections (Fig. 8.11), the *diphtheroid chancre,* characterized by the presence of a whitish pseudo-membrane covering the chancre (Fig. 8.12), and the *diffuse primary syphilitic balanitis (or vulvitis* in female patients) *of*

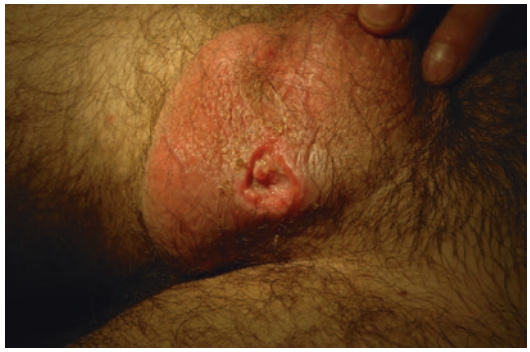

Fig. 8.10 Ulcerative syphilitic chancre

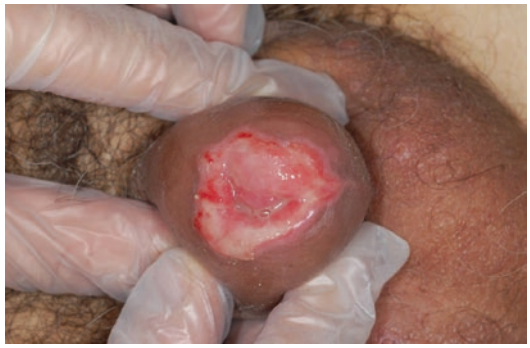

Fig. 8.11 Gangrenous syphilitic chancre

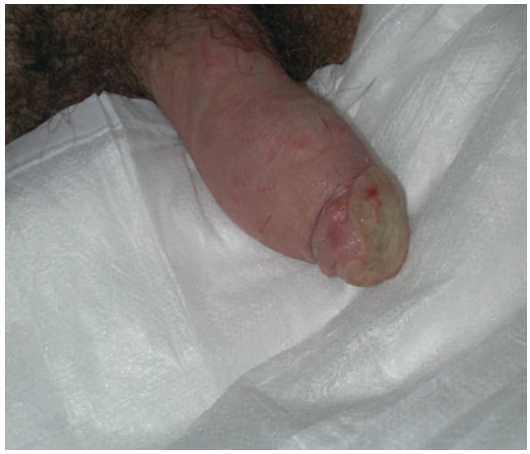

Fig. 8.12 Diphtheroid syphilitic chancre

Follmann, characterized by a diffused inflammation accompanied by superficial erosions secreting a serous exudate (Fig. 8.13) [21].

Localization Even if syphilitic chancres have been described in any part of the body, the most frequent localization is the genital area; only 5–10% of chancres are extragenital (this percentage may be underestimated), mainly involving oral cavity (Fig. 8.14) and anus where it can assume rhagadiform aspect (Fig. 8.15) [23–25].

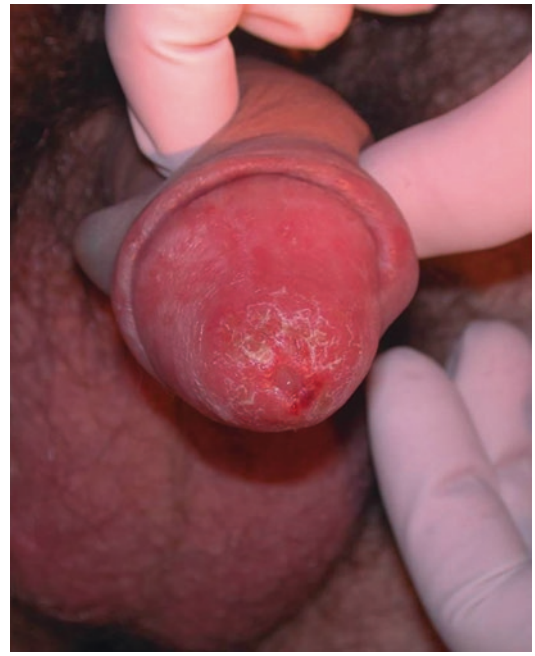

Fig. 8.13 Balanitis of Follmann

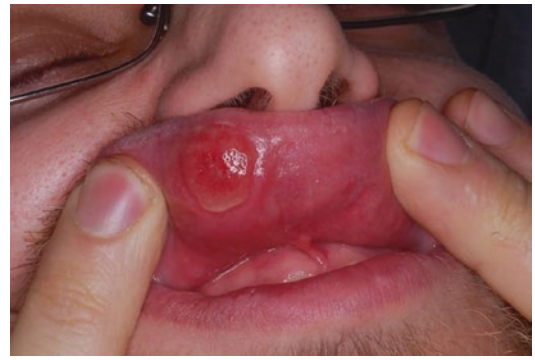

Fig. 8.14 Syphilitic chancre of the lip

Dimensions Typical syphilitic chancre is about one-centimeter in diameter but there are *dwarf chancres* (<1 cm) which can be confused with genital herpes, mainly in case of multiple lesions (Fig. 8.16), and *giant chancres* (>1 cm), more easily ulcerated and painful (Fig. 8.17) [21].

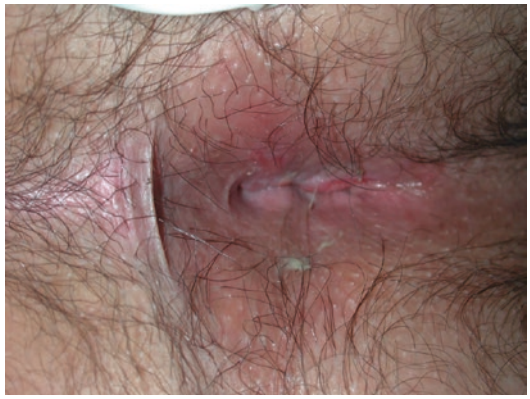

Fig. 8.15 Syphilitic chancre of the anus with rhagadiform aspect

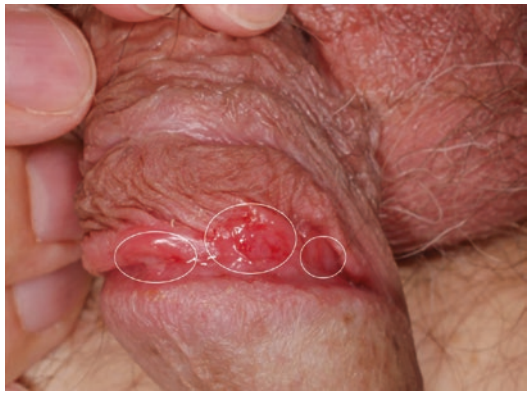

Fig. 8.16 Multiple dwarf syphilitic chancre mimicking a genital herpes

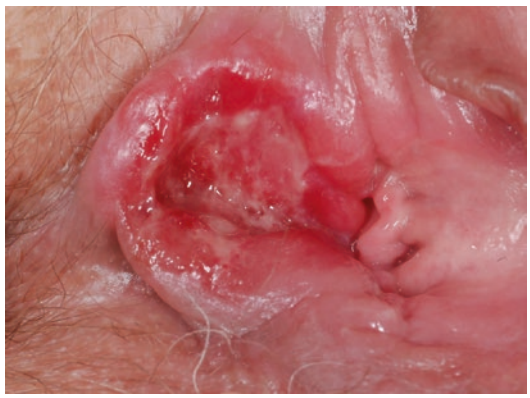

Fig. 8.17 Giant syphilitic chancre

Number of Lesions Rarely primary syphilis can present with multiple chancres (Fig. 8.18); they can be the consequence of multiple inoculations during the same sexual intercourse (in this case the lesions have the same localization) or of autoinoculation starting from the first chancre (in this case the lesions can be found in different sites of the body) [21].

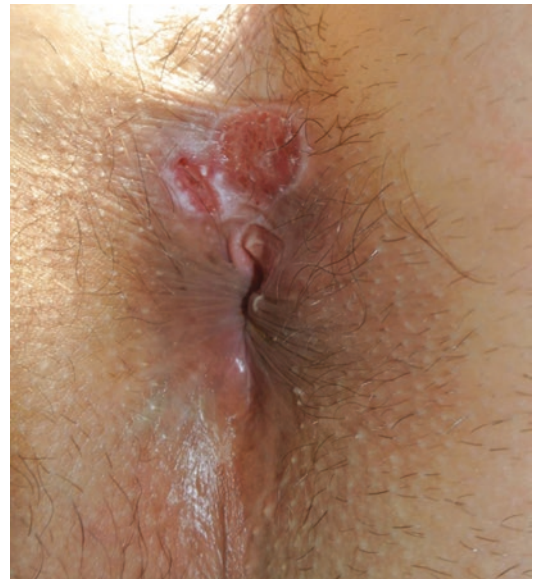

Fig. 8.18 Multiple syphilitic chancres

Clinical Course Syphilitic chancre is usually self-healing, without sequelae, in about 4 weeks; ulcerated and gangrenous chancres can result in scars. Syphilitic chancre is accompanied by local adenopathy: lymph nodes become painful palpable masses, separated from each other and non-adherent to adjacent tissues, with one of them larger and raised on the skin. The association of the chancre and the local adenopathy represent the so-called *primary complex of syphilis*. After treatment, regression of satellite adenopathy can take several months [26]. With the resolution of the chancre, a period of second incubation begins. It can be asymptomatic or characterized by fever, headache (attributed to meningeal irritation or to periostitis of the skull's inner surface), and osteocopic pains; these symptoms are accompanied by the enlargement of various superficial lymph nodes which are aflegmasic, mobile, and painless.

Differential Diagnosis

Differential diagnosis of primary chancre includes both STIs (such as genital herpes, lym-

phogranuloma venereum, and chancroid) and non-infectious diseases (such as bacterial balanitis, drug reactions, and carcinomas). A complete anamnesis and an accurate clinical examination are essential for the diagnosis that will be confirmed by laboratory.

Secondary Syphilis The second stage of the disease is very polymorphic in clinical features, this legitimating the name of "great imitator" used in the past for designating syphilis [27]. It is characterized by eruptive, disseminated, asymptomatic, and self-healing mucocutaneous lesions called secondary syphiloderms. Based on the time of onset, they are divided into syphiloderms of first eruption, relapsing syphiloderms, and transition syphiloderms (the last ones appearing during the transition period towards the tertiary stage of the disease) [21]. Syphiloderms of first eruption appear from 2 to 6 months after the infection and they can be classified according to the morphology of the lesions. The most frequent syphiloderms are the *erythematous syphiloderm* or *syphilitic roseola* (ES) and the *papular lenticular syphiloderm* (PLS). ES is the earliest manifestation of secondary syphilis, appearing 60–70 days after the infection. It is characterized by an eruption of small pale erythematous asymptomatic not palpable patches, variable in size from 1 mm to 1 cm, diffused on the trunk, abdomen, and arms (Fig. 8.19). These lesions remain stable for 1–2 weeks and then resolve without sequelae.

PLS appears 70–90 days after the infection and it is characterized by asymptomatic red-copper palpable papules with a peripheral collarette of desquamation called Biett's collarette; these kind of lesions can be disseminated all over the body but the most typical localization is the palmo-plantar one where the Biett's collarette is more evident due to hyperkeratotic aspects (Fig. 8.20).

In the cutaneous folds, the papular lesions are larger, coalesced in plaques, with superficial erosions secreting a serosity very rich in treponemas and possible vegetating aspects; these kinds of lesions are called condylomata lata (Fig. 8.21) and they can be detected in every cutaneous fold, mainly the genital ones.

Other possible variants of syphiloderms are the *impetiginoid one,* characterized by wet eroded lesions covered by yellowish scales (Fig. 8.22), the *acneiform one* (characterized by purulent fol-

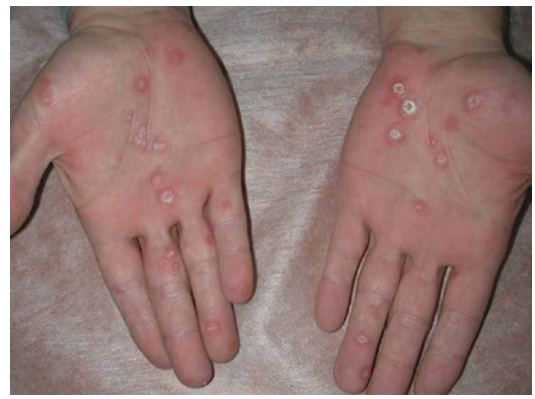

Fig. 8.20 Popular lenticular syphiloderm with palmar involvement

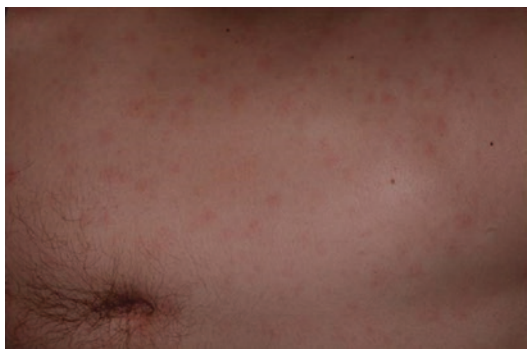

Fig. 8.19 Typical syphilitic roseola

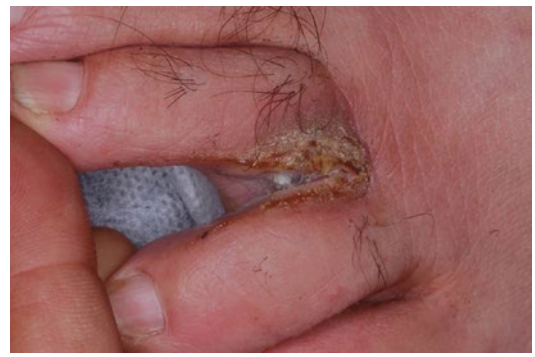

Fig. 8.21 Atypical condylomata lata

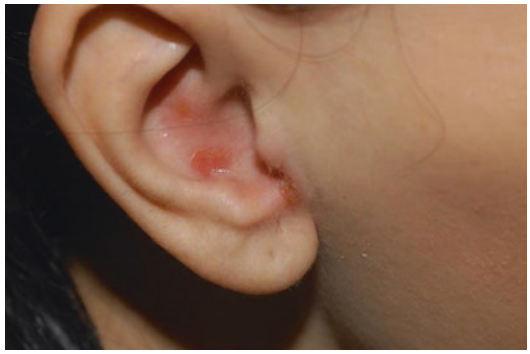

Fig. 8.22 Impetiginoid syphiloderm

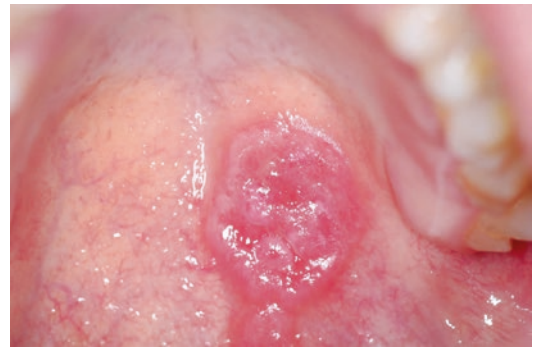

Fig. 8.24 Mucous plaque during secondary syphilis

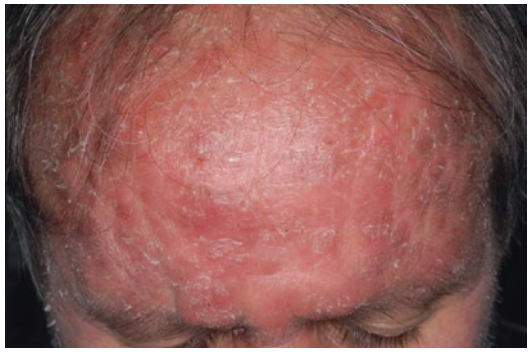

Fig. 8.23 Psoriasiform syphiloderm

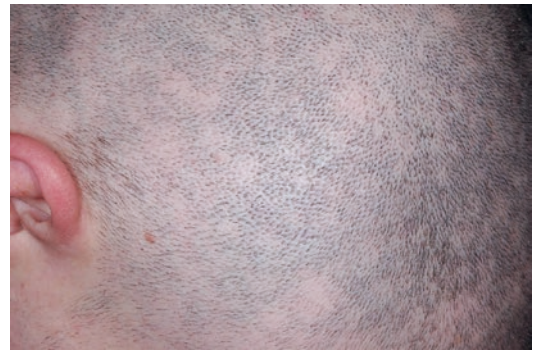

Fig. 8.25 Areolar alopecia of the scalp during secondary syphilis

licular lesions), the *psoriasiform one,* characterized by erythemato-scaling lesions resembling psoriasis—(Fig. 8.23), and the *lichenoid one* (characterized by diffused erythematous polygonal papules [27].

Relapsing syphiloderm can appear from a few months to a few years after the infection; they are characterized by a smaller number of lesions grouped in different shapes (corymbiform, ring shape, "S" shape, or polycyclic shape). The transition syphiloderms are nowadays very rare and they present with a few lesions, larger, more infiltrated, and darker than the classical syphilitic papules; moreover, they are more persistent and resolve very slowly living atrophic scars. Also mucous membranes can be affected during SS, with erythematous or erythematous-erosive patches; more characteristics are opalescent lesions with an erythematous halo, called *opaline plaques* when small papules and *mucous plaques* when larger lesions (Fig. 8.24).

Fig. 8.26 Alopecia of the eyebrow

Other possible manifestations of SS can involve the scalp with areolar or diffuse alopecia (Fig. 8.25) and the eyebrow (Fig. 8.26) with an alopecia most often localized to the outer third.

During the second sage, syphilis becomes a systemic infection due to haematic diffusion of treponemas, and so every organ and apparatus

can be involved. Patients can develop generalized adenopathy, hepatitis, pancreatitis, bones lesions (osteitis, osteomyelitis, periostisis), pulmonary involvement, and ocular diseases. Ocular syphilis is found in 4% of cases. Even if posterior uveitis and panuveitis are the most common features, every ocular structure can be involved; possible presentations are iritis, iridociclitis, optic neuropathy, anterior uveitis, interstitial cheratitis, and loss of visus [28]. So, in case of SS, a complete ocular visit with examination of fundus oculi should be performed and, in case of positive results, a liquor examination should be ruled out; ocular syphilis must be managed as neurosyphilis. Moreover, TP shows a particular tropism for the nervous system, so signs of neurological damages (such as neuritis and paralysis of cranial nerves, intercostal and sciatic nerve neuralgia, headache for meningeal involvement, acute meningitis, and early meningomyelitis with flaccid paraplegia) can be observed already in the secondary stage of the disease. However, the involvement of the nervous system only rarely manifests clinically and more often the damage is revealed only through a liquor examination.

Differential Diagnosis

"The great imitator" is very polymorphic in clinical presentation and so many differential diagnoses should be discussed. Considering only the most frequent presentations (ES and PLS), the principal differential diagnosis are pityriasis rosea, eruptive psoriasis, drug reactions, and viral exanthems. An accurate anamnesis can be helpful.

Early Latent Syphilis

Latent syphilis is an asymptomatic stage diagnosed with serological tests performed as routinary examinations (for at-risk people) or performed for other reasons (pregnancy, blood donation, etc.). Depending on the duration of the infection, latent syphilis can be considered "early" (if acquired within the last 2 years) or "late" (if acquired before the last 2 years or unknown). Early latent syphilis is considered contagious and so it is managed like the primary and the secondary stage of the disease.

Tertiary Syphilis Syphilis evolves in the third stage only in 30–40% of untreated patient, many years after the infection (period of late latency); late latency can last from 2 years to 25 years, with an average period of 15–16 years. TS can involve both the skin and the internal organs. The typical cutaneous lesions are the tertiary syphiloderms and the syphilitic gummas. *Tertiary syphiloderms* are firm nodular red-brown lesions raised on the skin, usually ranging in size from a lentil to a pea. They can be singles, but more often are multiple and grouped in circinate figures; the typical localization is the face. *Syphilitic gummas* are subcutaneous nodules with a central softening, covered by bluish-red skin, and a necrotizing evolution.

All organs can be affected during TS, but the most common involved are the cardiovascular system and the nervous system. Cardiovascular involvement can present with diffuse myocarditis, gummatous lesion of the cardiac muscle with aneurysmal dilatations, and panarteritis of large and medium vessel, but the most characteristic lesion of cardiovascular syphilis is the aortitis; it can be asymptomatic or can cause aortic valve insufficiency, aortic aneurysm, and stenosing ostial coronaritis. Neurosyphilis manifests in 6.5% of untreated patients and it can be asymptomatic in about one-third of cases. There are different clinical presentations of neurosyphilis, depending on the anatomic structures affected. *Meningovascular syphilis* affect about one-fifth of patients; it is consequent to meningeal gummatous infiltrates and it is characterized by paresis or paralysis of cranial nerves, peripheral neuritis, and radicular neuralgia. Also possible is chronic diffuse meningitis with headache and pupillary alterations: the pupils are miotic with no reflex to light, while the accommodation reflex persists (Argyll Robertson sign). In case of direct impairment of the cerebral and spinal nerve tissue, we have the so-called *parenchymatous neurosyphilis,* which includes the two classic conditions of tabes dorsalis and progressive paralysis. *Tabes dorsalis* is the most frequent presentation of neurosyphilis; it manifests with disorders of motor coordination (ataxia) and of the standing position with eyes closed (Romberg's

sign). There are also sensory alterations with radicular topography and reduction or absence of tendon reflexes. It can evolve in a pre-ataxic or neuralgic stage, followed by constitution ataxia, which culminates in the paraplegic stage [26]. *Progressive paralysis* is the result of a chronic meningoencephalitis with atrophy of the brain, in particular of the anterior portion. It is characterized by psychological alterations with behavioural changes, memory loss, fatigue, insomnia, and impaired speech and writing. This condition evolves towards a complete dementia; death usually occurs for intercurrent infections after a progressive decline of physical conditions [26].

A nowadays very rare condition is malignant syphilis. This condition is typical of patients in poor general conditions for chronic infectious diseases, diabetes, alcoholism, etc. It is characterized by ulcerative and necrotic syphiloderms associated with an impairment of general conditions with malaise, insomnia, joint pain, anaemia, and fever; the involvement of lymph nodes is absent or mild and the positivization of serology is delayed [21].

After 2000

With the recurrence of syphilis observed since the year 2000, also the unusual clinical presentations have become more frequent; the incidence of ocular syphilis and congenital syphilis has rised up as well. Fortunately, TS remains a rare condition and this is the consequence of the wide use of antibiotics for other conditions that can avoid the evolution of the disease. If the epidemiological trend will be confirmed in the next years, physicians will have to face more and more frequently with syphilis and with its unusual presentations, so they have to maintain a high index of suspicion in order to avoid diagnostic mistakes.

8.5 Syphilis in the HIV Patient

There are not substantial differences between the clinical presentation of syphilis in HIV-positive and HIV-negative patients. In HIV is more frequent the coexistence of manifestations of the primary and secondary stage of the disease; moreover, atypical clinical features are more likely observed in HIV infected people, notably in case of low CD4+ cells count.

8.6 Diagnosis

According to Merriam Webster English Dictionary Diagnosis is *"the art or act of identifying a disease from its signs and symptoms"* [28].

A correct diagnosis is fundamental in medicine and should include the understanding of aetiology, of the state of activity of the disease, of all pathologic processes ongoing.

Syphilis is known as the great imitator, because of the polymorphism of the disease, and clinical diagnosis is not always simple. A laboratory confirmation is therefore almost always needed (www.merriam-webster.com).

Syphilis laboratory diagnosis can be made both by direct and indirect methods.

Direct methods allow the demonstration of *Treponema pallidum* (TP) in a clinical sample, indirect methods work through the demonstration of specific antibodies, produced by the infected subject.

8.6.1 Direct Diagnosis

The story of the direct diagnosis of syphilis begins in 1905 when Fritz Schaudinn a German zoologist and microbiologist and Erich Hoffmann a German dermatologist discovered the aetiologic agent of syphilis [29]. On March third 1905, Schaudinn examined a fresh preparation of a material obtained by Hoffman from an eroded papule in the vulva of a woman with secondary syphilis. The exam was done in a then modern Zeiss microscope with apochromatic objectives, which allowed Schaudinn to observe various very light thin spiralled microorganims, turning around their largest length and moving back and forth. Schaudinn showed Hoffman this finding, and named it Spirochaeta pallida. This organism was demonstrated in various syphilis lesions, on both fresh preparations and Giemsa dyes.

In 1909 Alfred Charles Coles an English physician working at the Royal National Sanatorium in Bournemouth first described the use of dark field microscopy (DFM) for the detection of TP [30].

In 1964 Anne Roof Yobs working at the Venereal Disease Research Laboratory, in Atlanta, Georgia described the technique of Direct Immune Fluorescence for the demonstration of TP [31].

PCR was used for the diagnosis of syphilis at the beginning of the 1990s and is still the most sensitive and specific technique [32–35].

Dark field microscopy allows the observation of live TP collected from syphilitic lesions. This method is easy, rapid, and specific for genital primary syphilis.

The method encompasses:

1. Gentle cleansing of the lesion with saline solution.
2. Squeezing of the lesions between two fingers to obtain an exudate avoiding contamination with blood that can interfere during the lecture.
3. Apposition of the exudate on a glass slide by pressing it on the lesion.
4. Addition of a drop of saline solution.
5. Immediate examination with a dark field microscope at 600–1000 × magnification.

TP is easily identifiable by an expert eye for its morphology and typical movements.

Sensitivity of DFM is about 80% and a minimum of 10^5/mL organisms are required, specificity for genital lesions is near 100%.

The limits of this test are:

– the need of a microscope and an exercised observer,
– the method is not available for oral lesions, since its specificity is much lower due to the physiological presence of other treponemes in the oral cavity.

DFM After the Year 2000 Our opinion is that DFM should still be performed for the diagnosis of syphilis in genital lesions. The method is quick and permits immediate therapy. The availability of quick multiplex NAAT test is under development and will replace DFM in many settings; nevertheless DFM must still be considered as a cheap method that can be used in poorly developed countries.

Nucleic Acid Amplification Techniques (NAAT)

NAAT for the detection of TP has been used on many samples type such as blood, urine, cerebrospinal fluid, and exudate from ulcers; the main field of application is anyway related to the use of NAAT for the diagnosis of primary and secondary syphilis from ulcers or mucous samples (www.merriam-webster.com).

The sensitivity of NAAT in primary syphilis varies from 78.4% to 95% [36].

NAAT for TP have better sensitivity than DFM in genital ulcer and can be also used for the diagnosis of oral lesions where DFM loses in specificity.

The use of NAAT on other samples type, such as blood, can give information on the activity of the disease and can be considered in some circumstances such as pregnancy.

In a study on 237 pregnant women with a diagnosis of syphilis it was shown that the positivity of PCR on blood was related to an increased risk of miscarriage and to the positivity of PCR also on the neonates [37].

NAAT After the Year 2000 Our opinion is that NAAT are the gold standard for the diagnosis of primary syphilis; new TP NAAT are under development and rapid PCR test that can give results within 2 h will bring great advantages to this diagnostic method.

Other Direct Methods

– Warthin–Starry staining on tissue samples is difficult to perform, has low sensitivity, and seldom useful.
– Immunohistochemistry with polyclonal antibodies against TP can be useful for identification in skin, mucosa, and other tissue [38].
– Subtyping of TP by PCR restriction and DNA sequencing are also available and very helpful for molecular epidemiology study [39].

Today routine direct diagnosis of syphilis is based on two methods: Dark field microscopy and demonstration of TP by nucleic acid amplification techniques (NAAT). Still an algorithm for the use of the two is lacking.

We believe that DFM should always be performed when possible in cases of genital lesions suspect for primary syphilis. NAAT should be done in case of negativity or absence of DFM. Molecular samples from lesion have great value for epidemiological study and to assess molecular resistance to drugs; costs are of course a major issue.

8.6.2 Indirect Diagnosis

The importance of indirect diagnosis for syphilis is sustained by the unavailability of cultures media for an easy growth of TP and by the absence of lesions in several stages of the disease.

Non-Treponemal Test (NTT)

The first serological test for syphilis was born almost together with the first observation of TP.

In 1906 Wasserman [40] adapted the complement fixation technique, introduced few year before [41], to the diagnosis of syphilis. Wassermann utilized a liver extract from newborns dead with syphilis as antigen. Landsteiner [42] demonstrated that other source, such as beef heart extracts could be used as antigens. The first flocculation test that did not require complement was introduced in 1922 by Kahn [43]. But was only with the isolation and the use of cardiolipin in 1941 that tests for syphilis became reproducible and could be standardized [44]. The VDRL test was first described in 1946 [45] and rapid plasma reagin test (RPR) in 1957 [46]. The test underwent several modifications and a test very similar to what is used today was described in 1980 [47].

Today several NTT are available such as VDRL, RPR, TRUST (toluidine red unheated serum test).

All these tests detect both IgG and IgM, are cheap, and simple to perform but are still manual. NTT usually becomes positive 10–15 days after the appearance of the primary lesion that means about 6 weeks after infection. NTT titres have a good correlation with activity of disease and the report of tertiary active syphilis with negative NTT is doubtful.

Treponemal Test (TT)

All the test utilizing cardiolipin or other extracted antigens are affected by low specificity in many circumstances.

After several unsuccessful attempts in 1949 Nelson and Mayer [48] developed the first treponemal antibody test the Treponema pallidum immobilization test (TPI); the test utilizes TP (Nicholls strain) as antigen cultivated in rabbit testes. Sera from syphilitic patients added with complement were able to immobilize live TP at observation in DFM.

The first specific and widely used treponemal test was developed in 1957 [49]. The fluorescent treponemal assay (FTA) then evolved to FTA-abs in order to increase sensitivity and specificity [50].

Tests utilizing red cells agglutination were developed in the late 1960s and the first automated microagglutination test appeared in 1969 [51].

Red blood cells were then substituted by particles in Treponema pallidum particle agglutination assay (TPPA) [52].

The development of specific linked immune essay is the last step in syphilis serology and these tests are now widely used as screening test [53].

Today many TT tests are available on the market. *T. pallidum haemagglutination test (TPHA), micro-haemagglutination assay for T. pallidum (MHA-TP), T. pallidum passive particle agglutination test (TPPA), fluorescent treponemal antibody absorption test (FTA-abs test), treponemal enzyme immunoassay (EIA), chemiluminescence immunoassay (CIA), IgG immunoblot test for T. pallidum*. Most of these tests use recombinant treponemal antigens and detect both IgG and IgM.

FTA-abs test is almost abandoned because is manual, time consuming, and not easy to per-

form. TPHA and TPPA are manual and subject to individual variations in interpretation, but they are cheap and widely used all over the world.

EIA and CIA tests are automated and are ideal test for large samples. Tests become positive in the 1st–2nd weeks of the chancre. Titres of TT are not helpful in the diagnosis or management of syphilis (with possible exception of congenital syphilis). TT should not be used to assess disease activity and treatment outcome and remain positive for life in most patients [54].

IgM Tests

Several tests to assess the presence of specific IgM are available. They have low sensitivity and are of little use in the clinical management of patients. IgM's main usefulness is in the assessment of newborns and CSF [55].

Point of Care Tests

- Many rapid point of care (POC) tests using treponemal antigens have been developed in the last 20 years. Initially tests had suboptimal sensitivity compared to traditional methods, but some of the latest assays have shown a substantially improved sensitivity [56, 57]. New POC tests have better performances or detection of both treponemal and non-treponemal antibodies [58]. Use of rapid POC tests is very important in the WHO strategy for global elimination of congenital syphilis and mother-to-child-transmission (MTCT) of both syphilis and HIV, because they permit screening and treatment at the same visit at field level or peripheral clinics remote from laboratories. Currently, where laboratory diagnostics is available for syphilis in Europe syphilis POC tests are not recommended for use.

Serologic Diagnosis of Syphilis After the Year 2000

Screening: IUSTI Europe guideline for syphilis [54] suggests to use two treponemal tests for screening and confirmation and a non-treponemal test to assess disease activity.

Other strategy for serological testing has been suggested. In particular in the USA a non-treponemal test (RPR) is used as a screening test: traditional algorithm has been recommended by CDC [59]. A reverse algorithm using a treponemal test for screening and a non-treponemal test for confirmation has also been proposed [60].

A study performed in 2014 [61] compared the three algorithms and showed a higher sensitivity of the IUSTI and reverse algorithm. Using CDC screening 665 out of 2749 syphilis diagnosis would have been missed: relevantly 52 in patients with early disease and 390 new diagnoses of syphilis.

Advantages and disadvantages of the three algorithms are reported in Table 8.2.

Our opinion is that the use of the two TT tests for screening is the best method to achieve higher sensitivity and specificity and that NTT should be used only to assess disease activity.

In order to reduce costs we think that TT tests should be performed just on diagnosis and no more utilized; this is not the case in many laboratories in our country and all over Europe.

Table 8.2 Comparison of three selected algorithm screenings for syphilis

Algorithm	Methods	Advantages	Disadvantages
IUSTI Europe algorithm	Different treponemal tests as screening and confirmation	Sensitivity also in early and late phases, specificity, large samples on automated platforms	Risk of overtreatment costs
CDC algorithm	RPR as screening and treponemal test as confirmation	Cheap, only active infections identified	Low sensitivity in early and latent syphilis. Patients with RPR negative disease left untreated
Reverse algorithm	Treponemal test as screening and RPR as confirmation	Suitable for large samples, higher sensitivity in early disease	Need of a high performance treponemal test

CDC Center for Diseases Control and Prevention, *IUSTI* International Union against STI, *RPR* rapid plasma reagin

Table 8.3 Diagnostic test values for syphilis by stage

Stage	Direct methods	Serology
Primary	Always positive (DFM and/or—PCR)	May be negative; positivity within 1–2 weeks from the appearance of lesion
Secondary	DFM and PCR positive usually positive on lesions	All serological test positive (*beware of prozone phenomenon*)[a]
Early latent	Not possible	All serological tests positive. Prozone phenomenon possible
Late latent	Not possible	TT test positive in 97–100%. NTT may be negative

[a]Prozone phenomenon occurs both in RPR and VDRL in case of secondary and early latent syphilis: in this case an excess of antibodies bring to false negative results at initial dilution. Further dilution will turn the test positive. Dilution of sera should be performed in all TT positive tests

Laboratory Diagnosis at Various Stages

In Table 8.3 we summarize the value of diagnostic test for primary, secondary and latent syphilis.

Disease Assessment

Quantitative RPR or VDRL are the best test to assess disease activity and treatment efficacy. Test should be repeated at 1, 3, 6 months, and 1 year until titre negativization or stabilization to a low value (1:1–1:4). Serology testing should be continued in all individuals at risk. Follow-up must be performed also in patients with higher titres.

Diagnosis of Congenital Syphilis

Elimination of mother-to-child-transmission (MTCT) of syphilis is one of the major goals of WHO. WHO estimates that in 2012, 350,000 adverse pregnancy outcomes worldwide were attributed to syphilis, including 143,000 early foetal deaths/stillbirths, 62,000 neonatal deaths, 44,000 preterm/low-birth-weight babies, and 102,000 infected infants. Most untreated primary and secondary syphilis infections in pregnancy result in severe adverse pregnancy outcomes [62].

Diagnosis of congenital syphilis can be confirmed or presumed [54].

A confirmed diagnosis of congenital infection is achieved by the demonstration by DFM or PCR of TP in placenta or autopsy material, exudate from suspicious lesions or body fluids, e.g. nasal discharge.

A presumed diagnosis of congenital infection encompasses several possibilities:

- A stillborn neonate with a positive treponemal test for syphilis.
- Children with a positive treponemal test for syphilis in combination with one or several of the following:
  - persistent rhinitis, condylomata lata, osteitis, periostitis, osteochondritis, ascites, cutaneous and mucous membrane lesions, hepatitis, hepatosplenomegaly, glomerulonephritis, haemolytic anaemia;
  - radiological abnormalities of the long bones suggestive of congenital syphilis;
  - a positive RPR/VDRL test in the cerebrospinal fluid;
  - a fourfold increase or more of the TPPA/TPHA titre in the child's as opposed to the mother's serum (both obtained simultaneously at birth);
  - a fourfold increase or more of the titre of a non-treponemal test in the child's as opposed to the mother's serum (both obtained simultaneously at birth);
  - a fourfold increase or more of the titre of a non-treponemal test within 3 months after birth;
  - a positive anti-treponemal IgM EIA, 19S-IgM-FTA-abs test and/or IgM-immunoblot for *T. pallidum* in the child's serum;
  - a mother, in whom syphilis was confirmed during pregnancy, but who was not adequately treated either before or during pregnancy.
- In a child >12 months of age with a positive treponemal serologic test for syphilis and in whom sexual abuse has been excluded.

In these cases serological tests including IgM tests must be performed from infant blood since umbilical cord blood has lower sensitivity and specificity.

CSF examination and TPPA on CSF must be performed together with other clinical assessment according to the clinical status of the baby.

Laboratory Diagnosis of Neurosyphilis

Diagnosis of neurosyphilis is a difficult and debated task and must consider both clinical and laboratory data. The problem is even more complicate in the settings of HIV-positive individuals (www.who.int/reproductivehealth/publications/rtis/syphilis-ANC-screenandtreat-guidelines) [63–66].

The definition of asymptomatic neurosyphilis is still unclear and there is no consensus on it.

A complete clinical examination is usually recommended in any patient with positive syphilis test but has been demonstrated of little use in asymptomatic subjects [67]

- CSF assessment is not indicated in early syphilis both in HIV positive or negative individuals [68], unless there are neurological, ocular, or auricular symptoms.
- CSF assessment is indicated in patients with:- clinical evidence of neurological, ocular, and auricular involvement, whatever the stage of the disease—tertiary syphilis (cardiovascular, gummatous).

  Although robust data are lacking, CSF control may be indicated also in asymptomatic patients in the following situations for exclusion of asymptomatic neurosyphilis:
  - in HIV-positive patients with late syphilis and CD4+ cells ≤350/mm3 AND/OR a serum VDRL/RPR titre >1:32,
  - in case of serological failure,
  - in case of use of alternative treatment (tetracyclines) during late syphilis,
- Examination of CSF: must include total protein, number of mononuclear cells, a TT (TPHA/MHA-TP/TPPA), and a NTT (VDRL (preferably used)/RPR).
  - Normal protein level is possible in neurosyphilis.
  - The number of mononuclear cells in CSF can be normal in neurosyphilis, especially in parenchymatous neurosyphilis [69, 70]. On the contrary in several situations such as HIV infection mononuclear cells in the CSF can be increased also in the absence of neurosyphilis.
  - A positive CSF VDRL test has low sensitivity (about 33% of cases) but, in the absence of blood contamination has high specificity in late syphilis. In early syphilis the significance of a positive CSF VDRL test is less clear.
  - A positive CSF TT (TPHA/TPPA) does not confirm the diagnosis of neurosyphilis, but a negative CSF TT result is highly unlikely in neurosyphilis [71].
  - Several indexes taking into account blood–brain barrier (albumin) aiming at evaluation of intrathecal synthesis of immunoglobulins have been produced, however, none have been of real practical use.

  CSF PCR for the presence of *T. pallidum* to help establish a diagnosis of neurosyphillis is currently considered of little value since tests to date have shown low sensitivity and specificity [36, 72].
- In case of an abnormal CSF examination (high protein level and/or hypercytosis), repeat CSF examination must be performed after treatment (6 weeks to 6 months).

Treatment

Fundamental issues for a correct management of syphilis are prompt treatment with an effective antibiotic regimen, treatment of sex partners of subjects with infectious syphilis [73]. For latent syphilis there is no risk of transmission and treatment is aimed to treat tertiary manifestations or prevent progression of latent disease to tertiary stage.

Another important point is that people with syphilis are at higher risk of acquiring other STIs and screening for other STIs including HIV is part of the management.

WHO (http://www.who.int/reproductivehealth/publications/rtis/syphilis-treatment-guidelines/en/) and European guidelines [55] are identical. CDC guidelines are slightly different since they do not mention procaine-penicillin as a treatment [74].

General considerations:

A penicillin level of >0.018 mg/L is considered treponemicidal and this level is achieved

during standard treatment but is lower than the maximally effective in vitro level of concentration (0.36 mg/L).

- Duration of treponemicidal level of antimicrobials should be at least 7–10 days to cover a number of division times (30–33 h). In late syphilis there is a lower division time; therefore, longer duration of treatment is needed. Treponemes have been shown to persist despite apparently successful treatment [75]. The significance of this finding, if any, remains unknown.
- In general, long acting BPG 2.4 million units is the treatment of first choice, which provides a treponemicidal penicillin level in blood for up to 21–28 days. However, well-controlled clinical data are lacking on the optimal dose, duration of treatment, and long-term efficacy of all antimicrobials, even for penicillin.
- Treatment recommendations are based mainly on laboratory considerations, biological plausibility, practical considerations, expert opinions, case studies, and past clinical experience.
- Parenteral rather than oral penicillin treatment is the treatment of choice because parenteral therapy is supervised with guaranteed bioavailability. However, amoxicillin, given orally in combination with probenecid appears to be effective and results in treponemicidal drug levels within the CSF [76].
- Among non-penicillin antibiotics doxycycline has been widely used; it has good efficacy and good penetration into the CSF and is taken orally [77]. Newer treatments include intramuscular or intravenous ceftriaxone [78, 79]. Ceftriaxone has good CSF penetration, but dose and duration are not standardized However, like oral doxycycline, daily ceftriaxone injected intravenously or subcutaneously may be an alternative in patients with bleeding disorders. In case of penicillin allergy, use of ceftriaxone may be an option with risk although cross allergies are not frequent. History of anaphylaxis is an absolute contraindication [80]. Azithromycin should not be used since resistance can easily develop and clinical failures have been described in several studies [81–87].
- The importance of the host–immune response is demonstrated by the fact that about 60% of untreated patients will not develop further lesions after the primary stage [88]. CSF involvement is common in early syphilis [65, 89] but the prevalence of neurosyphilis remains low also if treatment does not reach treponemicidal levels in the CSF [66, 90] confirming the active role of host–immune response in the early phases of the disease.
- The control of syphilis over the past 50 years has been excellent compared to the pre-penicillin era. Late complications of syphilis and/or failures of treatment are uncommon, even in patients with concomitant HIV infection.
- It does not seem that immune-suppression alters the severity of the course of syphilis. A closer follow-up is usually recommended for HIV-positive individuals with low CD T cells count (<350/mm^3) and/or for subjects not treated with antiretroviral therapy. HIV coinfection does not appear to increase the risk of developing a more aggressive course of early syphilis [69]. Differences in the presentation of early syphilis in HIV are related to a major number of: (a) multiple chancres; (b) concomitant chancre and secondary eruption and, (c) Herxheimer reaction, in patients infected with HIV. Risk of ocular and neurological involvement is not increased in HIV-positive patients with early syphilis [65]. Data are lacking in late syphilis. Some specialists recommend routine-CSF examination in HIV-positive patients with late syphilis to exclude asymptomatic neurosyphilis, although there are no robust data to support it. Some experts limit the indications of CSF examination to HIV-positive patients with late syphilis and CD4+ cells ≤350/mm^3 and or a serum VDRL/RPR titre >1:32 [64] although there are no robust data to support it [91]. In Tables 8.4 and 8.5 below are listed the penicillin regimen treatment suggested by CDC guidelines 2015 [74] and by IUSTI Europe guidelines 2014 [55].

Management of Syphilis in Pregnancy

As said before congenital syphilis has still a high epidemiological relevance (see page 31) and morbidity is still very high in women with active

Table 8.4 CDC 2015 guidelines

Early syphilis: duration of the disease <1 year (primary, secondary, and early latent)	Benzathine penicillin G 2.4 MUI im as single dose
Late latent (duration of the disease >1 year) or unknown duration	Benzathine penicillin G 7.2 MUI, administered as 3 doses of 2.4 MUI im each at 1-week intervals
Tertiary syphilis with normal CSF examination	Benzathine penicillin G 7.2 MUI, administered as 3 doses of 2.4 MUI im each at 1-week intervals
Neurosyphilis and ocular syphilis	Aqueous crystalline penicillin G 18–24 MUI per day, administered as 3–4 MUI iv every 4 h or continuous infusion, for 10–14 days

syphilis [92]. Standard treatment is widely used but in some countries, (i.e. Russia) a more aggressive course has been suggested [93].

Prevention of congenital screening should be performed through serological screening first antenatal visit (first trimester). Serology should be repeated in case of high risk and local epidemiology.

Adverse Reaction to Treatment

Jarisch–Herxheimer Reaction

- Jarisch–Herxheimer reaction is common after penicillin therapy of early syphilis and is characterized by fever headache, myalgia, chills, and rigors, resolving within 24 h.

Table 8.5 IUSTI Europe 2014 guidelines

Early syphilis (primary, secondary, and early latent, i.e. acquired ≤1 year previously)	First-line therapy option	Benzathine penicillin G (BPG) 2.4 million units intramuscularly (IM) (one injection of 2.4 million units or 1.2 million units in each buttock) on day 1 [Ib; A]
	Penicillin allergy or parenteral treatment refused	Doxycycline 200 mg daily (either 100 mg twice daily or as a single 200 mg dose) orally for 14 days [III; B] or azithromycin 2 g orally single dose [I; B]
Late latent (i.e. acquired >1 year previously or of unknown duration), cardiovascular and gummatous syphilis	First-line therapy option	Benzathine penicillin G (BPG) 2.4 million units IM (one injection 2.4 million units single dose or 1.2 million units in each buttock) weekly on day 1, 8, and 15 [III; B]
	Penicillin allergy or parenteral treatment refused	Desensitization to penicillin or doxycycline 200 mg daily (either 100 mg twice daily or as a single 200 mg dose) orally during 21–28 days [III; B]
Neurosyphilis, ocular and auricular syphilis	First-line therapy option	Benzyl penicillin 18–24 million units IV daily, as 3–4 million units every 4 h during 10–14 days [III; B]
	Second-line therapy option (if hospitalization and IV benzyl penicillin is impossible)	Ceftriaxone 1–2 g IV daily during 10–14 days [III; B] Procaine penicillin 1.2–2.4 million units IM daily AND probenecid 500 mg four times daily, both during 10–14 days [IIb; B]
	Penicillin allergy	Desensitization to penicillin followed by the first-line regimen [III; B]
Syphilis in pregnancy		Pregnant women should be treated with the first-line therapy option appropriate for the stage of syphilis and if allergic to penicillin should be desensitized
Syphilis in HIV		Treatment should be given as for non-HIV infected patients, although there are very few data on the use of second line options
Congenital syphilis	First-line therapy	Benzyl penicillin 150,000 units/kg IV daily (administered in six doses every 4 h) during 10–14 days

- It has usually a benign course unless there is neurological or ophthalmic involvement, in neonates or in pregnancy when it may cause foetal distress and premature labour.
- Prednisolone can prevent the febrile episode [94] the drug should be administered at a dose of 20–60 mg daily for 3 days, starting antitreponemal treatment after 24 h of commencing prednisolone. Also antipyretics can be used to treat the symptoms of Jarisch–Herxheimer reaction.
- Characterized by fear of impending death, may cause hallucinations or fits immediately after injection. Lasts less than 20 min.

Anaphylactic Shock

- Anaphylactic shock is a very rare event but penicillin is one of the most frequent causes. For this reason facilities for treatment of anaphylaxis should be available.

Contact Tracing, Management of Sexual Partners, and Notification of Syphilis Cases

- All cases of syphilis should be interviewed for sexual contact notification and health education [95].
- Sexual contact notification has great importance in reducing the disease burden. Sexual contacts should include all those individuals who have had oral, vaginal, or anal intercourse with infected individuals, whether or not barrier protection was used.
- For primary syphilis partner notification should include partners of the past 3 months. For secondary syphilis and early latent syphilis period should be prolonged to 2 years. Longer periods may be required in those with late latent and late syphilis.
- 46–60% of traced sexual contacts, including pregnant women, of patients with early syphilis are likely to be infected [55].

Follow-Up and Test of Cure

Serological test are the base of follow-up to ascertain cure and detect reinfection or relapse. Globally many studies confirm that follow-up is poor [96].

- Early syphilis, minimum clinical and serological (VDRL/RPR) at 1, 3 months then at 6 and 12 months.
  - After treatment of early syphilis the titre of a NTT (e.g. VDRL and/or RPR) should decline by two dilution steps (fourfold) within 6 months. However, about 15% or more patients with early syphilis and no HIV infection do not have a fourfold decrease of titre at 6 months, the significance of which is unknown.
  - A negative NTT can be obtained in a substantial (but not in all) number of patients treated for early syphilis after 1–2 years. A negative NTT after treatment is considered as the best test of cure.
  - A TT often remains positive for life following effective treatment; proper documentation is necessary to prevent unnecessary retreatment.
- In late (latent) syphilis the serological response of NTTs is often absent. In non-HIV-infected late latent syphilis patients with a reactive NTT, which remains stable in the lowest titre range, follow-up after treatment is generally not indicated.
- An increase in ≥2 dilution steps (fourfold) in a NTT suggests reinfection or reactivation. Treatment should be therefore given.
- Follow-up examination of cerebrospinal fluid should be performed 6 weeks to 6 months after treatment of neurosyphilis [97].

8.7 Conclusions

The epidemic of syphilis started in 2000 and involving mainly MSM has brought to light clinical pictures that we call “atypical” because we have lost the habit to recognize them, but they were well described in old dermatological books. If the epidemiological trend will be confirmed, in the next years physicians will have to confront more and more often with syphilis and its uncommon presentations. A high index of suspicion is necessary in order to avoid diagnostic mistakes and a complete sexual anamnesis should be performed in at-risk people, notably MSM patients.

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Neisseria gonorrhoeae Infections

9

Antonio Cristaudo and Diego Orsini

9.1 Introduction

The *Neisseria gonorrhoeae* is the etiologic agent of gonorrhea and is a human pathogen, which is transmitted during sexual intercourse or by contact with infected biological fluids (e.g., genital secretions or saliva) [1]. The most common gonococcal infections in males affect the urethral mucosa, but rectal, pharyngeal and conjunctival epithelia can also be involved. In women, *N. gonorrhoeae* affects primarily the cervical epithelium but also vaginal tissue can be colonized. The urethral infection in males, or gonorrhea, is also known colloquially as *the clap* or *chaude pisse* (terms that underlined the characteristic burning during urination) in the French language. In men, urogenital infection is generally symptomatic, with the appearance of urethritis in 80% of cases. On the other hand, in women, when the infection affects the cervix, it causes vaginal symptoms, such as discharge, burning sensations, and discomfort. However, in over 50% of women, the infection can persist with unspecific or mild symptoms or be asymptomatic. In both genders, rectal and pharyngeal infections are asymptomatic in 90% of cases. The incubation period of urethral infection has been reported to range from 2 to 4 days after exposure. The brief incubation period of gonorrhea is an effective, hard biological proxy of a very recent sexual behavior at risk, and gonorrhea rates are commonly used as an outcome of effectiveness of preventive behavioral interventions [2].

Since the introduction of antibiotic therapy, *N. gonorrhoeae* has shown a great ability to modify its genome, creating different defense mechanisms against antimicrobial therapies. Some of these are not exclusively based on mechanisms of antimicrobial resistance, but also on an increase in adaptability [1, 3].

9.2 Historical Background

Gonorrhea was known from the earliest times and descriptions in Hebrew, Greek, Roman, and Arabic literature are reported. Firstly interpreted as an impurity, it was clinically and etiologically defined only in the second half of the nineteenth century, after the identification of the specific bacterium by Albert Neisser (1855–1916) in 1879. In the Nei-Ching-Su-Wen (Canon of the Yellow Emperor's internal medicine), drafted as a dialogue between Emperor Huang Ti (2698–2559 BC) and the doctor Tchi Pa, there are references to similar infective urethritis, treated with

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soybeans poultry [1]. In Genesis (Chap. 12), there are some references to a sexually transmitted disease, which could be gonorrhea, which affected Sarah, Abraham's wife, who probably derived her inability to conceive until later age. Leviticus (Chaps. 15 and 22) denounces the contagiousness (even moral) of a disease characterized by continuous emission of semen and painful erection. In Numbers (Chap. 12), a similar illness is described, which had struck thousands of Jews by divine punishment after they had traded with Moabite maidens, who adored the god Baal-Fegor (the Greek-Latin Priapus). Further references to gonorrhea can be found in the c.d. Papyrus from the 18th dynasty (1500 BC), which was discovered in 1862 in Luxor by Georg Moritz Ebers (1837–1898), in which the symptoms of acute urethritis are described, together with the treatment through endo-urethral instillations of sandalwood oil [4].

Hippocrates (458–370 or 356 or 351 BC) described the typical purulent secretion in Aphorisms (I, 56c) and Epidemie (III, 7). Celsus, in De Re Medica (IV, 21, VI, 18), and Pliny, in the Naturalis Historia (XXXI, 23), defined the "profluvia" (drain) from the male genital organs, which Galen (De locis affectis, VI) considered an involuntary loss of sperm, secondary to a weakness of the natural secretory faculty. Lucio Celio Aureliano (third to fourth century AD) coined the term "gonorrhea" ("draining seed") in "*De debilitate seminalium viarum of De morbis acutis et chronicis.*" The methodical Sovereign of Ephesus (II d.C.) in De artem medendi, in the chapter De vitium vesicae, observed the presence of curved mucopurulent filaments in the bottom of the matula (Fuerbringer's comma) [5].

No new knowledge came to light in the following centuries, although there are many authors who seem to mention gonorrhea itself with different denominations (i.e., Inflation virgae, "rheumatisatio virgae," "passio virgae"). The infection seems to re-emerge in London in 1162, where the prostitutes suffering from "burning urine," were forced to be visited by the barber-surgeon [6]. In 1376, the English physician John Arderne (1307–1380), in the manuscript De Arte Phisicali et de Cirurgia, related the term "Incendium virgae" with the popular "chaude pisse" and recommends the use of a jockstrap for testicular swelling [7].

The syphilitic emergency of 1494–1495 generated considerable confusion: the frequent comparison of both affections in the same patient led them to believe that they were connected, considering gonorrhea as an early symptom of syphilis. Only when Philippe Ricord (1800–1889) subdivided the clinical manifestation of syphilis into three stages in 1848, did gonorrhea become clearly distinct. In 1879, the gonococcus was discovered by Neisser, while Ernest Wertheim (1864–1920) determined its specific growth on a blood agar plate inoculated with pus taken from the tubes of infected women.

9.3 Epidemiology

In 2014, 66,413 cases of gonorrhea were reported in 27 European countries, with a 25% increase in the number of cases, compared to 2013 (n = 32,493). The UK reported 58% of all cases in 2014 (Table 9.1). The highest rates observed in 2014 (>15/100,000 inhabitants) were in the UK (60 of 100,000), Ireland (28), Denmark (20), and Latvia (18). The lowest rates (≤1 per 100,000) were observed in Croatia, Cyprus, Luxembourg, and Romania, probably due to a lower efficiency of the national STI surveillance system in these countries [3].

In Europe, the man-woman ratio in 2014 was 2.7: 1 and the incidence rate was 35 per 100,000 among men (45/328 cases) and 10 per 100,000 among women (16/490 cases). Only Estonia reported a male-female ratio of less than 2 (0.7: 1), while the highest was reported in Greece (14.1: 1).

In 2014, information about the most affected age was available for 23 countries, but not available for Bulgaria, Poland, and Spain (8% of all cases). The majority of cases reported in 2014 were among young adults aged 15–24, representing 38% of total cases, while the age between 25 and 34 represented 34% of all cases.

In countries with complete surveillance systems, the rates of cases reported in 2014 were

Table 9.1 Number of reported confirmed cases of gonococcal infections per 100,000 population in 2013 and 2014, by country, EU/EEA

	2013		2014	
Country	Cases	Rate	Cases	Rate
Austria	1148	–		
Belgium	1011	–	1119	–
Bulgaria	96	1.3	170	2.3
Croatia	14	0.3	22	0.5
Cyprus	2	0.2	4	0.5
Czech Republic	1407	13.4	1385	13.2
Denmark	817	14.6	1141	20.3
Estonia	133	10.1	134	10.2
Finland	267	4.9	286	5.2
France	1349	–	1330	–
Germany				
Greece	219	2	245	2.2
Hungary	1526	–	1620	–
Iceland	19	5.9	38	11.7
Ireland	1273	27.7	1304	28.3
Italy				
Latvia	554	27.4	365	18.2
Liechtenstein				
Lithuania	190	6.4	165	5.6
Luxembourg	4	0.7	5	0.9
Malta	62	14.7	51	12
Netherlands	4171	–	10,729	–
Norway	506	10	682	13.4
Poland	549	1.4	495	1.3
Portugal	121	1.2	201	1.9
Romania	340	1.7	178	0.9
Slovakia	378	7	423	7.8
Slovenia	62	3	61	3
Spain	3315	7.1	4562	9.8
Sweden	1110	11.6	1337	13.9
UK	32,493	50.8	38,361	59.7
EU/EEA total	53,136	17	66,413	20

Modified from [3]

higher between 20 and 24 years (107 per 100,000 inhabitants), while for women the rate between 15 and 19 years was slightly lower. The highest age and gender rates were among males between the ages of 20 and 24 (145 out of 100,000).

In 2014, in the group of 15 countries which reported data on the mode of transmission, the heterosexual population represented 49% of all cases; 44% of the cases concerned men who have sex with men (MSM), while the remaining 7% of cases were reported as "unknown." The cases diagnosed in MSM represented 65% (n = 24,960) of all male cases diagnosed in these countries in 2014.

Among European MSMs with gonococcal infections (23,906 cases), 24% (5659 cases) were HIV-infected, 63.0% were HIV negative and there was no data for 13.0%.

In 2014, for the first time since ECDC collected data, the number of cases of *N. gonorrhoeae* infections among women was higher than the number of cases among heterosexual men. These increases are worrying considering the risk of reproductive tract complications among women and the perinatal transmission of gonorrhea. These increases need to be further assessed because test patterns, such as the increased use of Nucleic Acid Amplification Tests (NAAT) that allow screening for chlamydia and gonorrhea, may have contributed to this increase.

The increase in the number of cases of gonorrhea reported by the Member States in recent years is also worrying due to the development of resistant strains. The latest data from the European gonococcal antimicrobial surveillance program suggest stable levels of cefixime resistance and no significant increase in ceftriaxone resistance. Resistance to azithromycin, however, appears to be increasing, and the development of resistance to third-generation cephalosporins is only a matter of time.

9.4 Physiology and Structure

The Neisseria genus includes ten species that have colonized humans and two of these are particularly pathogenic: *N. gonorrhoeae* and *N. meningitidis* includes several distinct groups based on their capsules, and some of them are more common (A, B, C, Y, and W135). They are aerobic bacteria (the pathogenic species require an environment with 5–10% CO2), gram-negative cocci, arranged in pairs (diplococci). They are immobile and do not form endospores. All species are oxidase-positive and most of them produce catalase. *N. gonorrhoeae* produces acid oxidizing glucose, while *N. meningitidis* oxidizes both glucose and maltose [8, 9]. *N. meningitidis*

shows variable growth on agar plates, while *N. gonorrhoeae* is a demanding microorganism, which requires a complex plate for growth. All *N. gonorrhoeae* strains require cysteine and a source of energy (glucose, pyruvate, lactate) to grow and many strains require a supplementation of the plate with amino acids, vitamins, purines and pyrimidines. For this reason, *N. gonorrhoeae* does not grow on blood agar plates but rather on an agar-chocolate plate and other enriched preparations. The optimal growth temperature ranges from 35 °C to 37 °C.

The major virulence factor for *N. meningitidis* is the polysaccharidic capsule and antigenic differences are at the basis of the subdivision into serogroups of these bacteria.

They also possess pili that mediate a range of functions, including adherence to host cells, transfer of genetic material, and motility (Fig. 9.1). They are composed of repeated protein subunits called pilin [9–14].

Porin proteins form pores on the external membrane and *N. gonorrhoeae and N. meningitidis* have two genes which codify porins, porA and porB. However, porA is silent in *N. gonorrhoeae*: porB is the most expressed external membrane protein and it must be functional in order for bacterial survival. PorB is important for the virulence of *N. gonorrhoeae*. PorB proteins can interfere with neutrophil degranulation and facilitate epithelial cell invasion.

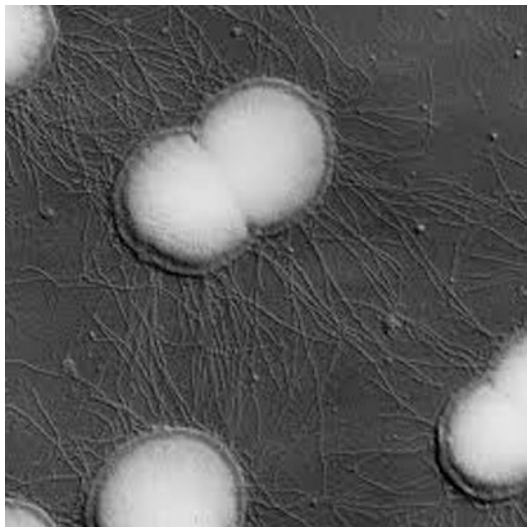

Fig. 9.1 *N. gonorrhoeae* magnified by electronic microscopy

Opa proteins (opacity proteins) are a series of membrane proteins that mediate binding with epithelial cells and phagocytes. *N. gonorrhoeae* appears opaque in culture when it expresses these proteins and their presence indicates a disease index. A third group of proteins of the external membrane is represented by Rmp proteins, which stimulate antibodies that block bactericidal activity against Neisseria.

Iron is required for their growth [14–16].

N. gonorrhoeae and N. meningitidis are able to compete with iron, binding the transferrin of host cells to specific surface bacterial receptors. The specificity of these bonds explains why these bacteria are pathogenic.

Another antigen in the cell wall is lipooligosaccharide (LOS), which is composed of lipid A and a central oligosaccharide but without the O-antigenic polysaccharide observed in LPS.

The portion of lipid A has endotoxin activity.

Both *N. gonorrhoeae* and *N. meningitidis* release external membrane vesicles during growth. These vesicles contain LOS and surface proteins and can enhance endotoxin-mediated toxicity and protect bacteria in replication, binding antibodies to proteins.

N. gonorrhoeae and *N. meningitidis* produce an IgA1 protease, which splits the hinge region of the IgA1. The gonococci adhere to the mucous membranes, penetrate into the cells and multiply, then pass through the cells in the subepithelial space where the infection is established [16–20].

9.5 Adherence, Colonization, and Invasion

After transmission, *N. gonorrhoeae* can replicate within the epithelium of the mucosa and it can be subsequently transmitted to new humans. *N. gonorrhoeae* is primarily a mucosal colonizer.

The primary event that determines infection and the first step in pathogenesis is bacterial adherence to the mucosal epithelium, which is mediated by distinct surface bacterial structures that include type IV pili, opacity proteins (Opa),

lipooligosaccharide (LOS), and the main porin protein of the external membrane (also known as PorB) [8–14].

During early infection, the *N. gonorrhoeae adheres to the host epithelial cells through type IV pili* (phase 1), which enables epithelial interactions with other surface structures**.** After initial adhesion, it *replicates and forms microcolonies* (phase 2), *biofilm*, and probably competes with the resident microbiota.

During this early phase of infection, *N. gonorrhoeae releases fragments of peptidoglycan, lipooligosaccharide (LOS), and external membrane vesicles (OMV) (phase 3), which* activate the Toll-like receptor (TLR) and the protein containing the nucleotide binding oligomerization (NOD-mediated signaling) in epithelial cells, macrophages, and dendritic cells (DC). The signal mediated by binding with NOD and TLR leads to *the activation of inflammatory transcription factors and to the release of cytokines and chemokines (phase 4)*.

N. gonorrhoeae also releases heptose-1,7-bisphosphate (HBP), which *activates the protein that interacts with TRAF and with the protein A (TIFA) containing the FHA115 domain (step 5).*

The release of proinflammatory cytokines and chemokines by these immune signaling *pathways creates gradients of cytokines and chemokines that recruit a large number of neutrophil polymorphonuclear leukocytes at the site of infection (phase 6). The neutrophil flow creates a purulent exudate that facilitates the transmission (phase 7)* [20–25].

The interactions between the Opa proteins and the receptors of the CEA-related cell adhesion molecules (CEACAM) are fundamental for bacterial adherence [26–30]. This phenomenon occurs immediately after contact with the type IV pili and the subsequent immune evasion which occurs through the antigenic variation [30–32]. Type IV pili and Opa proteins are expressed during the infection of women and men and are considered essential for the colonization of the epithelium of the genital tract. The role of *N. gonorrhoeae* in the development of biofilm during infection has yet to be clarified. In fact, it is not clear whether mucosal colonization during infection is mediated by bacterial microcolonies, biofilm formation or a combination of both.

The surface factors, porin and LOS, also influence colonization. Porin, which is a nutritive channel and one of the most abundant protein components of the external gonococcal membrane, binds the C4b-binding protein (C4BP) of the complement, the H factor, and suppresses the "oxidative burst" and the apoptosis of neutrophils [31]. The lipo-oligo-saccharide (LOS), located on the external membrane, is important for adherence and invasion of host cells. Changes in LOS influence the recognition of immune cells and LOS sialylation influences resistance through evasion of the complement [31, 32]. In addition to the colonization of the mucous epithelium, *N. gonorrhoeae* can invade the epithelial cells. Although mucosal invasion is less known than superficial colonization, *N. gonorrhoeae* invades the non-ciliary cervical epithelial cells and the urethral epithelial cells of men when LOS is declined [32].

The interaction between LOS and asialoglycoprotein receptors promotes epithelial invasion into the male urethra, while the complement receptor 3 (CR3) acts as a receptor that mediates the invasion of the lower cervical genital tract and the hormone receptor lutropin-choriogonadotropin acts as a receptor in the endometrium and in the fallopian tubes. This invasion of the epithelium and the consequent transcytosis of the epithelium lead to the dissemination of the gonococcal infection. Afterwards, *N. gonorrhoeae* adheres to the epithelium of the mucosa; Colonization requires extracellular bacterial replication and acquisition of nutrients from the surrounding extracellular environment. Microenvironments have not been precisely determined and, therefore, the exact nutritive composition of each ecological niche of *N. gonorrhoeae* during urogenital, rectal, and oropharyngeal infection is not known.

In culture, *N. gonorrhoeae* cannot grow without an integrated source of glucose, glutamine, thiamin, phosphate, iron, and carbon dioxide [32]. To satisfy its nutritional needs, *N. gonorrhoeae* must therefore interact and compete with the available nutrient microbiota.

To adapt to the urogenital, rectal, and oropharyngeal environment during infection, *N. gonorrhoeae* has a "regulation network." In particular, it has regulators that specifically respond to the amount of metal, antimicrobial peptides, oxygen amounts, and membrane stress. The main two-component regulation system consists of the histidine kinase sensor and the response regulator (MisR-MisS), which responds to perturbations and changes in membrane homeostasis [32].

9.6 Clinical Presentation

Gonococcal infections include urethral, anorectal, pharyngeal, and conjunctival infections in males and females. The main described risk factors for the acquisition of gonorrhea are summarized in Box 9.1. The differential diagnosis depends on the mucocutaneous site. Untreated infections can lead to pelvic inflammatory disease (PID), disseminated gonococcal infections, and, although rare, endocarditis and meningitis. Co-infection with *C. trachomatis* in the same site, is frequent.

Box 9.1 Main Described Risk Factors for Gonorrhea

Adults
- Age less than 25 years
- Black race
- History of gonococcal infection or other sexually transmitted infections (STI)
- Sex between MSM
- New or multiple sexual partners
- Prostitution
- Drug abuse or misuse
- Unprotected oral sex

Children
- Mothers with a history of STI or substance abuse
- Mothers without prenatal care
- Non-birth prophylaxis for neonatal ophthalmopathy
- Sexual abuse

9.6.1 Infection in Men

Men with gonorrhea are usually symptomatic, but asymptomatic urethral infections may occur in at least 10% of cases [33]

When symptoms occur, they typically appear in 2–4 days after contagion, but, sometimes, they do not appear for ore 30 days.

Common signs and symptoms include dysuria and white purulent penile secretion (Fig. 9.2). Unilateral epididymitis may also be present in the absence of secretions [34, 35].

9.6.2 Infection in Women

More than 95% of women with gonorrhea have no symptoms. The infection may be localized at the endocervix, Bartholin's glands, Skene's ducts, vagina, rectum or pharynx [33].

The most common manifestation is cervicitis, which usually occurs within 10 days after infection.

Ten to twenty percent of women with cervical gonorrhea have, also, a pharyngeal infection. If the symptoms occur, they are generally mild and can mimic acute cystitis or vaginitis. Metrorrhagia can also be a rare sign of acute gonococcal infection.

Untreated gonorrhea causes 10–20% cases of PID, and 15% of women with PID develop infertility due to tubal outcomes. Half of the patients who had three or more episodes of PID develop

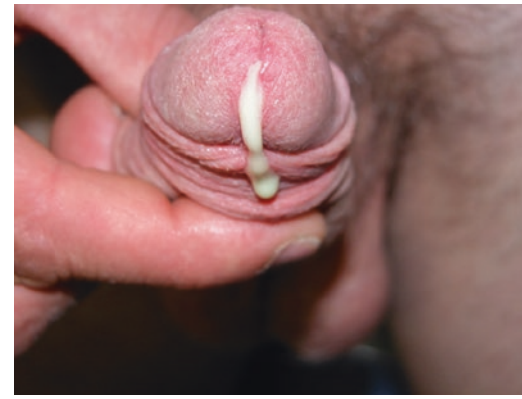

Fig. 9.2 Classical white discharge from urethra in male with gonorrhea

infertility. The CDC, for symptomatic women, recommends an accurate visit in order to test cervical or adnexal tenderness in suspected PID.

Gonorrhea, chlamydia, bacterial vaginosis, and trichomoniasis tests with endocervical swabs for optical microscopy and subsequent DNA amplification should be performed. Since optical microscopy presents only 50% sensitivity for Trichomonas vaginalis, CDC recommends the performance of culture tests [36].

9.6.3 Anorectal Infection

More than 50% of rectal *N. gonorrhoeae* infections in men and women may be asymptomatic.

The highest prevalence of anorectal gonorrhea is in men who have sex with men (MSM) [37].

Symptoms include anal itching, rectal pain, mucopurulent secretion, and tenesmus.

Untreated acute infections cause symptomatic proctitis. The CDC recommends DNA amplification as a gold standard.

9.6.4 Pharyngeal Infection

Gonorrhea can be transmitted through unprotected sexual contact with the oropharynx.

Approximately 90% of pharyngeal gonorrhea cases may be asymptomatic, both in men and women [37].

Twenty percent of women with cervical gonorrhea have a pharyngeal co-infection.

Pharyngeal infections are also common in men who have sex with men (MSM) and infected with the human immunodeficiency virus (HIV).

Physical examination may reveal oropharyngeal erythema or exudate and cervical lymphadenopathy.

Oropharyngeal gonorrhea is more difficult to treat than urogenital and endocervical infection.

The oral cephalosporins are effective in 90% of cases, compared to 99% for intramuscular ceftriaxone (Rocefin).

Patients are rarely coinfected with pharyngeal Chlamydia: however, since genital co-infection is possible, CDC recommends treatment for both pathogens.

9.6.5 Disseminated Infection

Disseminated gonococcal infection is rare and affects 0.4–3% of patients with gonorrhea 4, but it is the most common cause of infectious arthritis in sexually active and previously healthy patients [33]. Approximately 15% of patients have joint pain as a primary symptom. For this reason, gonococcal infection should be always considered. Acute reactive arthritis in a sexually active young adult is, in most cases, caused by disseminated gonococcal infection.

Skin lesions are present in 75% of patients with bacteremia and include petechiae, macules, papules, pustules, vesicles, and bullae.

At the onset, some patients have asymmetric arthropathies or polyarthralgia.

The joints that are commonly affected by tenosynovitis or septic arthritis include the wrists, ankles, hands, and feet. The progression of this disease can lead to peripatitis, meningitis, or endocarditis [33].

9.6.6 Neonatal Infection

Gonococcal infections may occur in newborns after neonatal exposure to infected cervical secretions during passage through the birth canal.

Sepsis, neonatal conjunctivitis (neonatal ophthalmia), meningitis, and arthritis are the most serious complications. Other manifestations include pharyngitis, rhinitis, vaginitis, urethritis, and, rarely, pneumonia.

Infants can develop localized scalp infections or skin abscesses. Up to 30% of neonatal conjunctivitis are caused by *C. trachomatis*, although gonorrhea still causes two or three cases per 10,000 live births. Other bacteria such as *Haemophilus influenzae*, *Escherichia coli*, *Staphylococcus aureus* can also be the cause.

Gonococcal conjunctivitis has an incubation period of 6 days [38].

Gram stains of conjunctival secretions show high white blood cell counts or intracellular gram-negative diplococci and they are sufficient to initiate treatment, although only cultures provide a definitive diagnosis. The cultural exam

must be performed in the child and in the mother, for gonorrhea and chlamydia. If sepsis is suspected, blood smears, cerebrospinal liquor, or joint aspiration may be required.

Treatment of neonatal ophthalmopathy is important to prevent globe perforation and blindness. The United States Preventive Services Task Force (USPSTF) recommends that all newborns receive the prophylactic topical eye drug against gonococcal ophthalmia of the newborn [39].

9.6.7 Infection in Children

In preadolescent children, gonococcal infection is probably indicative of sexual abuse. Vaginitis is the symptom that occurs in preadolescent girls. Pharyngeal and cutaneous infections can co-exist with vaginitis but they are often asymptomatic. Samples from all sites should be collected. Gram stains are inadequate for diagnosis in children. Culture techniques remain the preferred tests and nucleic acid amplification testing may be appropriate if culture swab collection cannot be performed. If gonorrhea is suspected, children should be evaluated for co-infection with HIV, chlamydia, and syphilis [38].

9.6.8 Screening and Prevention

High-risk patients and those that live in a high prevalence area should perform routine screening tests for ISCs17. USPSTF recommends routine screening for gonorrhea in all sexually active women with high risk of infection, including pregnant women, but it recommends screening tests for high-risk men and women [44]. Routine screening for gonorrhea and other sexually transmitted diseases should be performed at least once a year in sexually active patients with HIV infection. The CDC recommends that men who have sex with men should be screened at least once a year for gonorrhea in urethral, rectal, and pharyngeal sites for a history of anal receptive sex and oral-oral sex, respectively. Urinary tract infections should be performed with urinary nucleic acid amplification tests, while rectal or pharyngeal screening should be performed with a nucleic acid amplification buffer. Screening every three to 6 months is also recommended for men who have sex with men if they have multiple or anonymous partners because they are at higher risk of contracting STIs [43–45]. Condom use can reduce the risk of gonorrhea and other sexually transmitted infections. It also reduces the risk of PID by decreasing infections of the lower genital tract. According to observational studies, the use of a diaphragm can protect against cervical gonorrhea. Individual and group prevention counseling effectively reduces the risk of contracting gonorrhea. USPSTF recommends high-intensity behavioral counseling for all sexually active adolescents and adults at increased risk of STI. High-intensity behavioral counseling refers to multiple sessions in a context of primary care or clinical STI. Intensive counseling also increases adherence to treatment in women and contraceptive use in adolescent males, and reduces non-sexually risky behavior and pregnancy in sexually active adolescent females. Intensive behavioral interventions which are appropriate to culture may be more effective than standard prevention advice in reducing the re-infection rate with gonorrhea (number needed to treat = 12) [43–46].

9.7 Diagnosis

The diagnosis of gonorrhea is based on the identification of *N. gonorrhoeae* in genital, rectal, pharyngeal, or ocular secretions.

Gram-stained or methylene blue microscopy has good sensitivity (≥95%) and specificity as a rapid diagnostic test in symptomatic men with urethral secretion [34]. Microscopy has poor sensitivity (≤55%) in asymptomatic men and in the identification of endocervical (≤55%) or rectal infections (≤40%) and it cannot be recommended as a diagnostic test in these situations [34–36].

The culture test is a specific and cheap diagnostic test that allows a rapid identification of the pathogen and the antibiotic susceptibility test. The use of selective culture plates supplemented with antibiotics [47] (level of evidence III, grade

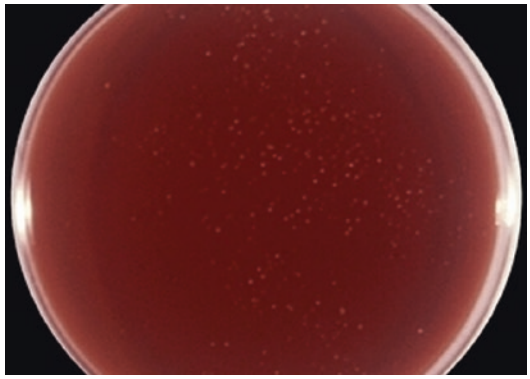

Fig. 9.3 Growth of colonies of diplococci of *N. gonorrhoeae* by selective Thayer-Martin medium

B) is recommended (Fig. 9.3). The culture is indicated for samples taken from endocervix, urethra, rectum, and the pharynx.

The sensitivity of the culture test is high for samples taken in the genital site, provided that the collection, transport and storage of the sample are adequate. Adequate quality control is necessary in view of the fact that commercially available culture plates vary in selectivity and sensitivity. The culture test should be performed in case of persistence of symptoms after treatment. NAATs are generally more sensitive (≥90%) than culture [48, 49]. They can be used on urine samples, vaginal tampons self-taken by the patient and endocervical and urethral swabs. In women, the sensitivity of NAAT tests on urine samples is less than that of tests performed on swabs taken from the genital tract: these tests are therefore not recommended. Samples that are positive for a NAAT test should be tested for confirmation [50, 51], for example, through the application of NAAT for the search for a different sequence (level of evidence III, recommendation level B). The positive predictive value of an unconfirmed positive NAAT result in low prevalence populations is sub-optimal. NAATs are significantly more sensitive than culture on samples obtained from pharyngeal or rectal swabs [52].

However, commercially available tests are not approved for use on the aforementioned materials. Their possible use should necessarily be accompanied by a confirmation test (level of evidence IIb, recommendation of grade B) [52]. Women may have a localized or urethral endocervical infection. A single endocervical or vaginal specimen assessed with NAAT has sufficient sensitivity (90%) when used as a screening test [53, 54].

A minority of MSM with gonorrhea (20–30%) has an infection in multiple sites [47].

Samples should be taken from the urethra or urine, rectum, pharynx, guided by the anamnesis related to sexual habits.

Test indications (level of evidence IV, grade C recommendation):

- Symptoms or signs of urethral secretion in men;
- Vaginal secretions associated with risk factors for sexually transmitted infection (age <30 years, new sexual partner);
- Mucus-purulent cervicitis;
- Sexual partner of person with recent diagnosis of sexually transmitted infection (STI) or pelvic inflammatory disease (PID);
- Epididymal acute orchitis in males aged less than 40 years;
- Acute PID;
- Screening for STI in young adults;
- Screening in subjects with new sexual partners or multiple sexual partners;
- Purulent conjunctivitis in the newborn. Management Information and advice for the patient;
- The patient should be advised to avoid sexual relationships until the patient and the partner have completed treatment and are asymptomatic (level of evidence IV, grade of recommendation C);
- A detailed explanation of the infection should be provided to the patient, accompanied by a clear written information document (level of evidence IV, grade of recommendation C).

9.8 Treatment

Antibiotic resistance is the main determinant of treatment efficacy and is responsible for severe limitations of treatment options [55–58].

The resistance of *N. gonorrhoeae* to antimicrobials is extended in Europe; in vitro resistance to penicillin, tetracyclines, and quinolones is more than 10% of isolates in many countries [59, 60]. Resistance to azithromycin is on the rise, with a high level recently reported in the UK [34–36, 40–62]. However, there is a significant geographical variability of the diffusion of resistant strains. For this reason, it is plausible to select different therapeutic options based on the resistance data provided by the local surveillance systems.

Thanks to the progressive tendency to the development of antibiotic resistance and in accordance with the European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults [63] (Box 9.2), gonococcus swab with antibiogram is recommended before starting therapy and a combined antibiotic treatment.

Box 9.2 Recommended Treatments for Uncomplicated *N. gonorrhoeae* Infections of the Urethra, Cervix, and Rectum in Adults and Adolescents When the Antimicrobial Sensitivity of the Infection is Unknown (Modified from Bignell and Unemo [63])

Recommended treatments for uncomplicated *N. gonorrhoeae* infections of the urethra, cervix and rectum in adults and adolescents when the antimicrobial sensitivity of the infection is unknown	Ceftriaxone 500 mg intramuscularly (IM) as a single dose *together with* azithromycin 2 g as single oral dose *Alternative regimens* 1. Cefixime 400 mg oral as a single dose together with azithromycin 2 g as a single oral dose. This regimen is only an alternative option if ceftriaxone is not available or administration of injectable antimicrobials is not possible or refused by the patient 2. Ceftriaxone 500 mg IM as a single dose. This regimen is only an alternative option if azithromycin is not available or patient is unable to take oral medication 3. Spectinomycin 2 g IM as a single dose *together with* azithromycin 2 g as a single oral dose. This regimen can be used if resistance to extended-spectrum cephalosporins is identified or suspected, or patient has history of penicillin anaphylaxis or cephalosporin allergy
Therapy for uncomplicated gonococcal infection of the pharynx	• Ceftriaxone 500 mg IM as a single dose together with azithromycin 2 g oral single dose *Alternative regimens* • Ceftriaxone 500 mg IM as a single dose. This regimen is only an alternative option if azithromycin is not available or patient is unable to take oral medication Alternative treatments for pharyngeal infection when there is a history of penicillin anaphylaxis or cephalosporin allergy and fluoroquinolone or azithromycin resistance are excluded by appropriate laboratory susceptibility testing – Ciprofloxacin 500 mg as a single oral dose or ofloxacin 400 mg as a single oral dose or azithromycin 2 g as a single oral dose
Therapy of genital, anorectal, and pharyngeal gonococcal infection when extended-spectrum cephalosporin resistance is identified	Ceftriaxone 1 g IM as a single dose *together with* azithromycin 2 g oral single dose Gentamicin 240 mg IM as a single dose *together with* azithromycin 2 g oral as single dose. This combination is currently under clinical study and may be valuable if infection persists after treatment with ceftriaxone. Gentamicin has been successfully used in Malawi, Africa for many years (mainly in syndromic management administered together with doxycycline) and high in vitro susceptibility in Europe has been proven. However, randomized, quality assured clinical trials need to confirm the efficacy of this treatment regimen
Therapy of gonococcal infections in pregnancy or when breastfeeding	• Ceftriaxone 500 mg IM as a single dose *Alternative regimen* • Spectinomycin 2 g IM as a single dose (has poor efficacy for treatment of pharyngeal gonorrhoea)

Therapy of gonococcal infections in patients with penicillin allergy	Third-generation cephalosporins show negligible cross-allergy with penicillins and allergy to these cephalosporins is rare. If allergy is not excluded and third-generation cephalosporins still need to be given, the patient should be under medical supervision for at least 30 min. Recommended treatment for patients with a history of penicillin anaphylaxis or cephalosporin allergy: Spectinomycin 2 g IM as a single dose together with Azithromycin 2 g oral single dose Alternative treatments in patients with known penicillin anaphylaxis or cephalosporin allergy when fluoroquinolone or azithromycin sensitivity has been confirmed by appropriate laboratory susceptibility testing • Ciprofloxacin 500 mg oral as a single dose or ofloxacin 400 mg oral as a single dose or azithromycin 2 g as a single oral dose
Therapy for upper genital tract gonococcal infection Gonococcal epididymo-orchitis	Ceftriaxone 500 mg IM as a single dose *together with* doxycycline 100 mg oral dose twice daily for 10–14 days Ciprofloxacin 500 mg as a single oral dose may be used as an alternative to ceftriaxone when sensitivity confirmed by appropriate laboratory susceptibility testing
Gonococcal pelvic inflammatory disease	Ceftriaxone 500 mg IM as a single dose *together with* doxycycline 100 mg oral dose twice daily *together with* metronidazole 400 mg oral dose twice daily for 14 days
Therapy for disseminated gonococcal infection	Initial therapy 1. Ceftriaxone 1 g IM or IV every 24 h 2. Spectinomycin 2 g IM every 12 h Therapy should continue for 7 days, but may be switched 24–48 h after symptoms improve to one of the following oral regimens a. Cefixime 400 mg oral dose twice daily or if fluoroquinolone sensitivity is confirmed by appropriate laboratory susceptibility testing b. Ciprofloxacin 500 mg oral dose or ofloxacin 400 mg oral dose, twice daily
Therapy for gonococcal conjunctivitis	A three-day systemic regimen is recommended as the cornea may be involved and is relatively avascular. The eye should be irrigated with sterile saline solution once. Ceftriaxone 500 mg IM as a single dose daily for 3 days If history of penicillin anaphylaxis or cephalosporin allergy • Spectinomycin 2 g IM as a single dose daily for 3 days • Or, if antibiotic susceptibility testing at laboratory has excluded resistance • Azithromycin 2 g oral as a single dose together with doxycycline 100 mg oral dose twice daily for 1 week together with ciprofloxacin 250 mg oral dose daily for 3 days
Therapy for ophthalmia neonatorum (gonococcal neonatal conjunctivitis)	The eye should be irrigated frequently with sterile saline solution • Ceftriaxone 25–50 mg/kg IV or IM as a single dose, not to exceed 125 mg

9.8.1 Partner Notification and Counseling

Sexual partners should be contacted and tests for gonorrhea and chlamydia infection should be offered, in addition to their treatment (level of evidence IV, grade of recommendation C).

In cases of gonorrhea, all sexual partners of the 60 days before diagnosis should be evaluated and treated [60] (level of evidence IV, grade of recommendation C). If the patient's last sexual relationship dates back more than 60 days before the diagnosis, the last sexual partner should be evaluated.

9.8.2 Follow-Up and Care Tests

A clinical evaluation after treatment is recommended to confirm adherence to therapy, resolution of signs and symptoms and communication and counseling to the partner (level of evidence IV, grade of recommendation C).

A treatment test is not routinely recommended for gonococcal anogenital infection if the patient has been treated with an appropriate therapeutic regimen [60]. Instead, the treatment test is indicated in the following situations (level of evidence IV, grade of recommendation C): persistence of symptoms; further exposure to infection; suspicion of resistance to the antibiotic used for the treatment; when recommended by local or national guidelines; pharyngeal infection.

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Novel Approach to *Chlamydia trachomatis* Infections in Female and Male

10

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10.1 Introduction

Chlamydial infection is a major public health concern globally [1]. The World Health Organization (WHO) estimated the global prevalence of several sexually transmitted infections (STIs) among individuals aged 15–49 years every 5 years. The study presented a pooled prevalence for chlamydia worldwide in 2012 of 4.2% (95% UI: 3.7–4.7%) in women, with regional values ranging from 1.8% to 7.6%, and 2.7% (95% UI: 2.0–3.6%) in men, with regional values that ranged from 1.3% to 5.2%. Regions of the Americas and Western Pacific were the most affected areas [2].

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Chlamydia is the most frequently reported infectious disease in the USA [3] and its estimated prevalence in Europe is statistically consistent with findings in other high income countries [4], despite the fact that most population based studies are considered at risk of participation bias [5].

The main risk factors for acquisition of *C. trachomatis* infection are young age (individuals aged <25 years tend to have the highest prevalence of the infection) and behavioural factors such as prior *C. trachomatis* infection, inconsistent condom use, and new or multiple partners per year. A consistent association was found between socioeconomic disadvantage, race, ethnicity, residence in deprived areas, and chlamydia infection, due to lack of access to screening programmes, awareness on safe sex and condom use, and higher prevalence of substance use [6].

A statistically significant difference in prevalence between the two sexes is reported. In 2016, case rate in females was about two times the one in males, most probably due to the larger number of women screened for this infection. The increasing use of urinary highly sensitive nucleic acid amplification tests (NAATs) shows a larger number of men infected, even though many men are still not receiving a diagnosis of chlamydia or being reported [7]. Different studies demonstrated that >53% of couples in which at least one partner had chlamydia were concordant for the infection and that the concordance correlated with higher bacterial loads [8, 9].

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Men who have sex with men are considered at high risk for STI. In 2016, the median site-specific prevalence of urogenital *C. trachomatis* among MSM tested at select STI clinics in the USA was 6% (range by site: 3–15%) [7].

Coinfections with *C. trachomatis* and other urogenital sexually transmitted pathogens have frequently been reported among high-risk men and women, especially *Neisseria gonorrhoeae*, *Trichomoniasis*, *Mycoplasma genitalium*, and HIV. Recent studies exhibited a possible role of *C. trachomatis* in enhancing the malignant potential of HPV, through the instauration of a chronic inflammatory environment [10, 11].

As asymptomatic infection is common among both men and women and immunity is short-lived, reinfection or persistent infection is also common.

10.2 Clinical Manifestations of *Chlamydia trachomatis* Infections

Chlamydia is known as a "silent" infection because most infected people are asymptomatic and lack abnormal physical examination findings. Estimates of the proportion of chlamydia-infected people who develop symptoms vary by setting and study methodology; two published studies that incorporated modelling techniques to address limitations of point prevalence surveys estimated that only about 10% of men and 5–30% of women with laboratory-confirmed chlamydial infection develop symptoms.

10.2.1 Clinical Syndromes in Women

10.2.1.1 Cervicitis

In women, the bacteria initially infect the cervix, where the infection may cause signs and symptoms of cervicitis such as:

- Purulent or mucopurulent vaginal discharge and/or
- Intermenstrual vaginal bleeding
- Post-coital bleeding
- Dyspareunia
- Vulvovaginal irritation
- Dysuria, urinary frequency

10.2.1.2 Urinary Symptoms

They are generally due to concomitant urethral infection, which occurs in approximately 15% of women with cervical chlamydia infection.

- Dysuria
- Urinary frequency

10.2.1.3 Pelvic Inflammatory Disease (PID)

Infection can spread from the cervix to the upper reproductive tract (i.e., uterus, fallopian tubes), causing pelvic inflammatory disease (PID), which encompasses a wide spectrum of clinical presentations.

- Lower abdominal pain is the cardinal symptom.

The abdominal or pelvic pain is usually bilateral and rarely of more than 2 weeks' duration [7]. The character of the pain is variable, and in some cases, may be quite subtle. The recent onset of pain that worsens during coitus or with jarring movement may be the only presenting symptom of PID. The onset of pain during or shortly after menses is particularly suggestive. On bimanual pelvic examination, physical examination, cervical motion tenderness or uterine tenderness or adnexal tenderness is the defining characteristic of acute symptomatic PID.

PID diagnostic criteria per CDC guidelines (2015)	
Minimal criteria[a]	Cervical motion tenderness
	Uterine tenderness
	Adnexal tenderness
Additional criteria[b]	Oral temperature greater than 101 °F (38.3 °C)
	Abnormal cervical mucopurulent discharge or cervical friability
	Abundant white blood cells on microscopic evaluation of vaginal fluid
	Elevated erythrocyte sedimentation rate
	Elevated C-reactive protein
	Laboratory documentation of cervical infection with *N. gonorrhoeae* or *C. trachomatis*

PID diagnostic criteria per CDC guidelines (2015)	
Specific criteria[c]	Endometrial biopsy with histopathologic evidence of endometritis
	Transvaginal ultrasound or magnetic resonance imaging showing thickened, fluid-filled tubes with or without free pelvic fluid or tubo-ovarian complex, or Doppler studies suggesting pelvic infection
	Laparoscopic findings consistent with PID

CDC US Centers for Disease Control and Prevention, *PID* pelvic inflammatory disease

[a]Initiate treatment if one or more of these criteria are met

[b]In addition to one or more minimal criteria, one or more of the additional criteria increases specificity of the diagnosis of PID

[c]One or more of these criteria provides the most specific diagnosis of PID

10.2.1.4 Perihepatitis (Fitz-Hugh–Curtis Syndrome)

Fitz-Hugh–Curtis syndrome is a rare disorder that occurs almost exclusively in women. It is characterized by inflammation of the membrane lining the stomach (peritoneum) and the tissues surrounding the liver (perihepatitis).

The syndrome is characterized by the onset of sudden, severe pain in the upper right area of the abdomen. Pain may spread to additional areas including the right shoulder and the inside of the right arm. Movement often increases pain. The upper right area may be extremely tender.

Additional symptoms may occur in some cases including fever, chills, night sweats, vomiting, and nausea. Some affected individuals may develop headaches, hiccupping, and a general feeling of poor health (malaise).

The pathogenesis of this entity is not fully understood but may involve either direct extension of infected material from the cul-de-sac through the peritoneum and/or lymphatics, or an immunologically mediated mechanism.

On laparoscopy or visual inspection, perihepatitis manifests as a patchy purulent and fibrinous exudate ("violin string" adhesions), most prominently affecting the anterior surfaces of the liver (not the liver parenchyma).

10.2.1.5 Tubo-Ovarian Abscess (TOA)

Tubo-ovarian abscess is usually a complication of PID. It is an inflammatory mass involving the fallopian tube, ovary, and, occasionally, other adjacent pelvic organs (e.g., bowel, bladder). This may manifest as a tubo-ovarian complex (an agglutination of those structures) or a collection of pus (tubo-ovarian abscess). These abscesses are found most commonly in reproductive age women and typically result from upper genital tract infection.

The classic presentation is the same as for PID alone, including acute lower abdominal pain, fever, chills, and vaginal discharge.

However, some cases of TOA differ from the classic scenario. Fever is not present in all patients and some patients report only low grade nocturnal fevers or chills. Also, not all women present in an acute fashion. These variations in clinical presentation were illustrated in one of the largest series of women with TOA ($n = 175$) [11]. As many as 40% of patients were afebrile upon presentation; 25% complained of chronic rather than acute abdominal pain; and 23% had normal white blood cell counts.

10.2.2 Clinical Syndromes in Men

10.2.2.1 Urethritis

C. trachomatis is the most common cause of nongonococcal urethritis in men. The proportion of cases that are asymptomatic vary by population and range from 40 to 96% [12–14]. When men do have symptoms, they typically present with a mucoid or watery urethral discharge, and dysuria is often a prominent complaint. The discharge is often clear and only seen upon milking the urethra. Sometimes the discharge is so scant that men only notice stained undergarments in the morning.

10.2.2.2 Epididymitis

Inflammation of the epididymis is known as epididymitis. *C. trachomatis* is one of the most frequent pathogens in epididymitis among sexually active men <35 years of age, along with *N. gonorrhoeae*.

Men with acute epididymitis typically have:

- unilateral testicular pain and tenderness
- hydrocele
- palpable swelling of the epididymis.

On physical examination, the affected testis has a normal vertical lie; the scrotum may be red and parchment-like (although this is an uncommon finding); scrotal oedema is present in at least 50% of cases. Sometimes an inflammatory nodule is felt with an otherwise soft, nontender epididymis. In contrast to patients with testicular torsion, patients with epididymitis usually have a normal cremasteric reflex (if they have one under normal conditions). Patients with epididymitis may experience pain relief with elevation of the testis (Prehn sign), but this is not a reliable marker for epididymitis.

10.2.2.3 Prostatitis

C. trachomatis may be an aetiology in some cases of chronic prostatitis, although this attribution remains highly speculative. Symptoms in these men included:

- dysuria
- urinary dysfunction
- pain with ejaculation
- pelvic pain.

10.2.2.4 Proctitis

Chlamydial proctitis, defined as inflammation of the distal rectal mucosa, occurs primarily in men who have sex with men (MSM) who engage in receptive anal intercourse.

The clinical presentation of chlamydial proctitis depends on the infecting chlamydial serovars.

The L1, L2, and L3 serovars of *C. trachomatis* cause the disease known as lymphogranuloma venereum (LGV), which can present as anorectal disease and has been reported in outbreaks among European and North American MSM, particularly those who are HIV-infected [15].

Symptoms occur in the vast majority of cases. These include:

- anorectal pain
- discharge
- tenesmus
- rectal bleeding
- constipation

Systemic symptoms of fever and malaise are also often present. Left untreated, rectal infection with the L1, L2, and L3 serovars can lead to rectal fistulae and strictures.

The non-LGV serovars that cause genital infection (serovars D through K) can also cause infection of the rectum, particularly in MSM, but in contrast to LGV, these infections are usually asymptomatic.

10.2.2.5 Reactive Arthritis/Reactive Arthritis Triad (RAT)

Approximately 1% of men with urethritis develop reactive arthritis, and approximately one-third of these patients have the complete reactive arthritis triad (RAT) formerly referred to as Reiter syndrome (arthritis, uveitis, and urethritis).

Patients with reactive arthritis typically present with an asymmetric oligoarthritis, usually 1–4 weeks following the inciting infection. The several types of clinical manifestations of reactive arthritis include:

- Symptoms of preceding enteric or genitourinary infection (diarrhoea or urethritis)
- Musculoskeletal signs and symptoms:
  - Arthritis: acute-onset asymmetric oligoarthritis, often affecting the lower extremities
  - Enthesitis: inflammation around the enthesis (site of insertion of ligaments, tendons, joint capsule, or fascia to bone). Common sites of heel involvement are at the insertions of the Achilles tendon and of the plantar fascia on the calcaneus. Pain, swelling, and local tenderness are suggestive clinical features. Estimates of the frequency of enthesitis in patients with reactive arthritis have ranged from 20 to 90%

- Extraarticular signs and symptoms
  - Conjunctivitis,
  - Genitourinary tract symptoms, such as dysuria, pelvic pain, urethritis, prostatitis
  - Oral lesions, including mucosal ulcers
  - Cutaneous eruptions and other skin changes, such as keratoderma blennorrhagica (hyperkeratotic skin lesions on soles and palms) and erythema nodosum
  - Nail changes that resemble those seen in psoriasis
  - Genital lesions such as circinate balanitis
  - Cardiac manifestations, which are uncommon, include valve disease, particularly aortic insufficiency, with greater chronicity of illness. Pericarditis has been reported very rarely.

10.3 Screening in Adult Men and Women

Detecting chlamydial infections among asymptomatic individuals is an important public health tool. Its main rationale is to identify and treat the infection to avoid complications, particularly in women, and to prevent transmission and reinfection among sex partners.

Women: As CT shows its highest prevalence in young women, an annual screening of all sexually active women aged <25 years is recommended, as well as screening of all women with behavioural risk factors such as new or multiple sex partners, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection. The main advantage of chlamydia screening programs is to reduce the rates of PID in women [16], whereas their effect on prevalence in the general population is uncertain [17].

Modern approach to women screening for chlamydia includes endocervical or vaginal swabs or first-catch urine samples. Nucleic acid amplification tests (NAAT), specific for nucleic acid (DNA or RNA), are considered the gold standard for the detection of *C. trachomatis* because of their high sensitiveness, specificity, and speed, thus replacing less-sensitive methods, such as isolation in cell culture or identification by direct fluorescence assays (DFA) [4, 18]. Serologic tests that detect a systemic immune response to infection are not recommended because of the lack of precision for the detection of an active infection. The test can be run on endocervical swabs collected during a vaginal speculum examination or on vaginal swabs or urine samples. The CDC considers vaginal swab to be the preferred specimen type for its efficacy and ease of collection [19]. A recent review on ten studies involving 10,479 participants showed that home-based specimen collection can be a valid alternative to clinic-based collection, encouraging more people to be tested and reducing costs, discomfort, and time of a clinical consultation [20]. A first-catch urine specimen is also acceptable but less sensitive.

According to CDC guidelines, a routine screening for chlamydia should be offered at the first prenatal visit to all pregnant women aged <25 years at increased risk. Retest during the third trimester is supposed to prevent maternal postnatal complications and neonatal infection. Positive women should undergo NAAT 3–4 weeks after treatment to assess eradication and be retested within 3 months [3].

Screening for chlamydia in the rectum and pharynx with the use of laboratory validated assays can be considered in persons who are at risk for infection at those sites, even if no definitive data on the group of women to screen or the harm of infection in these sites are available [21].

Men: Targeted chlamydia screening in men aims to reduce infection, reinfection, and transmission among sex partners and should only be considered when prevalence is high. Efficacy and cost-effectiveness of routine screening for *C. trachomatis* in sexually active young men are not clear [18]. However, it must be proposed in specific clinical settings with a high prevalence of infection such as adolescent clinics, correctional facilities, and STD clinics.

Strong evidences support the relevance of screening in populations at risk, such as men who have sex with men (MSM) [4, 22]. Since CT infections are often asymptomatic, accurate early diagnosis through an annual test in MSM is

critical to hinder the transmission and to reduce the risk of acquiring HIV infection. Nucleic acid amplification tests (NAAT) are now recommended by international guidelines to screen also MSM for extra-genital CT infections. Rectal prevalence in MSM for chlamydia is estimated 8.9%, with a pharyngeal prevalence of 1.6% and 5.3%, respectively. Oropharyngeal chlamydia screening could be useful to detect infections in this site, which would have been missed and not treated with just urogenital test. However, it is still unclear whether oropharyngeal chlamydia contributes to the overall prevalence of chlamydia in MSM [23].

10.3.1 Diagnostic Considerations

Diagnostic techniques for *C. trachomatis* infection have been ever developing during the last decades; NAAT replaced other classical methods such as culture, antigen detection, and genetic probes.

Nucleic acid amplification—NAAT methodology consists of amplifying *C. trachomatis* DNA or RNA sequences using polymerase chain reaction (PCR), transcription-mediated amplification (TMA), or strand displacement amplification (SDA). It is nowadays considered the benchmark for diagnosis, due to its superior sensitivity, specificity, and ease to collect specimens [24]. It can be performed on either endocervical, vaginal, or urethral swabs with a plastic or wire shaft and a rayon, dacron, or cytobrush tip [20]. Diagnosis of *C. trachomatis* urethral infection in men can be made by testing a urethral swab or first-catch urine specimen, which is preferred as non-invasive. In women, a swab of vaginal fluid is the preferred approach for diagnosing chlamydial infection, as this specimen provides the highest sensitivity [25]. First-catch urine can also be used with sensitivity and specificity of non-invasive testing comparable to invasive testing. Urine sample should be collected from the initial stream (approximately the first 10 mL) without pre-cleansing of the genital areas. Purulent material or blood does not affect the performance of the test. Commercially available NAATs are not yet licensed by FDA for the diagnosis of extra-genital (rectal or oropharyngeal) samples but have shown to be more reliable than culture for detection of chlamydial infection in persons engaging in receptive anal or oral intercourse. For this reason, chlamydia infection might be detected in a single specimen in the occasion of gonorrhoea testing. Self-collection of rectal specimens is considered as reliable as clinic-based collection [3].

Liquid-based cytology specimens collected for Pap smears have been tested for NAAT, with the aim of running different tests on the same specimen collected for HPV screening, which is offered by most states worldwide. Recently, the *Chlamydia trachomatis* Qx Amplified DNA Assay (CTQ) has been FDA-cleared for the detection of *C. trachomatis* in liquid-based preparations. Test sensitivity has shown to be lower than on cervical or vaginal swab specimens. A confirmatory test is recommended in a population with a low prevalence of chlamydial infection to minimize the number of false positives [3, 20, 26].

Rapid tests for chlamydia—rapid point-of-care diagnostic tests (RDTs) are particularly valuable as they allow immediate diagnosis and treatment. RDTs are useful to reach high-risk individuals marginalized from the healthcare system or for patients who have problems in returning to the clinic for the results. Most RDTs are immune chromatographic tests based on lateral-flow-technology and detect chlamydia LPS antigen in genital swabs or urine. They cannot be recommended as screening tests due to lack of sensitivity. Currently available near-point-of-care NAATs have acceptable performance characteristics and results for chlamydia (and gonorrhoea) can be provided within 90 min. This test is approved in the USA for use on endocervical or vaginal swabs and urine. European CDC suggests caution in the use of the actual available tests [4]. Several immunoassay-based and PCR-based tests are in development. These rapid tests provide results within 30 min of testing and are less expensive to perform and easy to interpret [27].

Culture—culture methods used to be the gold standard for *C. trachomatis* detection. Their use

is today limited to research due to the expense and technical difficulties.

Serology—*C. trachomatis* serology needs complement fixation titres >1:64. It can support the diagnosis of chlamydia in pelvic chronic and invasive infections (PID, LGV) but it is useless in cutaneous and early manifestations. It may also not perform as well in diagnosing rectal infections in men as it does upper genital tract infection in women.

Antigen detection—Antigen detection requires invasive testing using a swab from the cervix or urethra. The sensitivity of this method is 80–95% compared with culture.

Genetic probe methods—probe methods require invasive testing using a direct swab from the cervix or urethra. The sensitivity of this assay is approximately 80% compared with culture. The main advantage of these tests is their low cost; however, because their sensitivity is considerably lower than NAAT and because NAAT became more cost-competitive, these tests are not used as frequently as in the past.

European guidelines establish clear indication about whom should be tested for chlamydia infection.

Asymptomatic people who present risk factors for *C. trachomatis* and/or other STI (age <25 years, new sexual contact in the last year, more than one partner in the last year, receptive anal intercourses, MSM, sexually active HIV-infected individuals) should undergo routine screening.

Any sexually active individual with symptoms or signs consistent with risk of chlamydia infection such as urethritis, acute epididymo-orchitis in men, cervical or vaginal discharge, acute pelvic pain and/or symptoms or signs of PID or purulent conjunctivitis need to be diagnosed and treated.

Any patient affected from gonorrhoea or other STI should be tested for *Chlamydia trachomatis*.

After sexual contact with persons with a potential or known STI or PID, American College of Obstetricians and Gynaecologists supports the use of expedited partner therapy as a method of preventing gonorrhoea and chlamydial reinfection when a patient's partners are unable or unwilling to seek medical care. Patients should be instructed to abstain from sexual intercourse for 7 days after they and their sexual partners have completed treatment [28, 29].

Women undergoing any intrauterine interventions or manipulations should be tested for chlamydia to lower risk of PID.

Recurrent symptoms after initial resolution represent an indicator of reinfection. For that, evaluation for chlamydia should be repeated with NAAT. It is important to investigate whether sex partner has been appropriately treated and to counsel the patient about safe sex opportunities. Repeated testing in 3–6 months should be offered to young women and men (<25 years of age) who test positive for *C. trachomatis* [4]. A recent RCT suggests that invitation for retesting 8 weeks after initial treatment would be the optimal management for both younger and older than 25 to detect new infections [30]. However, this point is still controversial, and more evidence is needed.

10.4 New Evidence Based Treatment Approach

Treating urogenital *C. trachomatis* infection prevents complications (PID, chronic pelvic pain, infertility, ectopic pregnancy) and decreases the rate of sexual and vertical transmission. Treating sex partners is critical to avoid reinfection and spreading of the infection.

Chlamydia should be diagnosed early, and people resulted positive should be treated promptly; treatment delays have been associated with complications.

- Treatment of uncomplicated urogenital chlamydia (urethritis in men, urethritis and cervicitis in women)
  - Recommended Regimens
    Azithromycin 1 g orally in a single dose
    OR
    Doxycycline 100 mg orally twice a day for 7 days

All the world most important centres for diseases control state that azithromycin and doxycy-

cline are the first line in CT treatment. Cure rates with these two regimens are similar and exceed 95%. Some authors suggest that doxycycline might be superior in terms of efficacy to azithromycin [31, 32], but the relevance of this finding in clinical scenario is still not clear [33]. The drug choice in different settings results from cost, quality, and equity considerations. Azithromycin is preferred for its convenience in administration and excellent tissue and intracellular penetration, doxycycline reduces costs of treatment, but a 7-day compliance is needed and it is contraindicated in pregnancy.

– Alternative Regimens
  Tetracycline 500 mg orally four times a day for 7 days OR
  Erythromycin 500 mg orally twice a day for 7 days OR
  Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days [3]
  Ofloxacin 300 mg orally twice a day for 7 days OR [24]
  Levofloxacin 500 mg orally once daily for 7 days [4]

Erythromycin and penicillin are inferior in their cure rates but are useful in pregnant patients who cannot tolerate azithromycin. The use of these drugs requires a test of cure to ensure microbiologic eradication.

Fluoroquinolones are highly effective against chlamydia and are recognized by CDC as alternative therapies. As drawbacks, they require compliance to a week-therapy; are more expensive; and are contraindicated in pregnancy, lactation, and children. They were used in the past for coinfections from Chlamydia and Neisseria gonorrhoeae, but are no more considerate adequate as Neisseria has developed drug resistance.

- Treatment of other chlamydia infections
  – Coinfections with gonorrhoea: empiric therapy for gonorrhoea is not indicated routinely in all patients diagnosed with Chlamydia. Men should be treated for both if intracellular gram-negative diplococci are detected on a Gram stain of urethral discharge. Women should be treated in accordance to risk factors for gonorrhoea or high local prevalence of this infection, as presence of non-pathogen endocervical Neisseria makes Gram stain less useful [3].
  – Proctitis and lymphogranuloma venereum: A meta-analysis of observational studies showed higher cure rates of rectal chlamydia after doxycycline therapy than after azithromycin therapy [33]. However, these studies have limitations, and prospective clinical trials comparing azithromycin versus doxycycline regimens for rectal *C. trachomatis* infection are needed. Due to high prevalence of coinfection in patients with acute proctitis, empiric therapy for both chlamydia and gonorrhoea is indicated. It should be started immediately if symptoms are highly suggestive or polymorphonuclear leukocytes are found on anorectal exudate sample. The recommended regimen includes ceftriaxone 250 mg IM in a single dose plus doxycycline 100 mg orally twice a day for 7 days. MSM with acute proctitis and either a positive rectal chlamydia NAAT or HIV infection with bloody discharge, perianal ulcers, or mucosal ulcers should be empirically treated for LGV with doxycycline 100 mg twice daily orally for 3 weeks [3, 34].
  – Epididymitis: CDC guidelines recommended regimens for acute epididymitis most likely caused by sexually transmitted chlamydia and gonorrhoea include ceftriaxone 250 mg IM in a single dose plus doxycycline 100 mg orally twice a day for 10 days. In case of risk factors for enteric organisms, such as insertive anal sex, ceftriaxone 250 mg IM in a single dose plus levofloxacin 500 mg orally once a day for 10 days or ofloxacin 300 mg orally twice a day for 10 days are preferred [3].
  – Pelvic inflammatory disease: [3, 35] All patients diagnosed with PID should be tested for Chlamydia, gonorrhoea, M. genitalium, syphilis, and HIV. Delayed treat-

ment should be avoided for the increase of risk of long-term sequelae. Regimens should be adjusted by the changing spectrum of antimicrobial resistance over time and in different geographical areas.

Outpatient regimens include

- ceftriaxone 500 mg single dose followed by oral doxycycline 100 mg twice daily plus metronidazole 500 mg twice daily for 14 days or
- oral ofloxacin 400 mg twice daily plus oral metronidazole 500 mg twice daily for 14 days or
- oral moxifloxacin 400 mg once daily for 14 days.

If severe sign, tubo-ovarian abscess or pregnancy are verified, inpatient regimens are preferred:

- i.v./i.m. ceftriaxone 1 g once daily plus i.v. doxycycline 100 mg twice daily followed by oral doxycycline 100 mg twice daily plus oral metronidazole 500 mg twice daily to complete 14 days or
- i.v. clindamycin 900 mg three times daily plus i.m./i.v. gentamicin 3–6 mg/kg as a single daily dose with renal monitoring followed by either oral clindamycin 450 mg four times daily to complete 14 days or oral doxycycline 100 mg twice daily plus oral metronidazole 500 mg twice daily to complete 14 days.

Alternative regimens

- i.v. ofloxacin 400 mg twice daily plus i.v. metronidazole 500 mg three times daily for 14 days
- i.m. ceftriaxone 500 mg single dose plus oral azithromycin 1 g single dose followed by a second dose of oral azithromycin 1 g after 1 week
- Oropharyngeal infection: it is still uncertain the clinical significance of oropharyngeal *C. trachomatis* infection and therefore routine oropharyngeal screening for CT is not recommended. However, evidence suggests oropharyngeal *C. trachomatis* can be sexually transmitted to genital sites [36, 37]; therefore, detection of *C. trachomatis* from an oropharyngeal specimen should be treated with azithromycin or doxycycline. The efficacy of alternative antimicrobial regimens in oropharyngeal chlamydia infection remains unknown.

- Treatment of chlamydia infection during pregnancy: [3, 4, 24] treatment of chlamydia infection in pregnancy is critical to avoid transmission to the newborn.
  - Recommended regimen
    Oral azithromycin 1 g as a single dose is considered the first line, as it is effective and safe, while doxycycline is contraindicated in the second and third trimesters of pregnancy
  - Alternative Regimens
    Amoxicillin 500 mg orally three times a day for 7 days OR
    Erythromycin base 500 mg orally four times a day for 7 days OR erythromycin base 250 mg orally four times a day for 14 days OR
    Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days OR erythromycin ethylsuccinate 400 mg orally four times a day for 14 days

Amoxicillin is safe and it used to be a first-line drug, but data suggest that it might cause persistence in culture at physiologically relevant concentrations [38], erythromycin is safe but compliance is reduced by gastrointestinal side effects.

Test-of-cure to document chlamydial eradication (preferably by NAAT) 3–4 weeks after completion of therapy is needed to assess infection eradication. In addition, all pregnant women who have chlamydial infection diagnosed should be retested 3 months after treatment.

10.5 Follow-Up Strategies [3, 4]

Testing for chlamydia after treatment is critical to assess the eradication of infection and avoid misknown recurrence or persistence and spread of infection among sex partners. After-treatment strategies include "test of cure" (TOC) and "retesting."

TOC is a diagnostic testing with the aim of assessing whether the pathogen has been eradicated by the antibiotic strategy used. It is performed 3–4 weeks after treatment is completed. NAAT method is the first choice and it is not reliable if done earlier for the risk of testing false-positive for the presence of dead microorganisms [20, 38]. It is not recommended to perform TOC routinely in patients treated with recommended first-line regimens, unless therapeutic adherence is in question, symptoms persist, or reinfection is suspected. Indications for TOC include pregnancy, complicated infections, persistence of symptoms, use of second-line or third-line regimens such as erythromycin or amoxicillin. It should also be considered in extra-genital infections, particularly when azithromycin 1 g stat has been administered for treatment of rectal infections. TOC of asymptomatic MSM with rectal chlamydia after treatment for uncomplicated chlamydial infection (azithromycin 1 g single oral dose or doxycycline 100 mg, 7 days) should be considered to ensure that any LGV infection is not missed.

Retesting is performed approximately 3 months after treatment of infection, or if this is not possible, at the first visit thereafter within 12 months of treatment. This is necessary because most post-treatment infections result from reinfection due to lack of notification and treatment of sex partners or the initiation of sexual relation with a new infected partner. International guidelines assert that young women and men (<25 years of age) who test positive for *C. trachomatis* should be retested, regardless of whether they believe that their sex partners were treated. A recent RCT suggests that invitation for retesting 8 weeks after initial treatment would be the optimal management for both younger and older than 25 to detect new infections [30]. However, this point is still controversial, and more evidence is needed. Beyond testing, persistence or recurrence of symptoms must be monitored.

10.6 Management of Sex Partners

Reinfection among asymptomatic sex partners is the most frequent cause of recurrence of chlamydia infection. The only way to avoid it is to assess and treat people who had sexual intercourses with the patient within the last 60 days preceding the onset of symptoms or chlamydia diagnosis, or the most recent sex partner. As most people are unwilling to be tested and counselled, a strategy termed expedited partner therapy has been proposed. Expedited partner therapy enables the obstetrician–gynaecologist or other provider to give prescriptions or medications to patients to take to their partners without first examining them. Compared with standard patient referral of partners, this approach to therapy has been associated with decreased rates of persistent or recurrent chlamydia [39]. Despite the effectiveness of expedited partner therapy, numerous legal, medical, practical, and administrative barriers hinder its routine use by obstetrician–gynaecologists [28]. EPT is not routinely recommended for MSM with chlamydia because of a high risk for coexisting infections (especially undiagnosed HIV) among their partners, and because data are limited regarding the effectiveness of this approach in reducing persistent or recurrent chlamydia among MSM [4]. To avoid reinfection, sex partners should be instructed to abstain from sexual intercourse until they and their sex partners have been adequately treated (i.e., for 7 days after a single-dose regimen or after completion of a 7-day regimen) and have resolved any symptoms [28].

10.7 Conclusions

C. trachomatis is the leading cause of bacterial sexually transmitted diseases and it is responsible for cervicitis, salpingitis, and endometritis. Nowadays, *C. trachomatis* infection continues to

be an important public health problem worldwide because of its increasing incidence [1]. Many infections are asymptomatic and result in delayed diagnosis and uninterrupted transmission. Targeted screening, opportunistic testing for asymptomatic infections, contact tracing, and mandatory notification could be prerequisites to improve the knowledge and the diffusion of this important infection.

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Genital Herpes: Clinical and Scientific Novelties

11

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11.1 Introduction

The first historical record of an herpetic disease was the description twenty centuries ago by the Roman physician Herodotus of the "herpetic eruptions," but both herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) encountered human beings much earlier in the history of humanity. Oral herpes, due to HSV-1, has been around since humans and chimpanzees split 6 million years ago and HSV-2 must have jumped from ancestral chimpanzees into the human lineage later. Somewhere between 3 and 1.4 million years ago, HSV-2 jumped the species barrier from African apes back into human ancestors, probably through an intermediate hominid species unrelated to humans [1]. A recent study identifies Paranthropus boisei as the most likely intermediate host of HSV-2, while Homo habilis may also have played a role in the initial transmission of HSV-2 from the ancestors of chimpanzees to boisei.

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Using phylogenomic analyses, Burrel et al. recently show that two main HSV-2 lineages exist [2]. One lineage is mostly restricted to sub-Saharan Africa, whereas the other has reached a global distribution. Interestingly, only the worldwide lineage is characterized by ancient recombination events with HSV-1. At present HSV-2 is among the most successful human viruses in terms of its global distribution, evolutionary coexistence with humans, and persistence in the individual host. Indeed, there is remarkable genetic conservation between primary and recurrent episodes of HSV-2 infection and implies that strong selection pressures exist to maintain the fidelity of the viral genome during repeated reactivations from its latent state.

HSV is a common cause of both genital and oral disease. HSV-1 primarily causes infection, and sporadic recurrent lesions, within the oral cavity and establishes latency in the trigeminal ganglia, while HSV-2 is associated with infection of the genitalia and surrounding skin, and establishes latency in the sacral ganglia. Both viruses can infect either body cavity; however, there are viral shedding and clinical recurrence data that indicates that HSV-1 is a more successful oral pathogen and HSV-2 a more successful genital pathogen [3, 4]. However, genital herpes can result from infection with either HSV-1 or HSV-2 and in some parts of the world genital HSV-1 infection is now predominant.

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11.2 Epidemiology of Genital Herpes

HSV-2 is estimated to be harbored by 417 million people aged 15–49 worldwide and causes an estimated 23 million new infections each year. HSV-1 is even more common, with an estimated 3.7 billion people under age 50 having the infection globally with a seroprevalence of >90% in many nations. HSV-1 is frequently acquired during early childhood, primarily through oral secretions. However, the epidemiology of HSV-1 is changing, such that the frequency of sexual transmission of HSV-1 has increased in many countries, including the USA. It can partially be due to a decline in the seroprevalence of HSV-1 and HSV-2, leaving more people susceptible to genital HSV1 or HSV-2 infections, as recently documented in Germany and Italy [5, 6]. The incidence of HSV-1 genital herpes in USA now varies between 10 and 30%. In UK and Japan HSV-1 causes as much as 50% of all initial genital herpes infections.

11.3 Clinical Presentation and Transmission

Genital herpes is a sexually transmitted viral disease, characterized by recurrent genital symptoms and signs, such as pain or discomfort, lesions in the genital and perianal areas that are self-limiting, but which can recur periodically after the initial episode.

Genital HSV infection often remains undiagnosed and is frequently misdiagnosed for other conditions. Skin splits, fissures, minor abrasions, furuncles, erythema, and pain are common manifestations of genital herpes but are often attributed to injury, allergies, insect bites, or other infectious agents [7].

Only an estimate of 20% of people with genital herpes are accurately diagnosed because approximately 20% of infected people have true asymptomatic infection and 60% have symptoms that are not recognized as genital herpes [8]. Even among experienced clinicians following patients in a clinical trial of herpes infection, genital herpes was both underdiagnosed and overdiagnosed. In the study of Langenberg et al. [9] among 74 persons given a clinical diagnosis of genital HSV-2 during the study, 60 had true positive test results and 14 had false positive results (ratio of true positives to false positives, 4:1). Thus, 60 persons with symptomatic HSV-2 infection among a total of 155 persons with HSV-2 seroconversion were identified, yielding a sensitivity of 39% for the clinical diagnosis of HSV-2 infection and a specificity of 99% [9].

HSV can spread through skin-to-skin or mucosal contact at any time there is viable virus present. Transmission is more likely to occur when large amounts of virus are present; suppressive valacyclovir therapy of the infected individuals reduced the risk of the susceptible sexual partner acquiring genital herpes by 48% [10]. HSV cannot be transmitted through the air and only rarely is transmitted by non-intimate contact. It is very unlikely that anyone would acquire genital herpes from a toilette seat or by sharing a towel with someone infected.

HSV is usually transmitted in the absence of symptoms, through asymptomatic shedding of the virus. The majority of HSV-2 seropositive individuals shed virus from their genital tract. The frequency of virus isolation in the absence of lesions in women with history of symptomatic recurrent disease ranges from 1 to 8% of days; however, using more sensitive molecular tests, virus shedding can be detected 28% of days. Moreover, asymptomatic shedding can occur at various sites in the genital area: asymptomatic HSV shedding has been detected on the vulva, cervix, and anus in women, and on the penis, and anus in men. As the virus travels within sacral sensory nerves it can reach other areas innervated by the nerves from sacral ganglia such as scrotum, buttocks, urethra, or other skin areas [11]. Unrecognized disease with asymptomatic HSV shedding is the most common cause of transmission of the infection [12]. Only one-third of individuals develop symptoms at the time of acquisition of infection with HSV-2. Incubation time of infection from exposure to first clinical signs and symptoms ranges from 2 days to 2 weeks. The first clinical presentation of the

infection may be primary, non-primary, or recurrent: primary genital herpes is observed in individuals seronegative to both HSV-1 and HSV-2, non-primary in individuals with pre-existing antibodies to the alternative type of HSV, and recurrent in subjects with pre-existing antibodies to the type of virus causing the outbreak. Following the initial herpes outbreak, subsequent disease episodes may be symptomatic or asymptomatic, the vast majority being asymptomatic or subclinical.

Prior infection with HSV-1 modifies the clinical manifestations of first infection by HSV-2, usually making symptoms less severe. After childhood, symptomatic primary infection with HSV-1 is equally likely to be acquired in the genital area or oral areas. Primary and initial genital herpes may be caused by either HSV-1 or HSV-2. Following primary infection, the virus establishes latency in local sensory ganglia, periodically reactivating to cause symptomatic lesions or asymptomatic, but infectious, viral shedding.

The median recurrence rate for genital herpes after a symptomatic first episode is 0.34 recurrences/month (i.e., approximately four recurrences per year) for HSV-2 and is four times more frequent than the recurrence rate for HSV-1. Recurrence rates decline over time in most individuals, although this pattern is variable.

The majority of individuals found to be seropositive for HSV-2 type-specific antibodies subsequently present with symptomatic lesions (once aware of the clinical manifestations of HSV-2). In some of these individuals, the number of days when virus is shed asymptomatically, usually in lesser amount, exceeds the number of days of symptomatic shedding associated with lesions. Virus can be shed asymptomatically from the external genitalia, the ano-rectal area, the cervix, and urethra.

The first episode of genital herpes infection can cause painful and distressing symptoms for more than 2 weeks. Classical signs include erythema, and painful vesicular and ulcerative lesions of the genitals and surrounding areas (Fig. 11.1). Less classically, there may be only a small fissure or raw area, a painless ulcer, a small area of erythema, or an area of irritation with no visible abnormality. Inguinal lymph nodes may become swollen and tender. Typical sites include the shaft and glans of the penis in men (Fig. 11.2), and the labia, clitoris, and perineum in women (Fig. 11.3). The anus and rectum may be also affected. "Hidden" sites, such as the cervix, may be harder to identify.

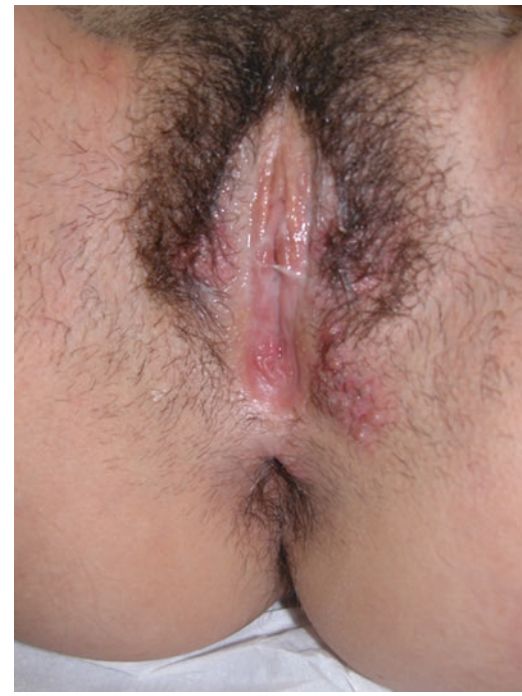

Fig. 11.1 Extensive, exudative, and vesicular vulvar lesions of primary genital herpes (Courtesy Dr. Giuliani)

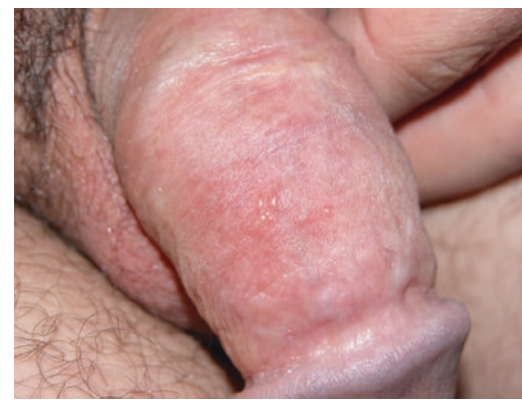

Fig. 11.2 Early form of recurrent herpes on shaft of the penis (Courtesy Dr. Giuliani)

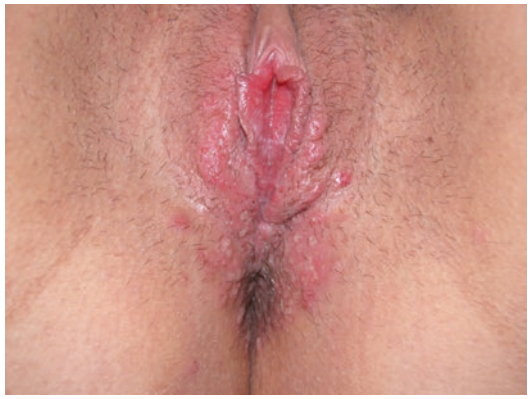

Fig. 11.3 Recurrent genital herpes (Courtesy Dr. Giuliani)

Fig. 11.4 Multiphase recurrent genital herpes (vesicular and scabs phases) (Courtesy Dr. Latini)

Ulcers and vesicles form dry scabs as they are healing, usually 2 weeks after initial symptoms of a first episode, or a few days after initial symptoms of a recurrent episode. Often vesicular and dry scabs lesions can coexist in the same anatomical area (Fig. 11.4).

Herpes can affect other areas of skin (e.g., buttocks, perianal area, and legs). If a person has recurring symptoms and signs anywhere in the region of the sacral dermatome, suspect recurrent episodes of genital herpes.

In recurrent symptomatic episodes prodromal symptoms occur in about half of people. These include itching, tingling, paresthesia, or irritation of the genitals and surrounding areas. Local symptoms include painful ulceration, dysuria, urinary retention, and vaginal or urethral discharge. Less typical symptoms include nonspecific vaginal or urethral discomfort, dull perineal pain, or areas feeling "raw" or irritated. Less frequent during recurrences are systemic symptoms that include malaise, fever, headache, and muscle aches. Rarely, systemic symptoms may be the only evidence of infection.

11.4 Complications and Sequelae

Complications of first-episode genital herpes are more common in women than men [13]. They can be short- and/or long-term. Short-term complications include spread to extra-genital areas, including the lips, buttocks, breasts, fingers, and eyes (possibly though self-inoculation). Pharyngitis due to HSV infection may be seen in people who have had orogenital sex [13]. Secondary infection can occur. Infection with *Staphylococcus* may give the appearance of folliculitis, and fungal or yeast superinfection may cause balanitis or vaginitis [13]. Acute urinary retention can occur, due to dysuria or (less commonly) autonomic neuropathy. Stiff neck, headache, and photophobia are relatively common systemic complications of first-episode genital herpes [13]. Less commonly, this can develop into full-blown meningitis symptoms [14]. Symptoms usually develop 3–12 days from the onset of genital lesions and settle over 2–3 days, usually with full recovery. Viremia [15] encephalitis or disseminated infection are rare complication that are more likely to occur in immunocompromised people [16].

Long-term complications include psychological and psychosexual problems that are common and can be severe [17]. Following a first episode of genital herpes, a survey found that 82% of respondents had feelings of depression, 75% had a fear of rejection, and 69% had feelings of isolation [18]. Vaginal adhesions after severe infection are rare but may result in obstructive dyspareunia [13]. Extra-genital lesions may occur in recurrent disease through neuronal spread. Typical areas affected include the buttock, groin, or thigh, and associated neuropathic

pain to the hips or legs may be severe. Neonatal herpes infection, following maternal transmission, is a rare but serious complication, with high mortality and long-term morbidity of the newborn [19]. Transmission of infection to a sexual partner occurs in up to 10% of people over a 1-year period. In at least 70% of cases, transmission occurs when the infected partner has no symptoms (i.e., asymptomatic shedding), and they may even be unaware that they have the infection. Although men and women shed the virus equally, women are more susceptible to acquiring it [20].

11.5 Genital Herpes Among HIV-1 Infected Individuals

Human immunodeficiency virus (HIV) has serious long-term implications in genital herpes [13]. This can be due to the fact that HIV increases the frequency and severity of recurrent episodes of genital herpes and to a slower response to acyclovir, even in the absence of overt acyclovir resistance. Moreover, in HIV-infected individuals and HSV-2 seropositive individuals, both symptomatic and asymptomatic shedding are increased, especially in those with low CD4+ cell counts and those who are also seropositive for HSV-1 [21]. Among HIV-1 infected individual with severe immunosuppression clinical presentation of genital herpes can show the characteristics of the chronic ulcerative HSV infection, which was included within the AIDS defining conditions by Centers for Diseases Control and Prevention (CDC) in 1993 [22]. In this case the herpetic lesions tend to be, in both gender, more extensive, deeply ulcerative, hypertrophic, very painful and they persist for over a month (Figs. 11.5 and 11.6). Thus, the same mobility of patient, due to the extent of the lesions and the associated pain, is greatly restricted. Moreover, there is evidence that HSV increases of shedding of HIV, effectively making the individual more contagious (cit.). The risk of acquiring HIV is increased in people who have genital herpes, especially when they are symptomatic with open lesions present.

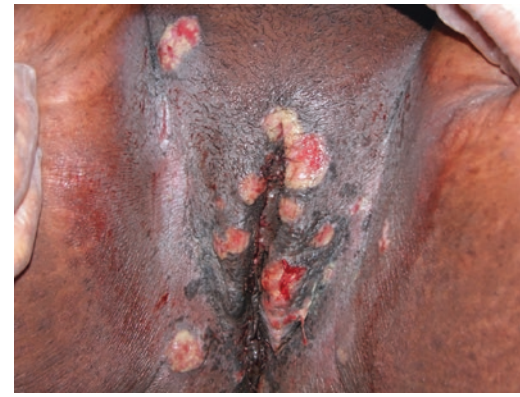

Fig. 11.5 Chronic ulcerative HSV infection in woman with AIDS (Courtesy Dr. Latini)

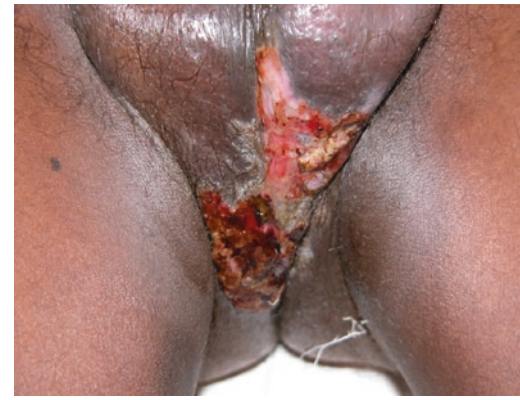

Fig. 11.6 Scrotal and perianal chronic ulcerative HSV infection in male AIDS patient (Courtesy Dr. Latini)

Recently Katharine Looker and colleagues [21] provided the first systematic meta-analysis in over a decade to assess the effect of HSV-2 infection on subsequent HIV acquisition. They analyzed 57 longitudinal studies and estimated the association between prevalent or incident HSV-2 infection and HIV acquisition. Their findings for prevalent HSV-2 infection showed that the risk of HIV acquisition was roughly tripled in general populations and almost doubled in high-risk populations. Their estimates for the effect of incident HSV-2 infection on HIV infection showed that incident HSV-2 infection was associated with a five times increase in the risk of HIV in general populations and a three times increase in the risk of HIV in high-risk populations.

11.6 Diagnosis

The prognosis is difficult to predict in individual cases, taking in to account that there is no definitive cure for genital herpes, although symptoms can be effectively treated. Indeed the natural course of the infection varies greatly between individuals. Symptomatic recurrence is likely in most people following a first clinical episode of HSV-2 genital herpes [23]. Recurrences are usually of shorter duration and are less severe than the first episode. Recurrence is more likely with HSV-2. The median rate of genital recurrence for HSV-2 is approximately once every 3 months, compared with once a year for HSV-1. Recurrences are most frequent during the first year following primary infection, and tend to decrease in frequency over time. Within a year of a documented first episode of genital herpes caused by HSV-2, 90% of people will have had at least one recurrent episode, 38% more than six, and 20% more than ten [23].

The laboratory diagnosis of genital herpes is recommended in various situations:

- Confirmation of clinically suspected genital herpes.
- Variable presentation of genital herpes.
- Extra-genital complications of genital herpes
- Differential diagnosis with other ulcerative STIs.
- Differential diagnosis with other genital ulcerative dermatoses (Crohn's disease, Behçet syndrome, or fixed drug eruption).

For active lesions, collection of vesicular fluid or exudate from small vesicles is the method of choice. Several tests with various specificities and sensitivities are used for the direct diagnosis of HSV infections.

Viral culture with herpes typing has been the gold standard of HSV diagnosis over the past two decades. Viral antigen can be easily detected by direct immunofluorescence using fluorescein-labelled type-specific monoclonal antibodies on smears, or by enzyme immunoassay on swabs. Although these assays lack sensitivity, they perform satisfactorily in symptomatic patients.

HSV DNA detection based on nucleic acid amplification, and polymerase chain reaction (PCR) in particular, has emerged as an alternative method because it is about four times more sensitive, less dependent on collection and transport conditions, and faster than viral culture. PCR increases diagnostic sensitivity in both early and late presentations and is emerging as the test of choice. PCR should be implemented, after local validation, as the preferred diagnostic method [24].

Type-specific HSV serological assays might be useful in the following situations:

- Recurrent genital symptoms or atypical symptoms with negative HSV cultures or PCR;
- Clinical diagnosis of genital herpes without laboratory confirmation;
- Partner with genital herpes.

HSV serology is most useful diagnostically in cases of recurrent genital ulceration. However, the value of test is inconclusive when HSV-1 antibody is found. HSV serology in first episode disease helps to stage (primary or initial) and to diagnose those patients that have primary disease that were culture or PCR negative. Serology can also be very helpful in the partners of patients with genital herpes even though interpretation of results can be complicated if the partners HSV type is unknown. HSV serologic testing should be included in a comprehensive evaluation for STIs among people with multiple sex partners, HIV infection, and men who have sex with men.

Detection of HSV-specific IgG antibodies can be done sensitively by several immunological methods. Accurate type-specific HSV serologic assays are based on the detection of HSV-specific gG1 (HSV-1) and gG2 (HSV-2) antibodies using native, purified, or recombinant gG1 or gG2 as antigens [24].

IgM antibodies can be detected in primary HSV infection (i.e., no prior exposure to either HSV-1 or HSV-2), but also found in individuals with an existing HSV infection of one serotype following infection with the other HSV serotype (e.g., an individual with HSV-1 who becomes infected with HSV-2). In addition, IgM antibod-

ies are sometimes produced following reactivation of latent HSV infection and IgM antibodies recognizing one HSV serotype may be produced in response to reactivation of the other HSV serotype. Hence, IgM detection cannot be taken as an indicator of primary HSV infection and the interpretation of HSV IgM is problematic. Thus its use should be discouraged.

The limitations of type specific serological tests should be understood by those who use them.

There are problems of reliability/reproducibility and variations in sensitivity and specificity. Among 1158 Thai military recruits with serum gathered at 6 month intervals (3116 samples) 6.6% of all HSV-1 positives and 14.9% of all HSV-2 positives lost antibody. Between 6 and 21% show a positive to negative shift in results by four different tests. Discrepancies in tests were not predicted by published validation data [25]. Also in a recent Dutch study 12–30% of patients with PCR proven recurrent HSV-2 were false negative using commercial type-specific gG HSV-1 or HSV-2 antibody assay [26].

11.7 Treatment

Antiviral treatment of primary and recurrent genital herpes can effectively shorten the severity and duration of the outbreak and when used prophylactically (chronic suppressive therapy) can reduce the frequency of recurrences and the likelihood of transmission of infection to a susceptible sexual partner [10, 27]. Unfortunately, antiviral treatment does not eradicate the latent infection. Acyclovir, valacyclovir, and famciclovir are the principal drugs used in the management of HSV infections and all three drugs are safe and well tolerated [27].

Table 11.1 summarized the treatments recently recommended by the International Union against STI (IUSTI)—Europe by different form of genital herpes [28].

A new drug, Pritelivir®, with a novel mode of action (inhibition of the viral helicase–primase enzyme complex) shows great promise in clinical trials but as yet has not received regulatory approval.

Table 11.1 Recommended treatments of genital herpes [28]

First-episode genital herpes
The recommended regimens—all for 5–10 days—are as follows
• Aciclovir 400 mg three times a day, or • Aciclovir 200 mg five times a day, or • Famciclovir 250 mg three times a day, or • Valaciclovir 500 mg two times a day
Recurrent genital herpes—episodic antiviral treatment
Short courses
• Aciclovir 800 mg three times daily for 2 days, or
• Famciclovir 1 g twice daily for one day, or • Valaciclovir 500 mg twice daily for 3 days
Alternative longer 5-day courses include
• Aciclovir 400 mg three times daily for 3–5 days, or • Aciclovir 200 mg five times daily, or • Valaciclovir 500 mg twice daily or • Famciclovir 125 mg twice daily
Suppressive therapy
• Aciclovir 400 mg twice daily (for all frequencies of disease recurrence)
• Valaciclovir 500 mg daily (if fewer than 10 recurrences/annum)
• Valaciclovir 1 g daily (if more than 10 recurrences/annum)
Second-stage therapy for poorly controlled patients
• Aciclovir 200 mg four times a day
• Valaciclovir 250 mg twice a day
• Valaciclovir 500 mg twice a day
• Aciclovir 400 mg three times a day
HIV-infected patients
• Aciclovir 400 mg five times daily, for 7–10 days (IV,C) • Valaciclovir 500–1000 mg twice daily, for 10 days (IV,C) • Famciclovir 250–500 mg three times daily, for 10 days (IV,C)

11.8 Future Prospective

Therapeutic vaccines are another strategy for the control of recurrent genital herpes. Animal studies and small clinical trials have shown that subunit HSV vaccines administered can reduce clinically apparent genital herpes outbreaks. Presently there are four therapeutic vaccines that have been tested in humans. All of the studies have looked at the effect of the vaccine on viral shedding, the safety profile, and some measures of immunogenicity. A summary of the trials is shown in Table 11.2.

Table 11.2 Therapeutic HSV vaccines in development

Company	Vaccine	Trial results	Status
Genocea	GEN-003—contains HSV glycoprotein D (gD) and ICP4 with Matrix M2 adjuvant	Good safety profile Boosted humoral and cellular immune responses Reduced viral shedding and clinical recurrences	Development on hold, seeking a financial partner
Vical	Two constructs: VCL-HB01—contains 2 DNA plasmids encoding HSV-2 gD and UL46) with Vaxfectin® adjuvant; HM01—containing plasmid encoding gD + adjuvant	Good safety profile Boosted humoral and cellular immune responses Reduced clinical recurrences and had modest effect on viral shedding	Phase II clinical trial of bivalent vaccine underway
Admedus	COR-1—contains DNA plasmids encoding HSV-2 gD with and without ubiquitin; no adjuvant	Good safety profile Boosted cellular immune responses Reduced viral shedding and increased the time to next recurrence	Phase 2b study planned
Agenus	HerpV—contains rh-Hsc70 polyvalent peptide complex plus QS21 adjuvant	Good safety profile Boosted cellular immune responses in some subjects Reduced viral shedding	Development appears to have been discontinued

11.9 Conclusion

Genital herpes simplex virus infection is recurrent and lasts lifelong as once established, there is no presently available treatment which will eliminate it. The prevalence of HSV infection worldwide has increased over the last several decades, making it a major public health concern. Severe complications associated with genital herpes include: other sexually transmitted infections, including HIV and newborn infection from infected mother, that may result in brain damage, blindness, or death. Effective oral antiviral medications are available for initial, episodic, and suppressive therapy but are not a cure. The development of effective prophylactic and therapeutic vaccines are expected to improve the situation significantly in future.

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12 Clinical Management of Anogenital Warts and Intraepithelial Neoplasia

Alessandra Latini

12.1 Introduction

The Human Papilloma Virus is the most common sexually transmitted infection worldwide. More than 200 different genotypes have been identified so far, and they are classified in five genera (Alpha, Beta, Gamma, Mu and Nu) [1]. Most of the infections caused by these types are transient and asymptomatic, since they are rapidly cleared by an intact immune system. However, HPV may cause clinical manifestations of variable severity. About 40 mucosal genotypes have been associated with the development of anogenital lesions [2]. These span from benign warts (condylomata), mainly caused by the low-risk HPV 6 and 11, to cancerous lesions, caused by high- risk (HR) types (e.g., HPV 16 and 18). Indeed, HR-HPV are implicated in the development of most cervical (close to 90% of the cases), anal (>80%), vulvar (around 40%), vaginal (around 70%), penile (around 50%) and oropharyngeal (13–56%) cancers. Because of the ascertained link between anal HPV infection and anal cancer development, and, in particularly, the increasing incidence of this neoplasia over the last years, the interest on the burden of anal HPV infection has been growing. Indeed, about 88% of anal cancer cases worldwide are associated with HPV infection 2. HPV 16 (75–80%) and HPV18 (about 3.5%) represent the most prevalent types in this neoplasia [3]. Most sexually active persons will have detectable HPV at least once in their lifetime [4]. The estimated incidence of HPV infection is high, with 14 million persons infected annually and 79 million persons with prevalent infection [5]. In the last years it emerged that in Italy the anogenital warts were more frequent (40,871 cases, 39.7% of the total) among other sexually transmitted diseases as latent syphilis (9190 cases, 8.9% of the total), bacterial cervicovaginitis (8798 cases, 8.5% of the total) and genital herpes (7860 cases, 7.6% of the total). In particular the anogenital warts were the most frequent pathologies among men (30,092 cases, 41.5% of total men) (http://www.iss.it/ccoa/index.php?lang=1&id=55&tipo=4). HPV-associated diseases include anogenital and other mucocutaneous warts as well as cervical, anal, vaginal, vulvar, penile and oropharyngeal cancer [1]. Anogenital warts (AGWs) (also known as genital warts, condylomata acuminata, condylomas) are benign proliferative lesions caused by human papillomavirus (HPV) types 6 and 11, which are found in >95% of lesions [1, 6]. AGWs are often co-infected with "'high-risk" HPVs such as HPV 16–18. Genital warts are sexually transmitted, with transmission rates of about 60% between partners [7].

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12.2 Epidemiology

The epidemiology of condyloma is not well defined. In the literature it is reported that HPV infection anogenital affects about 40% of the population during the sexually active life. The peak prevalence is between 15 and 24 years with a slightly lower age for women. The rate of annual incidence has been recently calculated to be 0.15% on the adult population general [5]. An increase in genital warts throughout the Western world is reported in the literature. This phenomenon is attributable to an early onset of sexual activity, as well as to an increase in total number of partners [1].

12.3 Clinical Aspects

AGWs tend to appear in areas subjected to trauma; they can be unique but generally they are in a number between 2 and 15, with a variable diameter between 1 and 10 mm and can flow into larger plaques especially in individuals those with immunity deficits cell (i.e., diabetes, HIV infection) (Fig. 12.1a, b). The involvement of multiple sites is not uncommon. In women (Fig. 12.2a–c), condylomata are found predominantly on the wet surfaces of the genitals: vagina, vulva (Fig. 12.3), perineum and perianal region (Fig. 12.4a, b), while in male AGWs are preferentially seen on the mucosa of the anus (Fig. 12.5), of the penis and sometimes on the scrotum and in the inguinal region (Fig. 12.6). In not circumcised persons, the lesions are visible mainly at the level of the preputial cavities, while in the circumcised subject on the penis shaft. The urethral meatus is affected in 20–25% of cases in male and in 4–8% of women. Finally, interanal lesions are not found only in the subjects that report anal sexual intercourses but may also be present in subjects who do not report passive anal sex. From a clinical point of view the AGWs can be distinguished in warty *exophytic lesions*: commonly called condylomata acuminata, showing a warty appearance and uneven surface of greyish-white colour. *Papillomatous lesions* are AGWs in which the pedunculated aspect prevails. Because they are common in the epithelium keratinized, they are often hyperkeratotic and pigmented (Fig. 12.7). The AGWs with *papular* clinical aspect are slightly detected on the skin or mucosa, with a smooth surface, instead the *macular lesions* seem poorly detected, they are frequent on the mucous membranes with colouration variable. Clinically they are the most difficult to recognize (Figs. 12.8 and 12.9). In some cases AGWs must be differentiated from the squamous cell carcinoma (Figs. 12.10 and 12.11) and the following borderline forms: Buschke–Löwenstein tumour is histologically an in situ epithelioma

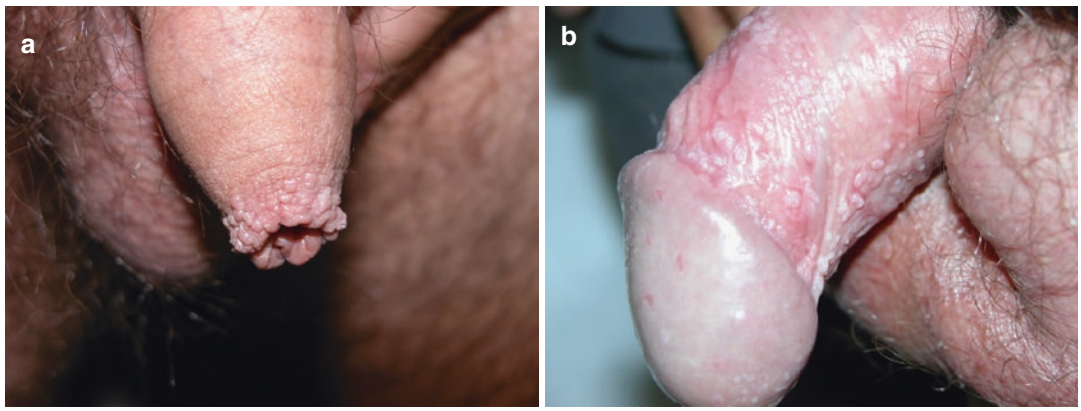

Fig. 12.1 Genital warts of the rod (**a**) and foreskin (**b**)

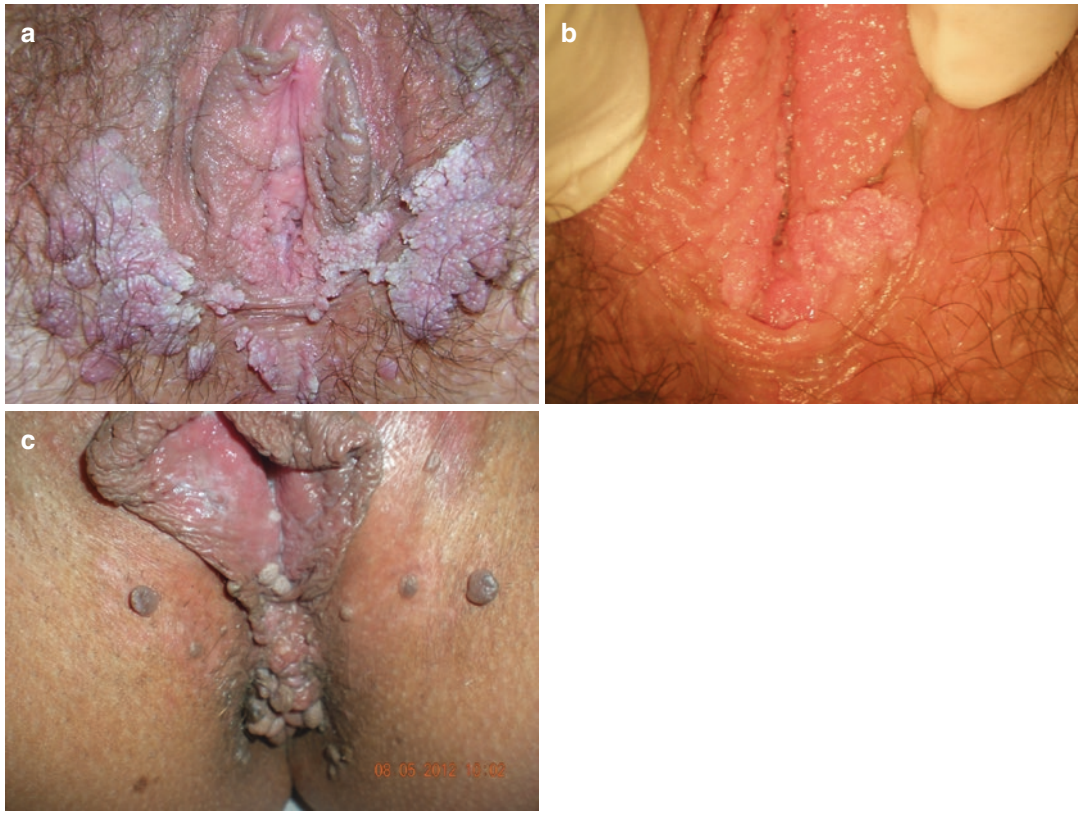

Fig. 12.2 Genital warts of the vulva and labia majora (**a**), labia minora (**b**) and perianus and gluteus skin (**c**)

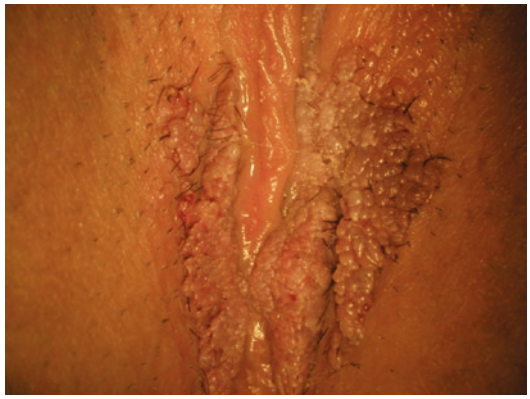

Fig. 12.3 Vulvar genital warts in a HIV-1 infected woman

caused by HPV 6 or 11 (Figs. 12.12 and 12.13); Bowenoid papulosis (PB) (Fig. 12.14) and Bowen's disease (MB) (Figs. 12.15 and 12.16) which are visible lesions associated with HPV 16; histologically, they are full-thickness intraepithelial neoplasms.

12.4 Diagnosis of AGWs

The physical examination is usually sufficient for the diagnosis of AGWs; the application of acetic acid of 5% it is not necessary because it has a low sensitivity and specificity. It is possible to use biopsy examination in doubtful cases or in suspicion of PB (papular lesion pigmented), in case of clinical deterioration or therapeutic failure in immunocompromised subjects (high risk of neoplastic lesions) (Figs. 12.17, 12.18, and 12.19). HPV typing does not appear to bring any diagnostic and therapeutic benefit. The diagnostic difficulty of genital HPV infection is represented by: multifocality (one or more lesions in a single anatomic site) and multicentricity (more lesions in different sites) (Figs. 12.10 and 12.11). About 25% of women with vulvar condylomatosis also have HPV-related lesions at the vaginal and cervical levels, while one-third of women with intraep-

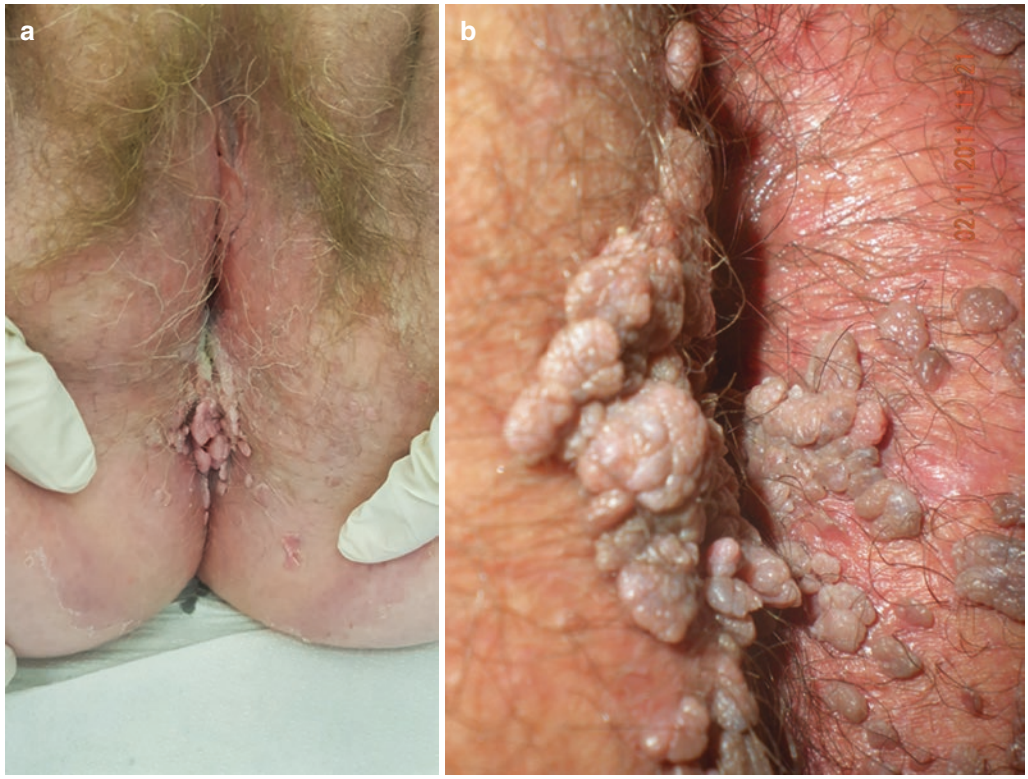

Fig. 12.4 Perianal genital warts (**a** and **b**)

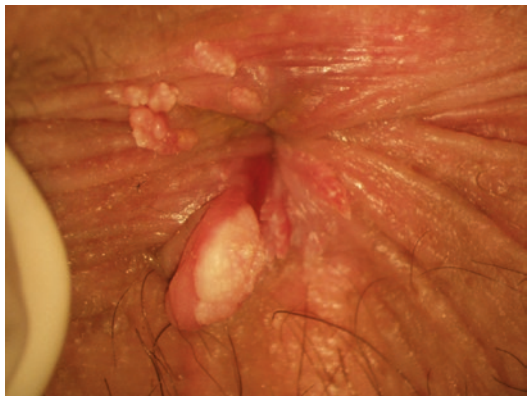

Fig. 12.5 Perianal genital warts

ithelial neoplasia of the vulva (VIN) (Fig. 12.20) presents in association an intraepithelial neoplasia of the cervix (CIN) and/or intraepithelial neoplasia of the vagina (VAIN). It is therefore advisable to perform always a cyto-colposcopic screening in all women diagnosed with condylomatosis genital. Because perianal localization is associated in 30% of subjects with localization

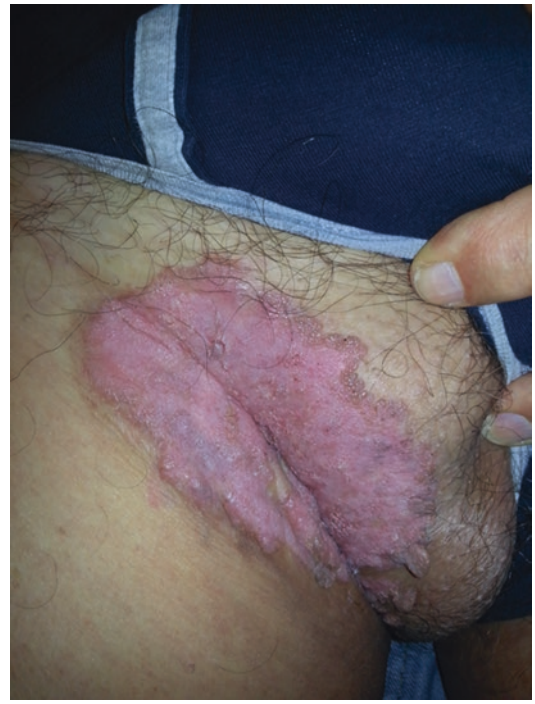

Fig. 12.6 Inguinal papular genital warts

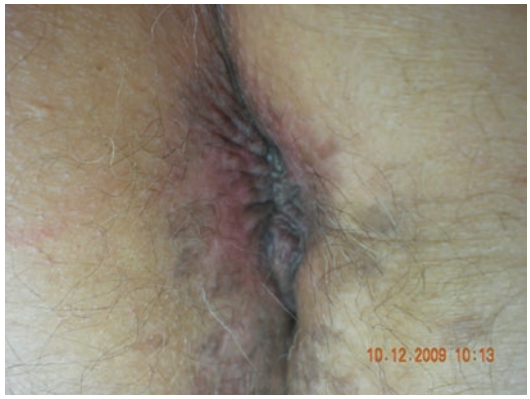

Fig. 12.7 Genital warts as perianal pigmentated lesions

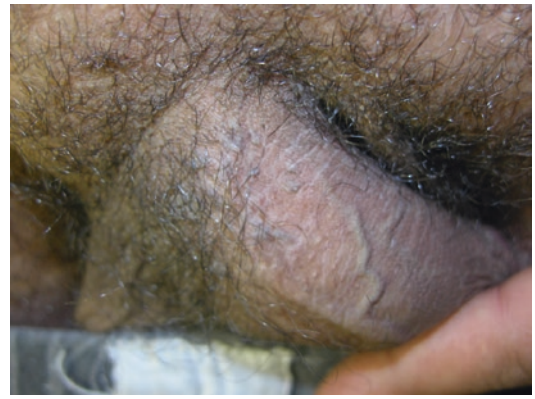

Fig. 12.8 Genital warts as papular lesions with smooth surface

Fig. 12.9 Genital warts as macular lesions

Fig. 12.10 Buschke-Lowenstein carcinoma in situ (HPV6-HPV11)

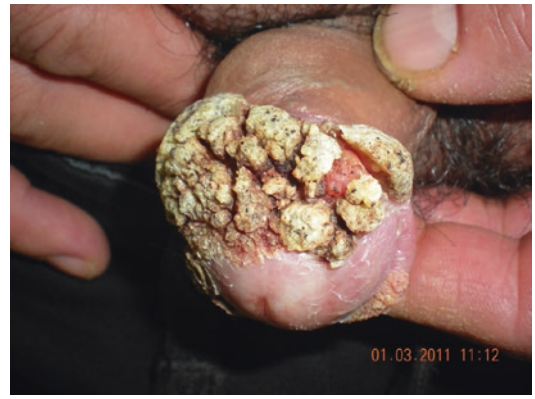

Fig. 12.11 Buschke-Lowenstein carcinoma in situ (HPV6-HPV11)

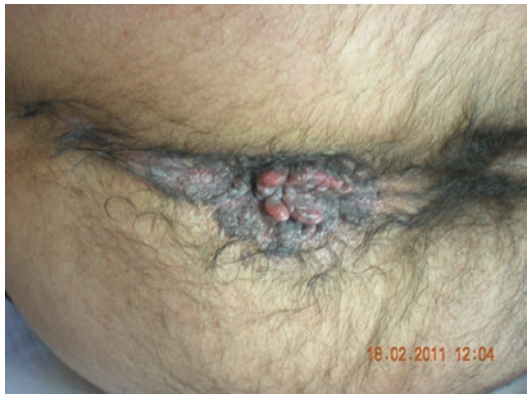

Fig. 12.12 Bowenoid Papulosis of the perianus

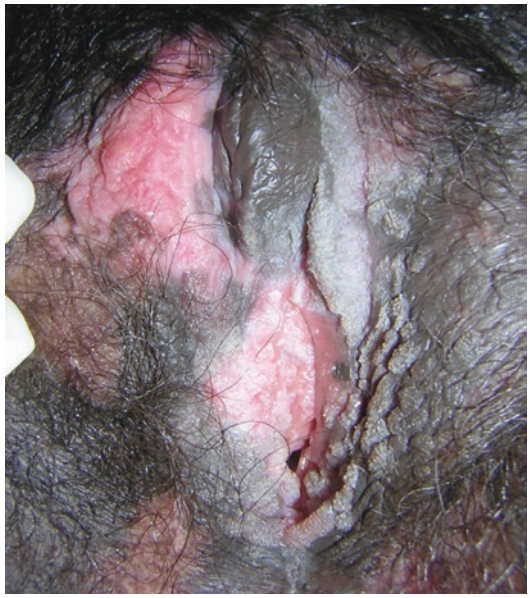

Fig. 12.13 Bowenoid Papulosis of the vulva

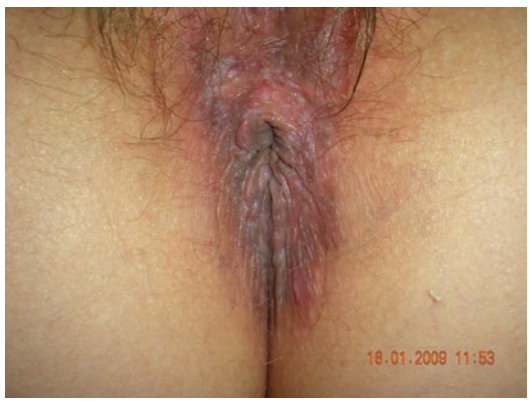

Fig. 12.14 Bowenoid Papulosis of the anus

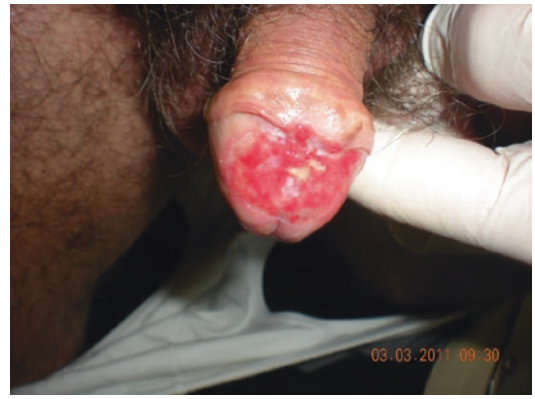

Fig. 12.15 Bowen disease (carcinoma in situ HPV16-associated)

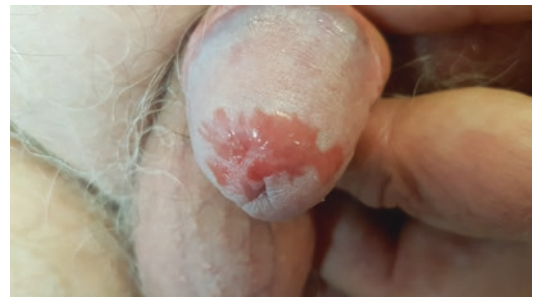

Fig. 12.16 Bowen Disease (HPV16)

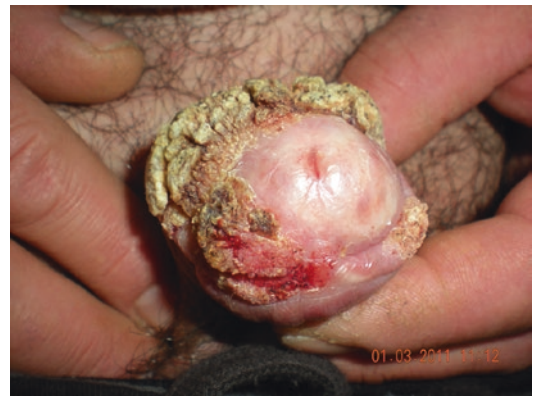

Fig. 12.17 Squamous Cell Carcinoma of the foreskin and gland

of HPV-lesions into the anal canal, anal endoscopy is recommended in individuals with perianal lesions and/or who report receptive anal sexual intercourses. Furthermore, since HPV infection is transmitted predominantly by sex, in all subjects diagnosed with condylomatosis genital it is advisable to carry out a direct screening for all other

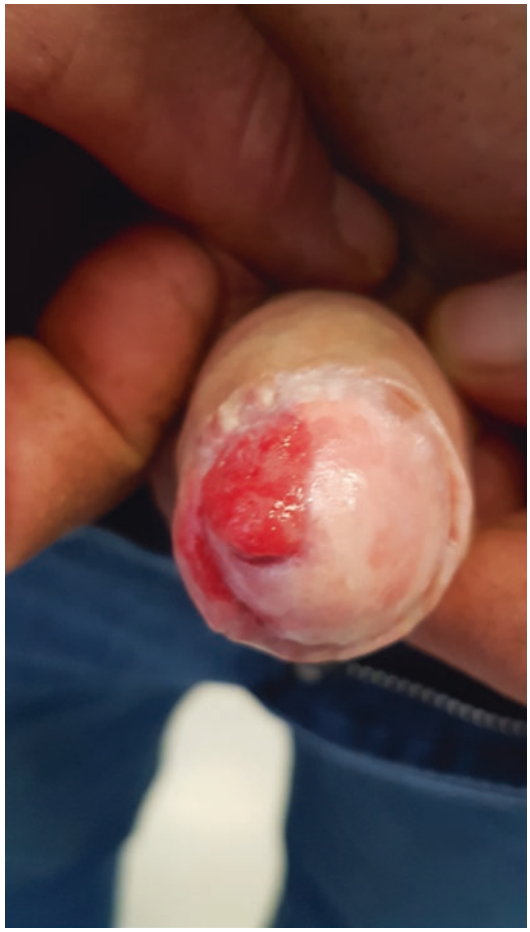

Fig. 12.18 Squamous Cell Carcinoma obstructing the urethral meatus

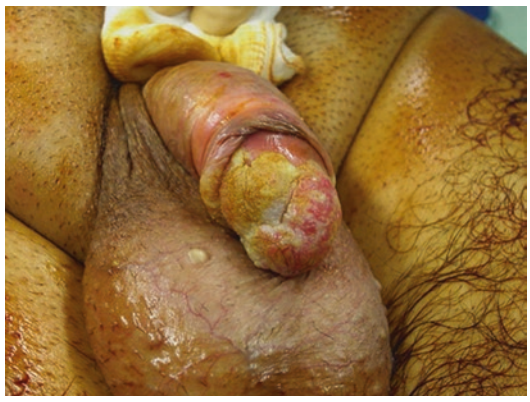

Fig. 12.19 Squamous Cell Carcinoma of the penis before the surgical excision

sexually transmitted diseases. The differential diagnosis of anogenital condylomatosis involves numerous dermatological conditions infectious

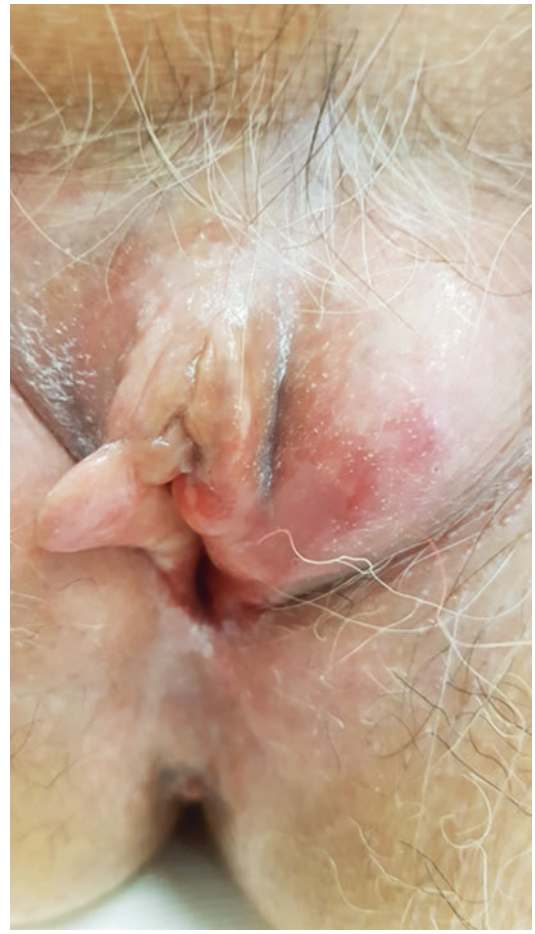

Fig. 12.20 Vulvar Squamous Cell Carcinoma in an elderly woman

(es contagious molluscum) and particular physiological genital pictures, in particular, the pearly papules in men and the papillomatosis of the inner surface of the small ones lips and vestibule (labial micro-papillomatosis) in women.

12.5 Treatment of AGWs

The primary goal of therapy of AGWs is the elimination of lesions responsible for physical or psychic symptomatology. No medical or surgical therapy is however able to eradicate HPV infection. The clearance of AGWs does not therefore eliminate HPV infection. The natural history of the disease in the absence of treatment includes: spontaneous resolution, clinical

stability, progression of lesions (increase in size or number of lesions (condylomata) and in 30% of cases recurrence [8]. All therapies are associated with local skin reactions including itching, burning, erosions and pain. Patients should be given a detailed explanation of their condition. This should be reinforced by offering them clear and accurate written information [9].

The treatment must be personalized according to the patient's preferences, the available treatments and to the doctor's experience, remembering that the ultimate goal is not just the removal of the injuries but the improvement of the patient's quality of life. In the absence of a response after at least 2 months or in case of relapses without reduction in the number of injuries it is advisable to change the therapeutic option or to consider also the therapeutic abstention possibility of spontaneous regression within six months described in 30% of untreated patients, a break therapy may be a choice when treatments prove to be an additional source of pain and stress. Treatments can be performed by the doctor (office therapy) or managed by the patient at home (home therapy) [8].

The following therapies are proposed

12.5.1 Home Therapy

1. Podophyllotoxin 0.5% solution and 0.15% cream: each course of podophyllotoxin treatment consists of self-application twice daily for 3 days, followed by four rest days. Use of 0.5% podophyllotoxin solution is convenient for penile warts. However, vulvar and anal warts are more feasibly and efficiently treated with 0.15% podophyllotoxin cream. Clearance rates of 45–83% after use of 0.5% podophyllotoxin solution for 3–6 weeks have been reported [10]. Clearance rates of 43–70% have been reported after the use of 0.15% podophyllotoxin cream at 4 weeks. Up to 65% of patients using podophyllotoxin experience transient and acceptable burning, tenderness, erythema and/or erosions for a few days when the warts necrotize. Recurrence rates of 6–100% have been reported with podophyllotoxin preparations between 8 and 21 weeks after clearance [11]. Podophyllotoxin is contraindicated during pregnancy, and women of childbearing age must use contraception or abstain from penetrative sexual activity during therapy.
2. Imiquimod cream 5%: Imiquimod cream is supplied as a package of twelve single use sachets. It is applied to the warts three times a week at the bedtime and the washed area with soap and water the next morning. Treatment continues until wart clearance, or for a maximum of 16 weeks. Local reactions at the treatment site may occur and may be managed by a rest period of several days, or by reducing the frequency of application. In clinical studies, wart clearance has been reported in 35–68% of patients with treatment courses up to 16 weeks. The reported clearance rates are higher in women than in men, and also women have a shorter median time to clearance than men. Erythema is often seen as a side effect with imiquimod therapy. Severe inflammation was frequently seen necessitating discontinuation of therapy. Phimosis and lichen sclerosis after therapy have also been reported. Relatively low recurrence rates (6–26%) after successful clearance have been reported [12–19]. Animal studies with imiquimod have not revealed any teratogenic effects in rats and rabbits. No adverse effects were found on reproduction in a second-generation rat study. Two case series of imiquimod use in pregnant women have been published, and no adverse pregnancy outcomes or foetal abnormalities have been reported [20, 21]. Nevertheless, more data is needed before imiquimod cream can be considered safe during pregnancy. The new formulation of a previously recommended medication, imiquimod 3.75% cream, has been approved by the Food and Drug Administration (FDA) for the treatment of external AGWs in patients aged 12 years and older. Instructions for use are similar to imiquimod 5.0% cream, with the exception that imiquimod 3.75% cream is applied once daily instead of 3 times/week. Safety and efficacy have not been evaluated in pregnant, breastfeeding, or immunosuppressed patients,

or in patients with intravaginal, cervical, rectal, or intra-anal warts [19]. FDA approval was based on two randomized, double-blinded, placebo-controlled trials involving 601 adult patients with external genital warts treated with vehicle or imiquimod 3.75% cream daily for up to 8 weeks. Sixteen weeks after the start of the study period, treated patients had a clearance rate of 27–29%, while patients receiving the vehicle had a clearance rate of 9–10%. Treatment-related adverse effects that occurred in >1% of those treated with imiquimod 3.75% cream included application site pain, pruritus, irritation, erythema, bleeding and discharge [26].

3. Sinecatechins (FDA pregnancy category C) are extracts of green tea leaves that are compounded as a 15% ointment. Sinecatechins are thought to decrease viral replication. Evidence suggests the mechanism of action of sinecatechins is through anti-proliferative mechanisms. Patients should apply a 0.5 cm strand of ointment onto each wart three times daily for up to 16 weeks. In a 16-week trial of 604 patients, clearance rates were 53.6% in the treatment group and 35.3% in the control group (number needed to treat = 5.5). Recurrence rates of 6.8% and 5.8% were noted at 12 and 16 weeks, respectively, after complete clearance. Adverse effects included erythema, itching, burning, pain, erosion, ulceration, induration and a vesicular rash. Up to 67% of patients experienced moderate to severe reactions, but only 2.3% discontinued therapy because of adverse effects. It cannot be used internally or in pregnancy [22–25].
4. Podophyllin (FDA pregnancy category X) is an herbal extract compounded as a 25% solution. In 1942 the first description of this practice was published in the medical literature, the first topical treatment of genital warts. The preparation causes wart regression and necrosis by stopping mitosis. The solution is applied to warts up to once weekly for 3–6 weeks. The solution is left on for 1–2 h, then washed off. Very rare side effects include bone-marrow suppression, liver dysfunction, neurological compromise, podophyllin hallucinations, psychosis, nausea, vomiting, diarrhoea, abdominal pain and genital burns. Because of reported toxicity, including foetal death, when podophyllin is overapplied or occluded, the Centers for Disease Control and Prevention recommends limiting the application area to less than 10 cm^2 of warts per treatment, and limiting the amount applied to less than 0.5 mL per treatment. Application to mucosal areas increases the risk of systemic absorption; open ulcers or wounds should be avoided. The clearance rate was 62%, with recurrence of 26% at 12 weeks. Podophyllin can cause significant toxicity if not applied and removed properly. Because of the risks of podophyllin and the safety of its purified form, podofilox, some experts have recommended discontinuing its use as a treatment for genital warts. However, it is inexpensive, widely available, and is likely effective [27–29].

12.5.2 Office Therapy

1. Trichloroacetic acid and dichloroacetic acid (also called bichloroacetic acid; both FDA pregnancy category N [not classified]) cause protein denaturing and cell death when applied to the skin. Trichloroacetic acid may be compounded in different strengths—generally 60–90%—whereas bichloroacetic acid comes in a standard strength. A thin amount is applied to each wart and allowed to turn white or "frost", indicating precipitation of denatured proteins. The solution has low viscosity and can spread readily, thereby destroying unaffected tissue. The acids are applied in the office, with repeated applications up to three times weekly until the warts have resolved. There are no placebo-controlled trials with these acids, but small comparison trials with cryotherapy have shown similar clinical effectiveness (64–88% clearance rate, 36% recurrence rate) [30, 31].
2. Cryotherapy eliminates warts by thermolysis. Liquid nitrogen (−196 °C) or a cryoprobe is applied centrally until a white halo of frozen

tissue reaches 1–2 mm beyond the lesion. As the tissue thaws, cells are lysed. Often, a second or third cycle of freezing is necessary at each session. Pedunculated lesions may be treated by grasping with tweezers or a hemostat that has been cooled in liquid nitrogen. Treatment sessions often must be repeated every 2 weeks, although no studies have systematically evaluated different treatment intervals. Clearance rates range from 71% to 79%, with recurrence rates of 38–73% at 6 months. Application techniques are difficult to standardize and there may be significant intra-operator differences. Cryotherapy has the advantages of being simple, inexpensive, rarely causes scarring or depigmentation, and is safe in pregnancy. Clinical studies have reported clearance rates in the range of 44–75%, 32–35 and recurrence rates of 21–42% 1–3 months after clearance [31, 32].

3. Electrosurgery effectively excises external genital warts—often in a single visit—but requires considerable technical skill and may result in pain and scarring if the excision is too deep. Local anaesthesia is required. Lesions on the penis or anal verge require additional caution and skill. The smoke resulting from this procedure may contain HPV particles, similar to that from carbon dioxide laser surgery of warts. Transmission to the oropharynx is unlikely, but use of smoke evacuation equipment and a mask is recommended. Clearance rates with electrosurgery range from 90% to 96%, and recurrence rates of 18% have been reported [31].
4. Surgical excision may be the most cost-effective treatment for genital warts. This method may be particularly helpful when warts are pedunculated or exophytic. After anaesthesia is administered, lesions are excised tangentially at the base. Haemostasis is obtained with aluminium chloride or light electrocautery. Sutures are rarely needed. This method preserves tissue for histologic examination and offers quick results. Pain, scarring, slow healing, and pigment changes are possible. Clearance rates with surgical excision range from 89% to 93%, and recurrence rates are 19–29% at 12 months. Curettage is a simple effective technique for small numbers of lesions, and either diathermy or silver nitrate is used for haemostasis. Carbon dioxide and YAG laser emissions result in very high power densities being delivered to small tissue volumes. Both electrosurgery and laser surgery should be performed with the use of surgical masks by the treatment team, and the use of a smoke evacuator. We were not able to locate any suitable RCTs evaluating curettage or laser therapy. Formal surgery bulky warts, extensive warts, anal /intra-anal warts and significant lesions in children are most conveniently removed under general anaesthesia by an appropriate surgical specialist. [32, 33].

12.6 Diagnosis and Management of Vulvar Intraepithelial Neoplasia (VIN)

Although spontaneous regression has been reported, VIN should be considered a premalignant condition. There are no screening strategies for the prevention of vulvar cancer through early detection of vulvar HSIL (VIN usual type). Detection is limited to visual assessment with confirmation by histopathology when needed [34].

12.6.1 Classification

Traditionally, squamous VIN was classified into three grades, analogous to the three-grade cervical intraepithelial neoplasia classification. In 2004, International Society for the Study of Vulvovaginal Disease (SSVD) replaced the previous three-grade classification system with a single-grade system, in which only high-grade disease is classified as VIN [35]. In that system, VIN is subdivided into *usual type VIN* (including warty, basaloid, and mixed VIN) and *differentiated VIN*. Usual type VIN commonly is associated with carcinogenic genotypes of HPV and persistence risk factors, such as cigarette smoking and immunocompromised status, whereas differentiated VIN usually is not asso-

ciated with HPV and is more often associated with vulvar dermatologic conditions, such as lichen sclerosus. Differentiated VIN associated with lichen sclerosus is more likely to be associated with a squamous cell carcinoma of the vulva than usual type VIN. Furthermore, it has a higher recurrence rate and decreased disease-specific survival from invasive squamous cell carcinoma [36].

The rationale for changing the terminology in 2015 was to unify the nomenclature of HPV-associated squamous lesions of the lower genital tract. The ISSVD recommends the terms *low-grade squamous intraepithelial lesion of the vulva* (*vulvar LSIL*) and *high-grade squamous intraepithelial lesion of the vulva* (*vulvar HSIL*) for histopathologic diagnosis of productive HPV infections, which includes external genital warts and precancer, respectively. The 2015 terminology is similar to the World Health Organization's classification and to the Lower Anogenital Tract Squamous Terminology classification that is used by the American Society for Colposcopy and Cervical Pathology and has been adopted by the College [37]. Based on the 2015 ISSVD terminology of vulvar squamous intraepithelial lesions, usual type VIN is now classified as vulvar HSIL, and differentiated VIN remains the same. Flat lesions associated with basal atypia and koilocytic changes (formerly termed VIN 1) are considered LSIL (condyloma or HPV effect) in the current 2015 ISSVD classification system [34].

Based on the level of involvement of the thickness of the epithelium by the dysplastic cells, VIN were graded in three grades (WHO terminology):

1. low grade (VIN 1) if the dysplastic cells involve the lower third of the epithelium;
2. moderate grade (VIN 2) when the dysplastic cells are present in the lower two-thirds of the epithelium;
3. high-grade (VIN 3) if there is full-thickness involvement of the epithelium by the dysplastic cells. VIN 3 is synonymous with carcinoma in situ. It is interesting to note that VIN 2 and VIN 3 confer the same risk and rate of progression to invasive carcinoma if untreated.

The International Society for the Study of Vulvovaginal disease (ISSVD) has proposed that VIN should not be graded but described as high-grade VIN lesions only (VIN 2 or VIN 3). The ISSVD has also recommended that the term low grade VIN (VIN 1, or mild dysplasia) should not be used anymore and that such lesions should be classified as flat condyloma acuminatum, or given an appropriate descriptive term.

Differentiated VIN (dVIN). Differentiated (simplex) VIN is classified as high-grade VIN (thus VIN 3) due to its associated high risk to progress into invasive SCC [38].

12.6.2 Diagnosis

There are no screening strategies for the prevention of vulvar cancer through early detection of vulvar HSIL (VIN usual type). Detection is limited to clinical assessment with confirmation by incisional biopsy and histopathology exam. The appearance of vulvar HSIL (VIN usual type) can vary. Biopsy should be performed in all clinically atypical lesions, especially in postmenopausal women with apparent genital warts and in women of all ages with suspected condyloma in whom topical therapies have failed. Indeed HIV positive patients and patients on immunosuppression after organ transplant may need biopsy of lesions when the level of suspicion is lower. Colposcopy is necessary in determining the extent of disease if lesions are not completely visible or not clearly demarcated.

12.6.3 Treatment

Treatment is recommended for all women with vulvar HSIL (VIN usual type). Because of the potential for occult invasion, wide local excision should be performed if cancer is suspected, even if biopsies show vulvar HSIL. When occult invasion is not a concern, vulvar HSIL (VIN usual type) can be treated with excision, laser ablation or topical imiquimod (off-label use) [38].

12.6.4 Surgical Therapy

Wide local excision is the preferred initial intervention to obtain a specimen for pathologic analysis for women in whom invasive cancer cannot be adequately ruled out from their clinical or pathologic findings, despite a biopsy diagnosis of only vulvar HSIL (VIN usual type). The excision should include gross margins of 0.5–1 cm around tissue with visible disease, but may be altered to avoid injury to the clitoris, urethra, anus or other critical structures. The presence of clear margins in the excised tissue specimens has a lower, although still significant, risk of recurrence compared with women with involved margins [39].

12.6.5 Laser Ablation

Laser ablation is acceptable for the treatment of vulvar HSIL (VIN usual type) when cancer is not suspected. It can be used for single, multifocal or confluent lesions, although the risk of recurrence may be higher than with excision [40]. As with excision, a 0.5–1 cm margin of normal-appearing skin should be treated. Extese vulvar HSIL (VIN usual type) lesionsover hair-bearing areas may be preferentially treated with surgical excision.

12.6.6 Medical Therapy

Randomized controlled trials have shown that the application of topical imiquimod 5% (that is considered an off-label use) is effective for the treatment of vulvar HSIL (VIN usual type) [41, 42]. Published regimens include three times weekly application to affected areas for 12–20 weeks, with colposcopic assessment at 4 to 6-week intervals during treatment. Residual lesions require surgical treatment. Erythema and vulvar pain may limit use. Experience with imiquimod in immunosuppressed patients is limited. Because it is believed to act through local immunomodulators, it may have decreased effectiveness in women who are immunocompromised. Photodynamic therapy has been effective in some trials, but requires specialized equipment and training [43].

12.7 Diagnosis and Management of Premalignant Penile Lesions

Premalignant penile lesions related to HPV infection include Bowen's disease (BD), erythroplasia of Queyrat (EQ) and Bowenoid papulosis (BP), which are associated with "high-risk" HPV types 16 and 18. Low-risk HPV types 6 and 11 are associated with other premalignant lesions, such as giant condylomata acuminate (GCA) or Buschke–Lowenstein tumours.

Several risk factors have been associated with the development of malignant penile lesions as the presence of a foreskin, phimosis, poor hygiene, smoking, chronic inflammation and having multiple sexual partners. Infection with human papilloma virus is one of the most important and widely studied risk factors in penile cancer development, with HPV DNA found in approximately 50% of all penile squamous cell carcinomas (SCCs) [44]. In a new proposed classification system the term penile intraepithelial neoplasia (PeIN) is used to describe all premalignant lesions. It is further subclassified into differentiated PeIN, the subtype most frequently associated with chronic inflammation and not HPV related, and three other subtypes (warty, basaloid, and mixed warty-basaloid), which are linked to HPV infection.

The immunohistochemical p16 overexpression is considered as a surrogate for high-risk human papillomavirus infection. It was demonstrated a significant association of the negative patterns and differentiated PeIN and of the positive pattern and warty, basaloid and warty-basaloid PeIN

[45].

Carcinoma in situ (CIS) is eponymously known as erythroplasia of Queyrat (EQ) and Bowen's Disease. Lesions in erythroplasia of Queyrat are usually sharply defined plaques, which have a smooth, velvety, bright red appearance. They are usually painless, but can have areas of erosion. The vast majority occur in

uncircumcised men with phimotic foreskins (Fig. 12.17).

Bowenoid papulosis lesions occur primarily on the penile shaft or mons, although they can also occasionally arise on the glans and prepuce. They are usually multiple, red, velvety, maculopapular areas, which can coalesce to form larger plaques. Associated pigmentation leads to a brownish appearance, and they often cause pruritis or discomfort. It is commonly associated with HPV 16. However, unlike the severe dysplasia of carcinoma in situ the moderate dysplasia of Bowenoid papulosis usually runs a more benign course, with malignant transformation in less than 1% of cases, primarily in immunocompromised patients [46]. Both are essentially the same histological premalignant condition, differing primarily only in location. Lesions arising from the mucosal surfaces of the genitalia, such as the inner prepuce and glans, are called EQ, while BD is essentially considered the same pathological process affecting the skin of the penile shaft. In Bowen's Disease lesions are usually solitary, well defined, scaly, dull-red plaques, often with areas of crusting. Lesions may also be heavily pigmented, resembling melanoma. Occasionally they may have associated leukoplakic, nodular or ulcerated changes. They occur primarily on the shaft, but may also be encountered in the inguinal and suprapubic regions.

Lesions in erythroplasia of Queyrat are usually sharply defined plaques, which have a smooth, velvety, bright red appearance. They are usually painless, but can have areas of erosion. These two entities have differing rates of progression to invasive disease. Malignant transformation has been reported in 5% of cases of Bowen's Disease [47], while erythroplasia of Queyrat has reported transformation rates of up to 30% [48].

12.7.1 Giant Condyloma Accuminatum (Buschke–Lowenstein Tumour)

Condyloma acuminata are warty, exophytic growths which can affect any part of the anogenital region. On the penis, they primarily occur around the coronal sulcus and frenulum, but can also be found as flat lesions on the penile shaft. They can occasionally extend into the anterior urethra, but more proximal urethral extension into the bladder is usually only seen in immunocompromised patients Confluence of these lesions can lead to the development of large, exophytic growths known as Buschke–Lowenstein tumours, after the original description of the condition by the authors in 1925 [52, 53, 57].

In recent years there has been a change of terminology used in the histopathological classification of premalignant penile lesions. In order to better understand the management of these lesions it is essential to be clear about these changes.

A different number of terms existed in the histopathological description of penile premalignant lesions. These include penile intraepithelial neoplasia (PeIN), Squamous Carcinoma in situ, High-Grade Squamous Intraepithelial Lesions (HSIL) and Low Grade Squamous Intraepithelial Lesion (LSIL). In addition PeIN has been further subdivided into grade I to III (low to high grade) [49].

Penile squamous cell carcinomas (SCCs) and their corresponding precancerous lesions can be classified in two major groups: human papillomavirus (HPV) related and HPV unrelated. In the former (warty and basaloid SCC), there is a predominance of undifferentiated basaloid cells. In the latter (usual, papillary and verrucous SCC), the predominant cell is larger with abundant eosinophilic cytoplasm. Based on these morphologic features, the new term, “penile intraepithelial neoplasia” (PeIN), was proposed. Macroscopically, PeIN subtypes are indistinguishable. Microscopically, differentiated PeIN is characterized by acanthosis, parakeratosis, enlarged keratinocytes with abundant “pink” cytoplasm (abnormal maturation) and hyperchromatic cells in the basal layer. In basaloid PeIN the epithelium is replaced by a monotonous population of uniform, small, round and basophilic cells. Warty PeIN is characterized by a spiky surface, prominent atypical parakeratosis and pleomorphic koilocytosis. Warty-basaloid PeIN shows features of both warty and basaloid PeIN. There is a significant association of subtypes of PeIN with specific variants of invasive

SCCs [50]. A simpler classification has recently been proposed and endorsed by the World Health Organization (WHO). This acknowledges the role of HPV in the pathophysiology of these lesions [49]. The change in classification has resulted in removal of all of the above terms (including the subgrouping of PeIN) to leave two categories, undifferentiated PeIN or differentiated PeIN.

Undifferentiated PeIN encompasses the clinically defined entities of Bowen's disease and Erythroplasia of Queyrat, as well as previously defined Squamous Carcinoma *in situ*. Since the recognition of the role of HPV, undifferentiated PeIN can be further subdivided into *basaloid and/or warty subtypes* [51]. These are frequently associated with HPV 16. The association between undifferentiated PeIN and invasive warty and basaloid type tumours encourages treatment undifferentiated PeIN as opposed to observation [57].

Differentiated PeIN is defined histologically by atypical squamous cells which are confined to the lower layers of the penile squamous epithelium. It is usually associated with architectural atypia, elongated rete ridges and aberrant intraepithelial keratinisation. It is not usually associated with high-risk HPV subtypes [51]. The association of lichen sclerosus and SCC is widely recognized and the risk has been estimated at between 2% and 12.5% [52–54]. Early microinvasive disease might be challenging to diagnose clinically against a background of LSc. A retrospective review by Barbagli et al. of 130 patients with penile lichen sclerosus, revealed 11 men (8.4%) with premalignant or malignant features (seven cases with SCC, two cases with verrucous carcinoma, 1 case of SCC associated with verrucous carcinoma and one case of erythroplasia of Queyrat) [55, 56]. The time interval between diagnosis of lichen sclerosus and the development of SCC was 14–30 years.

Clinically PeIN typically presents as a solitary lesion on the penis, and usually presents in men over the age of 35, with a peak incidence of 60–70 years [57]. According to the British Association for Sexual Health and HIV (BASHH) guidelines any suspicious persistent or atypical penile lesions should be managed with a full anogenital examination followed with a biopsy to confirm the diagnosis and exclude invasive malignancy [51].

12.8 Treatment of Premalignant Penile Lesions

Different options and treatment modalities are available for premalignant penile lesions [58, 59]. All men with suspected premalignant disease of the penis must undergo a diagnostic biopsy. The choice of treatment should be tailored to the type and site of the lesion, taking into account patient preference and the need for close follow-up with the more minimally invasive techniques. The two main topical treatments for non-invasive penile cancer are 5-fluorouracil (5-FU) and Imiquimod.

12.8.1 5-FU

5 Fluoro Uracil exerts its chemotherapeutic effects through inhibition of the enzyme thymidylate synthetase. The antimetabolite effects occur through increased uptake in rapidly dividing cancer cells. 5-FU was first recognized in the 1960s as a treatment for actinic keratosis. As this became a widely practiced way of topical and effective management it was trialed in other topical low-grade cancers, in particular penile cancer [60, 61]. Penile tissue is suited to topical 5-FU treatment as recurrence from secondary progression of bowenoid areas from hair follicles is common. This is a significant step as tissue preservation in penile cancer is very important for psychological and sexual preserving reasons. 5-FU is a topical agent and it is well tolerated by patients. Protocols vary among clinicians on how frequently the 5-FU is applied. It is usually applied topically for between 4 and 6 weeks on alternate days. Close follow-up is of particular importance in topical therapies for cancers. If the initial course of topical 5-FU fails, then this treatment is not usually repeated [62, 63].

12.8.2 Imiquimod (IQ)

IQ 5% is an immuno-modulating drug which acts on several levels of the adaptive immune system. It activates the cells of this aspect of the immune system through toll like receptor 7 (TLR-7) causing secretion of cytokines such as interferon alpha,

interleukin 6 (IL-6) and tumour necrosis factor alpha. The evidence for the use of topical IQ in PeIN is very heterogenous with most data coming from small case series and case reports. There has been more extensive use of IQ in extra genital CIS. A recent review picked up a total of 29 articles (22 case reports and 7 small case series) where IQ was used in the treatment of PeIN. In total this amounts to 48 patients, this is the largest aggregated number of patients in a review of IQ treatment to date. The majority of patients present with the clinical entity erythroplasia of query at ($n = 32$), the remaining present with Bowen's disease ($n = 8$) or bowenoid papulosis ($n = 8$). The small studies included in this review are quite heterogenous; the duration of treatment, frequency of application etc. is very variable. This makes drawing firm conclusions about the efficacy of topical IQ difficult. A review of cohort studies and case series has reported a complete response rate of 70% and a partial response rate of 30%. Treatment given less than 4 times weekly appears to give a longer duration of complete response (81% complete response), although, inevitably the treatment regime lasts longer (mean duration 113 days). This compares with a 68% complete response in those patients receiving treatment greater than 4 times weekly (mean duration 53 days). Toxicity data for the use of topical IQ is reported in extra genital disease. The most common adverse effect is local skin irritation at the application site. Other adverse effects include headache, flu like symptoms and myalgia. In our experience of a small number of patients treated with topical IQ for PeIN it is well tolerated. There is no evidence to suggest that any patients have ceased treatment due to toxicity [56].

12.8.3 Photodynamic therapy

Photodynamic therapy (PDT) for premalignant penile lesions is still in its infancy. This technique involves covering the affected region with a topical photosensitizing cream containing chemicals such as delta-5-aminolaevulinic acid for approximately 3 h, which are preferentially taken up and retained by malignant cells. The lesion is then treated by exposure to incoherent light from a PDT lamp, leading to photoselective cell death of sensitized cells. In a study of 10 patients, only 40% had a complete response after a mean follow-up of 35 months, but required on average four treatments [65].

The disadvantage of PDT and laser ablation is the lack of histological tissue for analysis. Evidence for these treatments once again is rather heterogeneous with small study populations. Filonenko et al. treated 10 patients over a 2-year period with PDT and achieved complete response in nine patients [64]. The follow-up period was short and this illustrates the difficulties in demonstrating durability of this treatment. PDT was used to treat seven out of ten patients successfully with a mean follow-up of 35 months by Paoli's group in Sweden. Three patients recurred with PeIN but no patient developed invasive cancer. Reported toxicity was minor with all patients experiencing a degree of pain, although this was not quantified. In addition superficial erosions were common but reportedly healed in a matter of days [65].

12.8.4 Laser Therapy

Carbon dioxide (CO_2) and neodymium:yttrium aluminium garnet (Nd:YAG) lasers have been used as first-line therapy with reasonable response rates and good cosmetic and functional results. The CO_2 laser has a tissue penetration of 2–2.5 mm and can be used as a scalpel to excise tissue for histological analysis by direct focusing of the beam. Treated areas generally take 3–4 weeks to heal. The Nd:YAG laser has a tissue penetration of 3–5 mm, but causes tissue coagulation preventing histological diagnosis, and runs a risk of understaging the disease. Larger lesions can be treated using this laser, but ablation sites can take up to 2–3 months to heal. Treatment with either of these lasers is usually well tolerated, with minor complications including minor pain and bleeding at treatment sites [66]. Laser has a higher retreatment rate and risk of progression compared with other treatment modalities. In one study of 19 patients treated with laser therapy, 26% required successful retreatment for his-

tologically confirmed Tis recurrence after a mean follow-up of 32 months, while one patient (5%) progressed to invasive disease [67]

12.8.5 Cryotherapy

Cryotherapy uses liquid nitrogen or nitrous oxide to generate rapid freeze/slow thaw cycles to achieve temperatures between −20 °C and −50 °C to cause tissue damage by formation of ice crystals, leading to disruption of cell membranes and cell death. A study of 299 patients with extragenital BD compared cryotherapy with topical 5-FU and surgical excision. The study showed that there was a greater risk of recurrence after cryotherapy (13.4%) compared with 5-FU (9%) and surgical excision (5.5%) [68].

12.8.6 Surgical Excision

Circumcision forms an essential part of the management of premalignant conditions, not only to remove the lesion if confined solely to the prepuce, but also to prevent persistence of an environment suited to HPV infection, chronic inflammation and progression to invasive disease. Some centres advocate the use of 5% acetic acid applied to the penis for up to 5 min to help detect occult areas of CIS using the "acetowhite" reaction and help guide resection [69] this technique is not advocated by all. All premalignant lesions are suitable for treatment by surgical excision. Primary surgical excision is advocated in patients who have extensive field change, and in those unlikely or unwilling to adhere to strict treatment and surveillance protocols. It also has a role in recurrent disease following other conservative therapies, where repeated topical therapies result in an unsightly scarred and denuded glans that can make clinical monitoring difficult.

12.8.7 Mohs' Micrographic Surgery

An alternative surgical approach is excision using Mohs' micrographic surgery. This involves removal of the entire lesion in thin sections, with concurrent histological examination to ensure clear margins microscopically. While this technique allows maximal preservation of normal penile tissue, it is difficult and time consuming, requiring both a surgeon and pathologist trained in the technique to ensure adequate oncological clearance. A recent review of this technique reported a high (32%) recurrence rate and the uptake and use of the technique worldwide has been very limited [70]

12.9 Anal Intraepithelial Neoplasia (AIN)

Anal cancer is a rare neoplasia, and represents 0.4% of all cancer diagnosed in USA and in UK [71]. A rise in incidence of Anal cancer has been observed in the last decades. It is associated with HPV infection in 70–90% of cases especially in presence of high-risk strain like HPV16 [72]. AIN is a potential precursor of anal squamous cell carcinoma and it is has been described by a variety of different classification.

Considering the connection between anal dysplasia and the HPV new criteria borrowing from the cervical pathology a new classification system was developed using the Bethesda System terminology borrowing from cervical pathology. AIN I is considered where nuclear abnormalities are limited to the lower third of the epithelium, AIN II where the cellular alterations are in the upper two third of the epithelium and in AIN III changes are found in over the entire epithelium. Cytology reports include the term "ASCUS", or "atypical squamous cells of undetermined significance", "low grade" and "high-grade squamous intraepithelial lesions" (LSIL and HSIL, respectively) [73]. Cytological and histological grading of anal lesions shows low concordance: one-third of cases of ASCUS and LSIL may correspond to high-grade lesions (AIN II-III). For this reason it is very important that all cases of alteration cytology should be examined in detail with anoscopy HD. Poor data exist about the correct incidence of AIN in the general population. The high prevalence of HR-HPV in cancerous cervical (about

90%), vulvar (around 40%), penile (around 50%), oropharyngeal (13–56%). In anal lesions the presence of HPV is very high (>80%) [74] and in particular anal HPV is very common among population at high risk for sexually transmitted infections as men who have sex with men and HIV-infected people. These population develop more frequently preneoplastic and neoplastic anal lesions due to their sexual habits. The prevalence of anal HPV infection is wide different in different study and depend on the characteristics of the HPV testing method used and those the target population. In HIV-negative MSM the overall prevalence of anal HPV was detected from 63.9% [75] to 72.8% [76] and 74.8% with 56.2% of the MSM being infected by high-risk (HR) types and did not significantly change across ages both for any and high-risk genotypes (77%). Among HIV MSM-positive patients anal infection is very common also in older individuals (about 60%) [77]. This in contrast to cervical HPV infection which is more frequent at 25–30 years and declines until the second age peak in women under 45 years. In MSM the anal HPV prevalence across all ages may be driven by multiple partnerships in association with sexual mixing across all age groups [78].

12.9.1 Risk Factors

Studies about the risk of acquisition of anal HPV infection showed that in MSM unsafe anal sex is significantly associated with a higher risk of anal infections [79], indeed the HIV seropositivity status was associated with a higher incidence and a low clearance of anal HPV infection especially in MSM HIV positive persons [80]. The risk of HPV anal infection in women increases with the number of lifetime sexual partners as in men [81]. Anal cancer development is preceded by precursor lesions defined as high-grade squamous intraepithelial lesions (HSIL or AIN2/3). The prevalence for these lesions is estimated from 29 to 50% for HIV-infected MSM patients and 21.5% for HIV-negative MSM [82]. Scarce data there are about the prevalence of HSIL in the general population, as the majority of the studies were conducted in MSM and MSM HIV positive considered at more risk to cancer progression. The direct progression of anal high-grade squamous intraepithelial lesions to invasive anal cancer was made on HIV positive MSM and demonstrated that anal HSIL represents the true precursor lesion of anal cancer [83]. The main risks factors for H-grade lesions are anal, cervical or any other genital diseases for women, being MSM, HIV infection, receptive anal intercourses [84]. Cigarette smoking was considered a risk factor for anal cancer in general population [85]. The risk for anal cancer is higher in HIV positive MSM and the antiretroviral therapy (HAART) for HIV does not reduce the risk for anal cancer, and the improvements in life expectancy of HIV positive persons thanks to HAART allow sufficient time to anal preneoplastic lesions to develop into cancer and could explain the increase burden of anal cancer in HAART era [86].

12.9.2 Screening for AIN

Anal cytology for cytopathologic examination is recommended screening for at risk populations as for cervical cancer screening test given the biological analogy of CIN and AIN [87]. The exam consists of the insertion of a rayon or polyester swab moister with tap water for more than 3 cm until it reaches the rectum. The anal swab is rotated in circular motion to obtain a sufficient number of cells from the mucosal epithelium. The collected cells are submerged in a vial containing a liquid preservative that is able to fix the cells that then are processed and stained. The Bethesda System categorizes cells as atypical squamous cells of undetermined significance (ASCUS), squamous intraepithelial lesions subdivided in Low and High SIL. SIL refers to mild dysplasia or AIN I and HSIL comprises moderate and severe dysplasia, carcinoma in situ, AIN II and AIN III [88]. The sensitivity of anal cytology is similar to the cervical one (ranging from 69% to 93%) however cytology showed less specificity (32–59%). The lower specificity of anal cytology in MSM respect to women could due to the high number of sexual partners and to the high prevalence of anal HPV infection in all age group [76].

12.9.3 High Resolution Anoscopy

Patients with cytological alterations are considered eligible for High Resolution Anoscopy (HRA). HRA is used to identify visible signs of dysplastic tissue, after application of acid acetic. Solution and Lugol's iodine abnormal tissue areas on that anal canal are coloured. Any alteration, ulcerations, irregular vascular patterns or acetowhite areas should be considered for biopsy [89]. HRA and biopsy of abnormal tissue of anal canal are considered the gold standard for the diagnosis of AIN.

12.9.4 HPV Testing

HPV testing is not considered to date a standardized clinical test. None of the currently available assays has been approved for use in the AIN screening because the high prevalence of HPV especially in the high-risk population HPV testing is not considered of value for primary screening [90].

Recent European AIDS and Clinical Society (October 2017) recommend screening with anal pap smear and anoscopy for all MSM HIV positive every 1–3 years but given the paucity of studies the guidelines consider that the evidence of benefit is unknown but advocated by some experts.

The Centers for Disease Control and Prevention last guidelines 2018 consider as cost-effectiveness evaluations indicate that in HIV-seropositive patients screening for lesions using anal cytology and treating anal precancerous lesions to reduce risk of anal cancer in patients with HIV may provide clinical benefits comparable to measures for prevention of other opportunistic infection [91].

No recommendations exist for routine screening for anal cancer. However, some specialists recommend anal cytologic screening or high resolution anoscopy for HIV-seropositive men and women; annual digital anal examination may be useful to detect masses on palpation that could be anal cancer. Screening for anal cancer with anal cytology should not be done without the availability of referral for high resolution anoscopy. If anal cytology is performed and indicates ASCUS, then ASC cannot rule out ASC-H, LSIL, or high-grade squamous intraepithelial lesion (HSIL), then it should be followed by high-resolution anoscopy.

Visible lesions should be biopsied to determine the level of histologic changes and to rule out invasive cancer.

12.9.5 Treatment of AIN

For AIN2-3, no adequate RCTs have been reported and data are insufficient to recommend a specific treatment approach. An RCT was recently initiated to determine if treatment of AIN2-3 reduces the incidence of anal cancer in patients with HIV. Definitive guidelines on anal screening and treatment in patients with HIV will likely follow from the results of this study. Until then, treatment decisions are based on assessment of the size and location of the lesion and its histologic grade. All treatment modalities are associated with high rates of recurrence. Topical treatment options including 5-FU, cidofovir, [91] intra-anal imiquimod, and provider applied TCA have demonstrated moderate efficacy for treatment of intra-anal AIN [92, 93]. Ablative therapies including infrared coagulation, cryotherapy, laser therapy, and electrocautery/hyfrecator are well tolerated.

Repeated ablative treatment or a combination of treatment methods are often required for long-term clearance of AIN2-3.

No indications exist for systemic chemotherapy or radiation therapy for patients with AIN in the absence of evidence of invasive cancer.

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Sexually Transmitted Hepatitis

13

Roberto Rossotti and Massimo Puoti

13.1 Introduction

The term "hepatitis" is a generic definition for "liver inflammation" that may be caused by many transmittable and non-transmittable conditions. Among infectious agents, there are at least five viruses for which hepatitis is the primary (or only) clinical manifestation: hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D (or delta) virus, and hepatitis E virus.

All viruses can cause acute hepatitis with jaundice, but symptoms are more common with acute hepatitis A and E infections than with acute C infection. In contrast, chronic infection is quite common following hepatitis C infection, while it is rare with hepatitis E. With hepatitis B virus, age plays a major role in the development of acute rather than chronic infection: in children, infection usually occurs without symptoms but often becomes chronic; in adults, symptoms are more common but chronic infection occurs in less than 5% of cases. Most of the mortality attributed to viral hepatitis is believed to occur from the long-term consequences of chronic hepatitis, i.e. liver cirrhosis and hepatocellular carcinoma, but even acute infection may lead to fatal fulminant diseases requiring liver transplantation.

Although viral hepatitis occurs worldwide, there are major differences in the global prevalence of these infections that match to the route of transmission (by percutaneous exposure, by sexual intercourse, from a mother to her infant, by oral intake, or by other means). In countries with limited sanitary conditions, hepatitis A infection from ingestion of contaminated food or water is common in childhood, but may occur in sporadic outbreaks in economically advanced settings. Similarly, hepatitis E infection has been reported worldwide, but the incidence is much higher in Asia and sub-Saharan Africa. In Asia and sub-Saharan Africa, nearly 80% of the population has been exposed to hepatitis B infection by adolescence, compared with less than 15% of people in some regions of Europe or the USA. In Egypt and other settings where percutaneous injections were common, hepatitis C infection can be found in nearly 20–30% of the population compared with below 1% in the general population in most other settings [1].

Besides traditional routes of transmission, each virus has showed the potential also for sexual spread. Hepatitis E and above all hepatitis A can be transmitted via oral-anal and digital-anal sex leading to several outbreaks in men who have

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sex with men (MSM) in Western countries. Hepatitis B is a well-recognized sexually transmitted infection (STI), involving both heterosexual and homosexual population. Hepatitis C has been long considered as non-transmittable via sexual intercourse, but several recent outbreaks among MSM—either HIV infected or uninfected—have redefined its epidemiology as a potential STI under specific conditions and risk factors.

The present chapter will describe virology, diagnosis, and prevention for each virus with a specific focus on epidemiologic infections as STIs. Treatment principles are mentioned for the acute phase, while a full description of the management of chronic hepatitis B and C diseases is beyond the purpose of this paper.

13.2 HAV

Hepatitis A virus (HAV) is an RNA virus that replicates in the liver and is shed into the stools, resulting in transmission by the fecal-oral route. According to the World Health Organization (WHO) estimates, there were 13.7 million of HAV-related infections and 28,000 deaths in 2010 [2]. The clinical course is generally benign, even though acute liver failure requiring transplantation may occur in rare cases in patients with concomitant chronic liver disease [3].

13.2.1 Virology

In 1973 Feinstone detected HAV by immune electron microscopy in stools obtained from patients with acute hepatitis A (AHA) [4]. The viral genome, belonging to the *Hepatovirus* genus of the family *Picornaviridae*, is a positive-strand RNA of 7470 nucleotides that encodes only a single open reading frame, which is translated into a polyprotein. The virus-encoded protease ($3C^{pro}$) cleaves such polyprotein into eight viral proteins (VP0, VP3, VP1-2A, 2B, 2C, 3AB, $3C^{pro}$, and the RNA-dependent RNA polymerase $3C^{pol}$). Three proteins (VP0, VP1-2A, and VP3) compose the virus: during the assembly of the capsid, 2A is removed from the VP1-2A, and, at the final stage of maturation, VP0 is cleaved into VP2 and VP4. Five copies of each protein are assembled to form a pentamer, and 12 copies of the pentamer form a virus capsid. There are some amino acid variations between different HAV strains, but the virus exists as a single serotype. HAV has seven unique genotypes (I–VII) that exhibit less than 85% of sequence identity, even though further analyses showed that the genotypes III and VII should be reclassified as subtypes A and B of genotype III, and genotypes I and III could also be divided into sub-genotypes A and B. Four genotypes (I, II, III, and VII) are of human origin, and 3 (IV, V, VI) are of simian origin (Fig. 13.1).

13.2.2 Epidemiology

HAV is transmitted through the fecal-oral route, either through person-to-person contact or through contaminated food or water. The incubation period of AHA is 2.5–5 weeks, but stool shedding of the virus starts about 20 days before the peak elevation of ALT and continues for at least 25 days, although it may last up to 80 days: thus, transmission may occur over a long interval of time. In countries with high endemicity due to poor sanitary conditions and hygienic practices, most people acquire HAV in their early childhood and are therefore immune to the infection. On the contrary, adults living in areas with low endemicity are usually exposed to HAV during travel in endemic areas, or through risky behaviors, such using illicit drugs, or being MSM practicing oral-anal and digital-anal sex.

Reports of sexual transmission of HAV started more than 30 years ago with several AHA outbreaks within the MSM community. In 1979, the incidence of AHA in the San Francisco area was noted to be six times higher in men aged 20–40 years than in women of similar age. This was the first demonstration of a high incidence of AHA in MSM and of the strong correlation with oral-anal sexual contact.

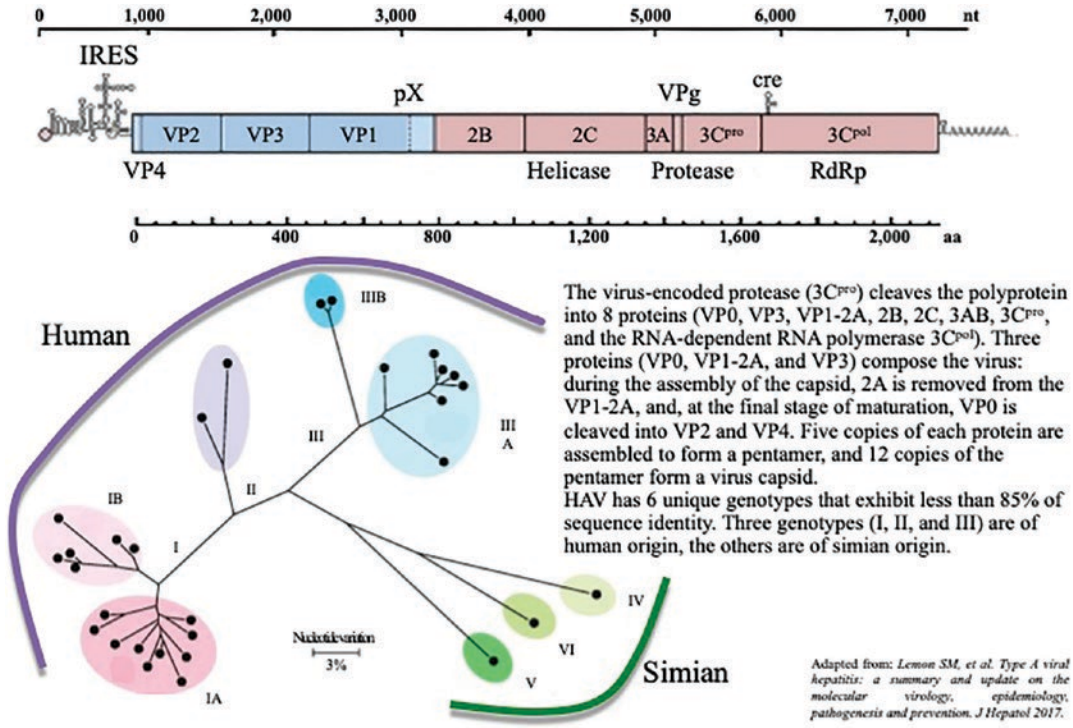

Fig. 13.1 Virological structure and subtype diversity of HAV

Improvements in sanitation and access to clean water reduced viral circulation and the overall rates of transmission. The consequent decrease of population immunity is deemed to be responsible for periodic outbreaks of AHA [5] (Fig. 13.2). Sexual spread of HAV is part of these epidemics: since oral-anal contact is not a risk factor for the transmission of HIV, it became more prevalent as an alternative to penetrative intercourse so the number of outbreaks of sexually acquired AHA in MSM strikingly increased in the following decades. The correlation between AHA, oral-anal contact, and the MSM community is so strong that one of the viral strains involved in the 2016–2017 outbreak—RIVM-HAV16-090, belonging to the IA subgenotype—was called "EUROPRIDE strain," named after the European Gay Pride held in Amsterdam in 2016 [6]. Additionally, the outbreaks of AHA in MSM observed in the last decade have been associated with a significant increase of STIs (recent or concomitant with AHA) and with a significant correlation with drug use (the so-called chemsex practices) in the previous 12 months [7].

13.2.3 Diagnosis

Diagnosis is made by the detection of specific serum immunoglobulin M (IgM) for HAV. Tests for IgM anti-HAV antibodies can distinguish between AHA and other forms of hepatitis with both sensitivity and specificity above 95% [8]. HAV begins to be excreted in the stool shortly after the ALT level begins to increase and just before IgM is detectable: serology becomes positive within 5–10 days from the onset of symptoms, but it does not detect the lower concentrations of anti-HAV IgM already present in the weeks soon after the contagion. False-positive results may occur; thus, the test should be performed only in symptomatic subjects [9]. After infection or immunization, anti-HAV immunoglobulin G test remains positive throughout the patient's lifetime.

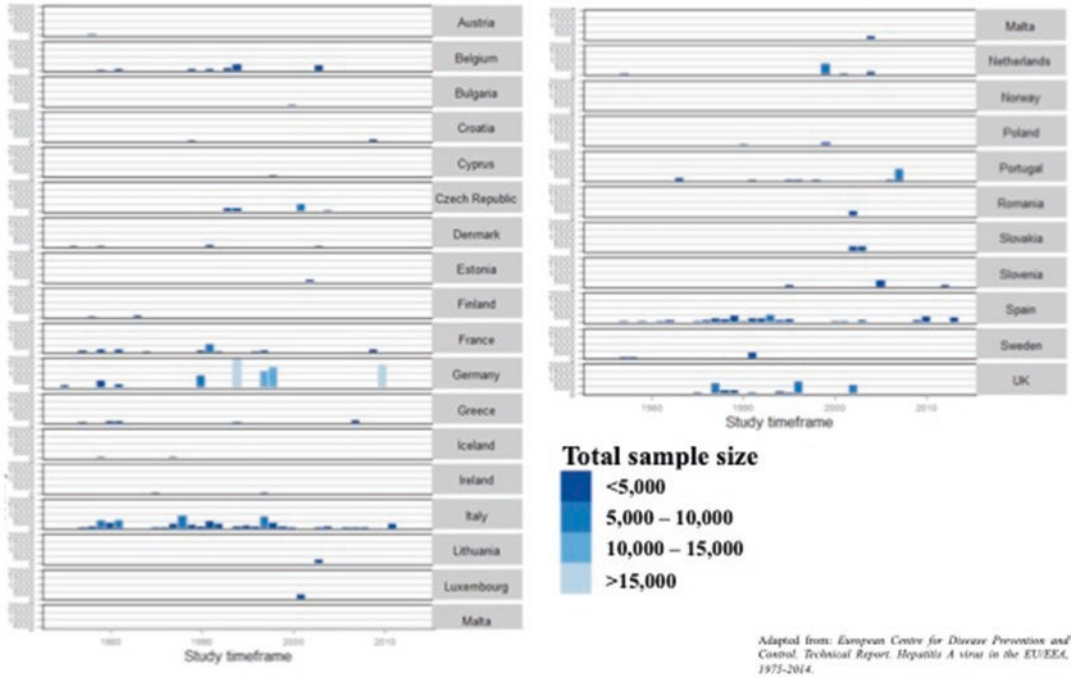

Fig. 13.2 Distribution and sample size of European acute HAV outbreak reports published in the period 1975–2014 (referring to the year 1975–2013)

13.2.4 Treatment

No specific treatment for AHA is available and only supportive treatment is indicated for the routine cases A. It includes bed rest (patients should not return to work or school until fever and jaundice have subsided); age-appropriate treatment for nausea and diarrhea; and alcohol avoidance (but nourishment might be normal if tolerated). Pregnant women who contract AHA should be carefully evaluated and assisted since they have an increased incidence of gestational complications and preterm labor [10]. Rare cases of fulminant AHA may occasionally require emergency liver transplantation [11].

13.2.5 Prevention

Since no specific treatment for AHA is available, preventive measures—including vaccination—play a pivotal role in the containment of outbreaks. In non-endemic countries, anti-HAV immunity is low and decreasing: in Europe, for instance, people living in areas of intermediate seroprevalence passed from 133,738,138 in 1980 (28.7%) to 22,455,485 in 2000 (4.6%). Consequently, the number of persons living in areas with very low seroprevalence was 22,502,299 in 1980 (4.8%) and increased to 394,754,781 2000 (80.6%) (Fig. 13.3) [12]. In MSM, anti-HAV immunity is low (around 39%), but with relevant differences across age classes: 54% in MSM older than 30 years, 32% in those aged 20–30, and 19% in those younger than 20 years [13]. Moreover, in HIV-negative individuals receiving pre-exposure prophylaxis (PrEP), considered at high risk for acquiring STIs, anti-HAV immunity is 37.5% [14].

The critical threshold of immunity beyond which sustained epidemics could not occur has been estimated at 70% (plausible interval 41–73%) in MSM, corresponding to a basic reproduction number (R_0) of 3.3 (plausible interval 1.71–3.67) [15]. A recent paper describing the incidence of AHA in a cohort of HIV-infected

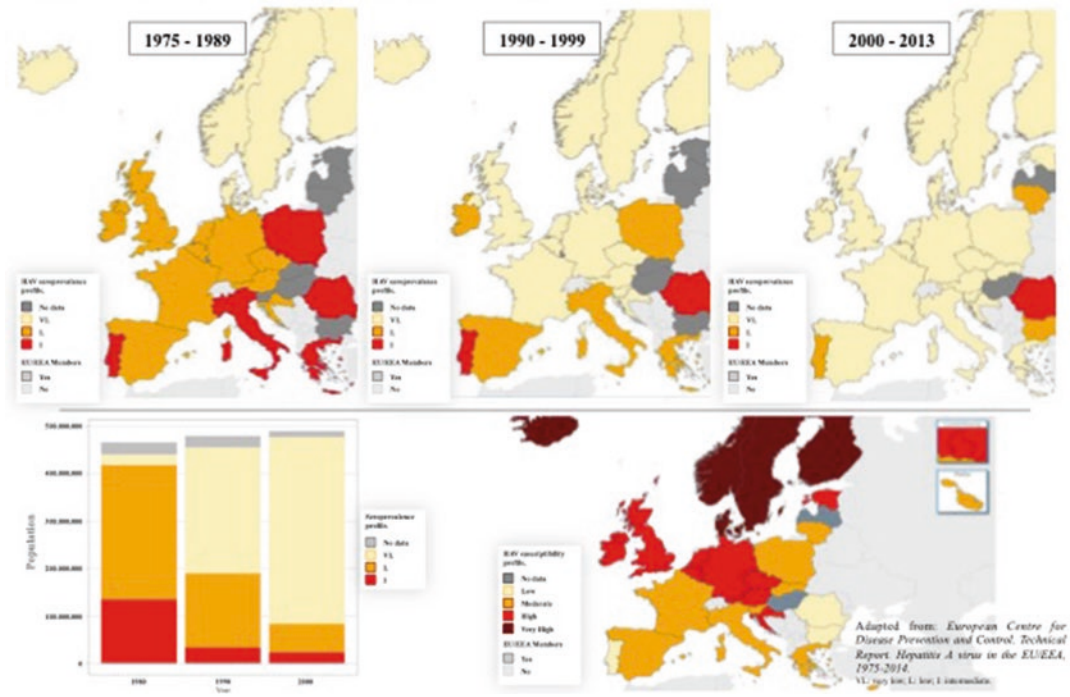

Fig. 13.3 Geographical distribution of HAV seroprevalence profile in the EU/EEU over time with inhabitants at potential risk

MSM during an outbreak in Taiwan showed the strong impact of vaccination on the epidemic: the immunization rate passed from 4.7% to 70.6%, when the proportion of immune subjects reached the threshold of 65%, a steep decline of the incidence of AHA was observed [16].

A vaccine against HAV is commercially available from 1992. A live attenuated vaccine is mainly used in China; most other countries use inactivated vaccines [17]. There are several monovalent inactivated vaccines licensed either for children aged 1 year or older and with different antigen content; however, all are considered safe and immunogenic so the WHO judges anti-HAV vaccines of different brand names as interchangeable. Current HAV vaccination schedules include two doses of inactivated vaccine (administered 6–12 months apart each other) that allows achieving long-term protection (up to 17 years).

Since infectivity starts before the symptoms onset, close contacts of an index case may require an approach as post-exposure prophylaxis (PEP). Traditionally, anti-HAV PEP was based on the administration of standard immunoglobulins (IGs) to exposed people within 2 weeks after exposure with an efficacy around 80–98%. Whether IGs administration completely prevents infection or only leads to an asymptomatic infection with the development of persistent anti-HAV antibodies depends on the length of time between the exposure and IGs administration [18]. On the other hand, if IGs are administered later than 2 weeks after exposure, the efficacy for the prevention of secondary cases has not been established. The use of IGs as PEP has been superseded by the availability of inactivated vaccines: they are highly immunogenic so that more than 95% of healthy vaccinated subjects achieve protective antibody levels 1 month after receipt of the first dose. One month after the second dose administration, the protective antibody level is achieved by more than 99% of individuals. Adults older than 40 years seem to respond less well to a single dose, but they reach equal response after two doses [19]. A recent paper compared the effectiveness of administering one dose of vaccine or

IGs in preventing AHA cases in susceptible exposed people during outbreaks in Catalonia during 2006–2012. The study showed high rate of protection after HAV vaccination (97.6%), which was similar to anti-HAV IGs (98.3%) [20].

13.3 HEV

The real global burden of hepatitis E virus (HEV) infection is not established, but it is supposed to be the globally most frequent acute viral hepatitis infection: in Western countries the seroprevalence within the general population is decreasing and esteemed to be between 4% and 52% in Europe and around 15–25% in the USA [21]. For many years, HEV has been considered as a self-limiting acute infection transmitted via a fecal-oral route, responsible for epidemics in tropical and subtropical countries. Nevertheless, some sporadic cases of locally acquired HEV in industrialized countries have been reported [22, 23], but so far with no overt outbreaks in developed countries.

13.3.1 Virology

HEV is a not enveloped RNA virus recognized in the early 1980s [24] and is grouped in the genus *Orthohepevirus* in the *Hepeviridae* family. The *Orthohepevirus* A species includes seven genotypes (HEV1–HEV7).

HEV is a small RNA virus with an icosahedral capsid that has been described in two types of fully infectious particles: the not enveloped virions shed in the feces (naked HEV particles), and the virions covered by host cell membranes circulating in the blood. The viral genome is a single-stranded, positive-sense RNA of ~7.2 kb consisting of a short 5′ noncoding region that is capped with 7 methylguanosine, three open reading frames (ORFs) and a short 3′ noncoding region that ends in a poly(A) tail [25]. ORF1 encodes non-structural proteins, ORF2 encodes the viral capsid (it is the target for humoral and cellular immunity), and ORF3 encodes a small non-structural phosphoprotein that interacts at various levels with the infected cell.

13.3.2 Epidemiology

In Western countries, the transmission of HEV occurs predominantly by the fecal-oral route, although parenteral and perinatal routes have been implicated. Seroprevalence data suggest that HEV may be endemic also in industrialized countries [26]: an increasing number of sporadic autochthonous cases have been reported, some with a lethal outcome [27]. Many HEV infections are subclinical, thus a continuous circulating pool of asymptomatic carriers is occurring. Recent European studies have assessed the risk of HEV seropositivity in MSM populations with conflicting findings. In an Italian study performed between 2002 and 2011 on 1116 serums from subjects who underwent HIV testing, overall HEV seroprevalence was found to be 5.38%, with MSM having an odds ratio of 1.9 (95% Confidence Interval 1.03–3.50, $p = 0.04$) for past infection compared with the general population [28]. Additionally, in a retrospective 3-year study performed in various sexual health clinics in the UK, 422 serums of MSM and heterosexual males were analyzed: a higher prevalence of previous HEV infection was found in both HIV-infected (7.5%) and HIV-uninfected MSM (10.4%) as compared with heterosexuals (3.5%) [29]. Despite these findings, other studies performed in European countries failed to detect any association between sexual behavior and HEV seropositivity [30].

13.3.3 Diagnosis

The diagnosis of acute HEV infection is generally based on the detection of HEV RNA by reverse transcription polymerase chain reaction or anti-HEV IgM antibodies. However, the positivity of anti-HEV IgM can be delayed in immunocompromised patients [31]. In addition, a negative HEV viremia does not necessarily exclude the possibility of an acute infection because the HEV RNA is detectable only in a short interval [32].

13.3.4 Treatment

In immunocompetent individuals, acute hepatitis E does not generally require antiviral therapy, as the infection clears spontaneously. In immunocompromised patients such as transplant recipients, spontaneous clearance occurs in 30% of cases when doses of immunosuppressive drugs are reduced [33]. The use of pegylated-interferon (Peg-IFN)-α-2a with [34] or without [35] ribavirin has been effective in chronic active hepatitis secondary to HEV. It has been shown also the possibility of a sustained virologic response after a 3-month course of ribavirin mono-therapy in kidney transplant recipients, but the dose remains to be determined [36]. The optimal duration of Peg-IFN-α-2a therapy and the role of ribavirin are still to be clearly determined.

13.3.5 Prevention

The most effective tool for hepatitis E prevention is a three-dose vaccine that showed high efficacy (>99% of protection) [37]. The HEV 239 vaccine was developed to elicit protective immunity across all HEV genotypes [38] and demonstrated cross-protection for both HEV1 and HEV4. It is licensed for sale and use in China since 2012, but it is not yet available for routine or emergency use elsewhere. Modeling studies of antibody persistence suggest long-lasting (up to 30 years) protective immunity after vaccination [39].

13.4 HBV

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus that can cause both acute and chronic liver infection. Potential consequences of chronic HBV infection include chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). It is estimated that there are globally 240 million people who are infected with HBV [40]. Chronic infection is the tenth leading cause of death worldwide [41]: 15–40% of infected subjects develop serious liver disease, resulting in an annual mortality around 1.2 million cases.

13.4.1 Virology

HBV is a small, enveloped, virus that belongs to *Hepadnaviridae* family and replicates in quiescent hepatocytes [42]. The interaction with the cell surface starts with a low affinity bond with heparan sulfate proteoglycans followed by higher affinity binding of the PreS1 domain to the sodium taurocholate cotransporting polypeptide: these reactions trigger the internalization of HBV through a clathrin-dependent endocytosis-mediated process [43]. The capsid passes the nuclear pore and the genome (in the shape of relaxed circular DNA, rcDNA) reaches the nucleus. The rcDNA is rearranged by host enzymes to form covalently closed circular DNA (cccDNA), which is assembled into a minichromosome that acts as transcriptional template for all viral messenger RNAs (mRNAs) [44]. The cellular RNA polymerase II is responsible for the production of viral transcripts, all characterized by features that make them structurally indistinguishable from cellular mRNAs. Transcription is driven by four viral promoters (preS1, preS2, core, and X) and two enhancers (Enh1 and Enh2) distributed throughout the viral genome. A total of seven viral proteins are encoded: viral polymerase (pol) and core (HBcAg) proteins produced from the pregenomic RNA (pgRNA); precore protein (e antigen or HBeAg) from the precore mRNA; large (L), medium (M), and small (S) envelope surface proteins from the PreS1, PreS2, and M/S mRNAs, respectively; and HBx protein from the X mRNA [45].

Besides its coding capacity, the pgRNA serves also as template for virus genome. pgRNA-bound viral polymerase primes the reverse transcription step in which the first DNA strand (minus strand) is generated along with the almost complete degradation of the pgRNA by the RNase H activity of the polymerase. Subsequently, the incomplete second DNA strand (plus strand) is produced

through the DNA-dependent DNA polymerization activity of the viral polymerase, generating the rcDNA [46]. Newly formed replication-derived capsids are transported back to the nucleus, thereby amplifying the cccDNA pool. On the other hand, infectious viral particles are produced upon envelopment of replication-derived capsids and they exit the cells [47]. Infected hepatocytes also release huge amounts of HBeAg and hepatitis B surface antigen (HBsAg) sub-viral particles (Fig. 13.4).

Based on the extent of genetic diversity, HBV can be divided into 10 genotypes (GT) and several subtypes [48]. GT A to D are the most common: A in sub-Saharan Africa, Northern Europe, and West Africa; B and C in Asia; D in Africa, Eastern Europe, the Mediterranean basin, and India. Other HBV genotypes are much less prevalent: E in West Africa; F in Central and South America; G in France, Germany, and the USA; H in Central America. Two recently identified GTs, I and J, have a less clear geographic characterization: I was found in Vietnam and Laos and is essentially a recombinant of A, C, and G [49]; J was limited to the Japanese archipelago [50].

13.4.2 Epidemiology

According to the WHO, the world can be divided into three areas according to the levels of endemicity: low endemicity (below 2%); intermediate endemicity (from 2% to 8%); and high endemicity (above 8%) [51]. Chronic HBV (CHB) is endemic in several regions including Southeast Asia, China, sub-Saharan Africa, and the indigenous populations of Alaska, Greenland, and Australia: around 45% of the global population lives in an area of high prevalence. In these high-prevalence regions more than 7% of the population is chronically infected [52]. Most infections are acquired early in childhood and the risk of

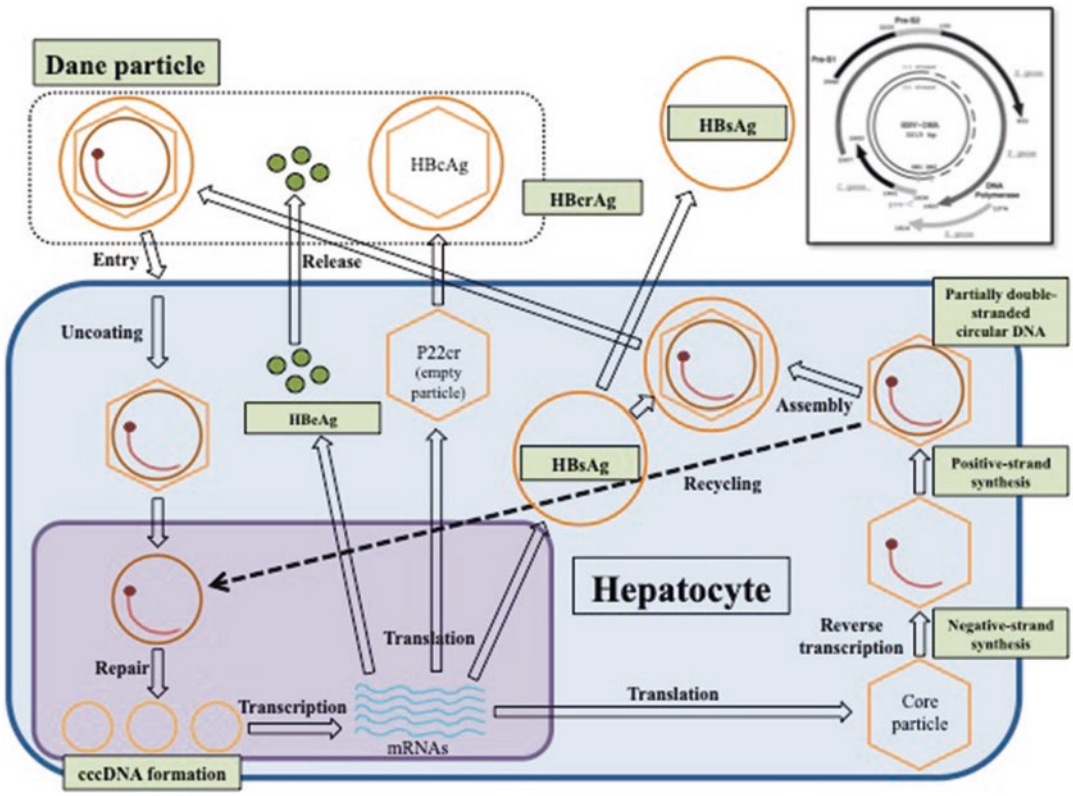

Fig. 13.4 Schematic representation of life cycle of HBV

chronicity is inversely related to the age of infection [53]. Moderate prevalence is reported in the southern regions of Eastern and Central Europe, the Middle East, and the Indian subcontinent, while North America, the United Kingdom, and Northern Europe are low-prevalence regions: in these countries, CHB is seen predominantly in immigrants from countries with high prevalence and their unvaccinated offspring as well as in specific groups with percutaneous and sexual risk factors. In the USA in 2015, for instance, 14,416 new cases of CHB have been reported, mainly in Asian Pacific islanders (27.9%) and in the age group between 25 and 54 years (66.1%). In Europe, the number of reported cases in 2015 was 15,595 with a rate of 9.9 per 100,000 inhabitants, mainly in the age group of 25–34 years [54]. In more than 60% of cases mother-to-child was the route of transmission.

On the contrary, acute hepatitis B infection (AHB) remains mainly a disease of adulthood. The availability of the HBV vaccine had a dramatic impact on the incidence of AHB. In the USA, the reported rate of AHB has declined since 1990, passing from 8.5 per 100,000 inhabitants to 0.9 per 100,000 in 2011, the lowest rate ever recorded [55]. According to the CDC report, in 2015 there were 3370 cases of AHB: with a rate of 1.1 per 100,000, incidence slightly declined compared with the previous decade. Unfortunately, few data about the route of transmission were available. Similar trends have been seen also in Europe: in 2015 there were 2505 cases of AHB (incidence rate 0.6 per 100,000), involving mainly males in the central age groups. Heterosexual transmission accounted for more than 30% of cases, followed by nosocomial, MSM, and IVDU transmission (Fig. 13.5).

Multiple countries have reported shifts in the epidemiology of AHB underlining the central role of sexual transmission. HBV is efficiently transmitted via sexual contact with infected individuals: HBsAg or HBV DNA has been detected in body fluids and mucosal surfaces of infected individuals, including semen, menstrual blood/vaginal discharge [56], saliva [57], feces [58], anal canal

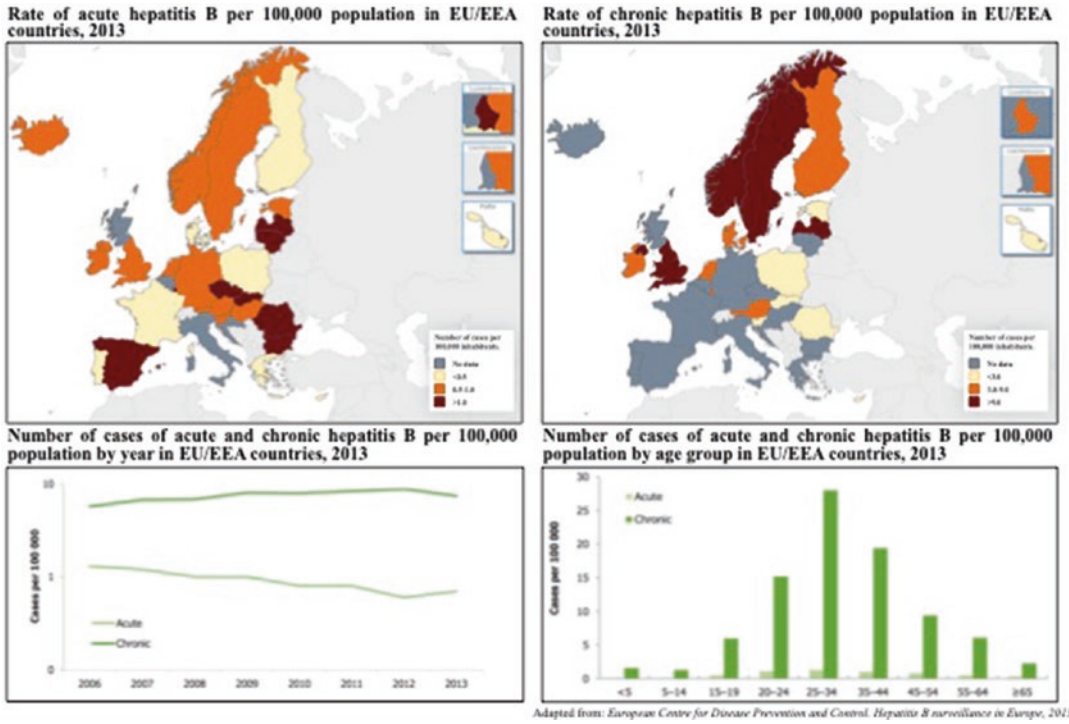

Fig. 13.5 Incidence of acute and chronic B infection, temporal trends, and stratification by age group in Europe

and rectal mucosa, and rectal mucosal lesions [59]. Sexual transmission has been confirmed in animal models showing the infectiousness of human semen by intravaginal instillation or inoculation [60], and case reports of HBV infection transmitted by artificial insemination support the role of semen in HBV transmission [61].

MSM have long been recognized as a group with higher seroprevalence of HBV: surveys performed in the 1980s found prevalence around 70% [62]. In a large group of 2946 Dutch blood donors, 4.8% MSM were found to be HBsAg positive versus 0.22% among controls. In a large group of MSM followed in MACS (Multicenter AIDS Cohort Study) in Pittsburgh, over a 30-month follow-up period seroconversion for HBV was 19.8%. Insertive anal intercourse was the major risk factor identified for HBV acquisition [63].

13.4.3 Diagnosis

The serologic patterns of acute and chronic HBV infection are wide-ranging and complex. Antigens and antibodies that can be detected during HBV infection include: HBsAg and antibody (anti-HBs); hepatitis B core antigen (HBcAg) and (anti-HBc); hepatitis B e antigen (HBeAg), and antibody (anti-HBe). Serologic assays are commercially available for all markers except HBcAg, because no free HBcAg circulates in blood. Testing includes also molecular biology to assess the presence and concentration of circulating HBV DNA.

The serologic markers typically used to differentiate among acute, resolving, and chronic infection are HBsAg, IgM anti-HBc, and anti-HBs. The presence of HBeAg and HBV DNA generally indicates high levels of viral replication; the presence of anti-HBe usually indicates decreased or undetectable HBV DNA and lower levels of viral replication. In newly infected persons, HBsAg is the only serologic marker detected during the first 3–5 weeks after infection. The mean time from exposure to detection of HBsAg is 30 days [64]. Molecular biology assay might detect HBV DNA in the serum of an infected person 10–20 days before detection of HBsAg [65].

Anti-HBc appears at the onset of symptoms or biochemistry abnormalities in AHB and persists for life in the majority of persons. Acute or recently acquired infection can be distinguished from chronic infection by the presence of the IgM class of anti-HBc: they are detected at the onset of the acute phase and persist for up to 6 months if the infection resolves. Nevertheless, IgM anti-HBc may reappear also in persons with exacerbations of chronic infection [66].

Sometimes, total anti-HBc is the only detectable HBV serologic marker. Isolated anti-HBc positivity can represent: resolved HBV infection in individuals with waning anti-HBs levels; chronic infection in which circulating HBsAg is not detectable by commercial serology, most commonly in HIV or HCV co-infected subjects [67]; false-positive reaction. Persons positive only for anti-HBc are generally not infectious [68].

In persons who recover from HBV infection, HBsAg and HBV DNA become no more detectable while anti-HBs antibodies appear. On the other hand, in persons with CHB HBsAg and HBV DNA persist. In persons in whom chronic infection resolves, HBsAg becomes undetectable; anti-HBc persists, and anti-HBs will occur in the majority of these persons [69] (Table 13.1).

13.4.4 Treatment

The goal of antiviral therapy would be the loss of HBsAg and the development of anti-HBs, but such objective is achieved only in a small number of individuals. As a consequence, more realistic goals in clinical practice are: suppression of HBV DNA replication, necro-inflammatory activity reduction, and prevention of progression to cirrhosis and HCC. At present, several therapeutic agents, including IFN, pegylated-interferon (peg-IFN), lamivudine (LAM), emtricitabine (FTC), adefovir (ADV), entecavir (ETV), telbivudine (LdT) and tenofovir, either in the disoproxil fumarate (TDF) or alafenamide

Table 13.1 HBV diagnosis according to serologic markers

Serologic markers				
HBsAg	Total anti-HBc	IgM and-HBc	Anti-IIBs	Meaning
–	–	–	–	No contact with IIBV
+	–	–	–	Early acute infection
+	+	+	–	Acute infection
–	+	+	–	Acute infection resolving
–	+	–	+	Previous infection (even though cccDNA persists within hepatocytes)
+	+	–	–	Chronic infection
–	+	–	–	Traditionally called "occult infection," may represent several different conditions, including a false-positive result
–	–	–	+	Vaccinated

(TAF) preparations, are approved for the treatment of CHB [70]. LAM, FTC, and TDF are active also against HIV.

In the setting of AHB, the main treatment goal is the prevention of acute or subacute liver failure, even though an improvement of quality of life by shortening the symptomatic disease could be taken into account. AHB will recover with seroconversion to anti-HBs without antiviral therapy in more than 95% of adults. Nevertheless, the potentially life-threatening evolution to severe or fulminant AHB should be carefully ruled out. Characteristics of severe AHB include: coagulopathy defined this as international normalized ratio (INR) above 1.5; protracted course (i.e., persistent symptoms or marked jaundice for more than 4 weeks); signs of acute liver failure [71]. There are no randomized controlled trials to assess the role of antiviral agents, but several cohort studies suggest that an early therapy might prevent progression to acute liver failure. This effect, however, is not seen if antiviral therapy is initiated late in the course of severe acute hepatitis B in patients with already manifested acute liver failure and advanced hepatic encephalopathy. Data achieved from large case series support that TDF, ETV, or LAM can be safely used in acute severe hepatitis B [72]. Older studies supported also the use of steroids, but they did not include current antiviral drugs, so the reliability nowadays is questionable [73]. Early treatment does not increase the risk of chronicity: observational data from a multicenter cohort indicated reduced rates of chronicity, if antiviral agents are initiated within 8 weeks of presentation [74].

13.4.5 Prevention

Currently available hepatitis B vaccines are highly effective for HBV prevention. The first HBV vaccine was licensed in 1982, while recombinant vaccine is available since 1986. A 3-dose course generates a protective response in more than 90% of healthy vaccinees and provides long-lasting protection [75].

Country-specific recommendations vary, although the WHO recommends universal HBV immunization of all infants starting at birth and supports additional target groups for catch-up vaccination appropriate to the epidemiologic setting and available resources [76]. Despite the acknowledgment that certain adult populations are at particular risk for HBV infection through sexual transmission and the availability of a highly effective vaccine to prevent HBV infection, immunization rates among at-risk adults remain low [77]. Among MSM aged 15–22 years surveyed in 1994 to 1998 in the Young Men's Health Study, for instance, only 9% had serologic evidence of having received hepatitis B vaccine, despite more than 96% of those susceptible to hepatitis B having reported contact with the health care system. Of note, 67% of them were susceptible to HBV infection, and 11% had prevalent infection [78].

Two single-antigen vaccines (Engerix-B and Recombivax HB) and one combination antigen vaccine (TWINRIX, combined hepatitis A and B) are licensed for adults. All of them use HBsAg as the antigen, which is generated through recombinant DNA technology.

13.5 HCV

Hepatitis C virus (HCV) is a major cause of liver disease: according to the WHO, an estimated number of 71 million subjects worldwide are chronically infected and approximately 399,000 people die each year from HCV-related cirrhosis and HCC [79]. Acute hepatitis C (AHC) is classically defined as the initial 6 months following the infection with HCV. After AHC, 20–30% of patients clear spontaneously HCV; therefore the majority develops chronic hepatitis C (CHC), which might progress to life-threatening conditions such as end-stage liver disease and HCC [80]. Indeed, the rate of spontaneous clearance varies considerably among the studies, especially when HIV-infected patients are taken into account: in cohorts focusing on treatment, viral clearance is estimated to be as high as 25% [81], while in studies focusing on diagnostics and epidemiology it drops to 15% [82–84]. In a cohort of predominantly black men from the Johns Hopkins University who acquired their infection through intra-venous drug use (IVDU), the rate of spontaneous clearance was lower, ranging from 7% in HIV-infected to 14% in HIV-uninfected individuals [85].

AHC differs significantly from CHC in terms of diagnosis, epidemiology, natural history, immunology, and treatment response.

13.5.1 Virology

HCV is a single-stranded RNA genome of positive polarity: it belongs to the *Hepacivirus* genus within the *Flaviviridae* family. The HCV life cycle is based on the concerted action of 10 viral proteins: core, E1, and E2 are the main constituents of the virion assembly with the involvement of p7 and the non-structural protein (NS) 2; furthermore, there are the replicase proteins (NS3, 4A, 4B, 5A, and 5B) [86]. In the infected cell, HCV causes profound membrane rearrangements (the so-called membranous web) that leads to a replication organelle derived from the endoplasmic reticulum and composed of single-, double-, and multi-membrane vesicles [87]. The double-membrane vesicles are the most abundant membrane structures present within the infected cells and the kinetics of their appearance correlates with the kinetics of viral RNA replication: it suggests that they are the site of HCV RNA replication. HCV employs cellular proteins and specific lipids to create a microenvironment supporting viral RNA replication (Fig. 13.6). For instance, unesterified cholesterol was shown to be a major structural component of the HCV replication organelle: the endoplasmic reticulum is the site of de novo cholesterol synthesis but has a low cholesterol content, thus the replication organelle has to obtain unesterified membrane cholesterol by both on-site de novo synthesized cholesterol and by direct lipid transport inside the replication organelle. Indeed, HCV has been reported to seize the function of the oxysterol-binding protein.

HCV exhibits a great genetic diversity: to date, seven major genotypes and more than sixty subtypes have been reported [88]. HCV strains show about 30% divergence at the genotype level, and 15% divergence at the subtype level. GT1 is the most common worldwide (83 million cases, 45% of all HCV cases), but the global geographic distribution is complex and dynamic. Many GTs are endemic strains: GT1 are prevalent in the Americas, Europe, and East Asia; GT2 are primarily present in West Africa and East Asia (infecting 17 million subjects overall), GT3 in Asia (54 million subjects), GT4 in the Middle East to Central Africa (20 million subjects), GT5 in Southern Africa (2 million subjects), and GT6 in South East Asia (10 million subjects) [89]. Nevertheless, the distribution of viral strains is very dynamic due to contemporary human migration trends (for example, the prevalence of GT4—especially GT4a and 4d—is significantly increasing in Europe) [90].

13.5.2 Epidemiology

Overall, the largest population with CHC resides in South and East Asia such as China and Pakistan, but data about incidence of HCV infection in this

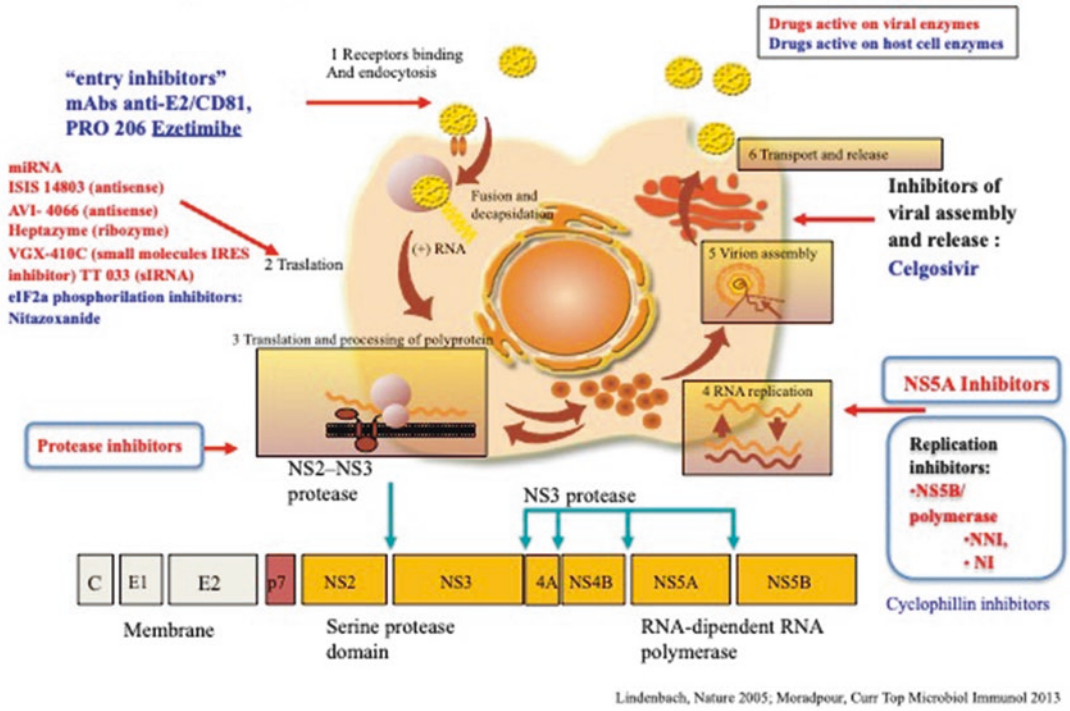

Fig. 13.6 Schematic representation of HCV life cycle with target identified for anti-HCV drug development

region are scarce. Egypt has the highest worldwide prevalence, with some studies reporting up to 15% of the population with a positive anti-HCV serology, and an estimated 10% of population with chronic CHC [91]. In the US, HCV infection has a prevalence of 4.1 million people, affecting 1.6% of the population [92]. Prevalence rates vary sharply by age, with the highest prevalence in people born in 1945–1965 (the so-called baby boomers) [93]. The incidence of HCV has fallen in the USA, from an estimated 7.4 per 100,000 people in 1982–1989 to 0.7 per 100,000 in 1994–2006, corresponding to approximately 17,000 new cases in 2007. This drop was driven by a decline in the iatrogenic spread [94]. On the other hand, a slight increase has been registered in the last few years: according to the official data from the Centers for Disease Control and Prevention, in 2015 there were 2436 reported cases of AHC, with a rate of 0.8 per 100,000 inhabitants, somehow higher than in 2011 when 1232 cases occurred (rate of 0.4 per 100,000 inhabitants). Males were mainly involved (around 60% of cases) with a rate of incidence of 7.31 per 100,000 inhabitants *versus* 2.71 per 100,000 inhabitants in females. The age groups principally involved are 45–54 (8.51/100,000), 55–64 (23.73/100,000), and 65–74 (14.60/100,000). Nevertheless, few data are available about route of transmission [95].

In Western Europe epidemiology is similar to what described in North America. Prevalence of CHC is estimated to be 7.3–8.8 million people (1.1–1.3% of the population) [96], while incidence of HCV was estimated at 6.2 per 100,000 people in 2005, varying widely from 0 to 39/100,000 residents in the region [97]. The official data from the European Centers for Disease Control and Prevention are characterized by a great heterogeneity within the reporting systems [98]. In 2015, 34,651 cases were reported with a crude rate of 8.6 per 100,000 inhabitants; of these, the large majority (69.5%) did not classify the disease as "acute" or "chronic." HCV is more common in men with a male-to-female ratio of 1.9, and half of cases involved people aged

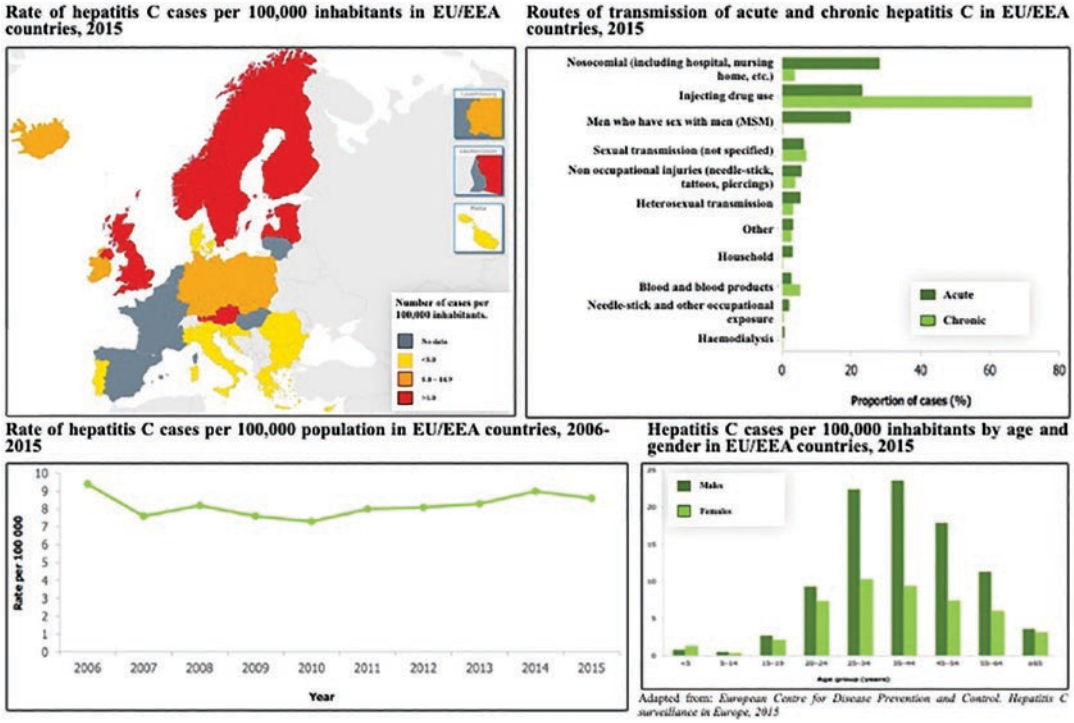

Fig. 13.7 Incidence of acute and chronic hepatitis C infection, temporal trends, and stratification by age group in Europe

between 25 and 44 years. Again, few data are available about route of transmission (14.4% of cases): the most commonly reported was injecting drug use (75.3%). Between 2006 and 2015 the overall number of cases reported increases by 26.5%, with a rate that passed from 7.3/100,000 in 2010 to 9.4/100,000 in 2014 (Fig. 13.7).

Several prospective studies examined HCV transmission in long-term heterosexual partnerships: three cohorts (including one with an extended follow-up to 10 years) did not find any confirmed case of HCV transmission between serodiscordant partners [99–101]. Anyway, the reported incidence of infection was low, ranging from 2.3 to 12.0 per 1,000 person years [102]. Heterosexual transmission may occur more efficiently in the presence of other biological and behavioral risk factors such as HIV co-infection and HCV prevalence. Within the Women's Interagency HIV Study, HIV-infected women with no history of IVDU showed that having sex with a IVDU male was associated with prevalent HCV, while among all participants being HIV infected was associated with a nearly twofold increased risk of acquiring also HCV [103]. Other identified potential risks for sexually acquired HCV include multiple sex partners [104], high-risk sexual practices (i.e., anonymous partners, sex in the setting of drug use, exchanging sex for money/drugs, sex with IVDUs) [105], presence or history of other STIs [106], and exposure to blood via partner violence [107].

Although sexual transmission is relatively uncommon, there have been increasing reports among HIV-positive MSM. Since the first publication of transmission of AHC in HIV-positive MSM from the United Kingdom [108], several other publications from Europe, the USA, and Australia indicated the presence of an ongoing epidemic within this population although limited to certain geographic areas (in Italy, for instance, the overall incidence in the ICONA Cohort was 1.2 per 100 persons-year: even though it was

higher in MSM, it remained stable over time) [109]. The only factor associated with HCV RNA detection in semen was the HCV viral load in blood, which suggests that a passive process determines seminal dynamics. Seminal HCV RNA levels were generally low (median, 2.1 log IU/mL), but it may well be infectious, since only 10–20 viral particles are required to establish a productive infection [110].

Temporal trends in HCV incidence show that the cases in HIV-positive MSM increased from 1–3 per 1000 person years to over 10 per 1000 person years after the introduction of highly active antiretroviral therapy (HAART) in 1996 [111, 112]. Molecular epidemiology analyses demonstrate that 90% of HIV-positive MSM with AHC is infected with genotypes 1a and 4d within a robust monophyletic transmission cluster among gay communities [113]. Evolutionary studies indicate that strains belonging to the IVDU population were introduced into the MSM community through multiple independent introduction of HCV, the first ones dating back to the 1980s [114].

Further evidences arose after the more recent outbreaks of AHC among HIV-positive MSM. In a prospective Swiss study published in 2005, unsafe sex and syphilis infection were significantly associated with acquisition of HCV [115], but still data on specific sexual practices were limited. In the analysis of another longitudinal cohort of MSM attending British STI clinics only fisting was associated with HCV after multivariate analyses [116]. A cross-sectional study from Amsterdam found that HIV infection, IVDU, fisting, and non-injecting recreational drug uses—especially gamma hydroxyl butyrate (GHB)—were independently associated with HCV infection [117]. A permucosal traumatic sexual practice, particularly when practiced in the context of group sex and/or non-injecting recreational drug use, seems to be associated with AHC. Taken together, these data suggests that HIV-positive MSM are at risk of AHC because of a combination of various risk factors such as ulcerative STIs, potentially high-risk, traumatic sexual practices and recreational drug use. It should be noted that intranasal and rectal drugs might favor HCV transmission via shared contaminated implements and increase risky behaviors due to loss of inhibition and sexual arousal.

Recent data changed the perspective suggesting that AHC is not a phenomenon limited to HIV-positive MSM, starting from a cohort of 36 HIV-negative MSM with AHC described in 2014 [118]. Even though the number of published cases in HIV-negative MSM is low, a bias should be taken into account: recent data from the UK have shown that among 3811 HIV-negative MSM attending a London clinic for sexual health screening, only 565 (14.8%) were tested for HCV [119]. Furthermore, HIV-negative MSM may consult with their general practitioner on a less-frequent basis, so acute ALT elevations may be missed in this population. Data recently published from pre-exposure prophylaxis demonstration studies highlighted the incidence also in HIV-negative MSM. In Amsterdam, at study entry 4.8% of participants had serology positive for HCV and 4.0% had detectable HCV RNA, prevalence significantly higher than what previously reported [120]. Additionally, in France the group by Charre and colleagues in Lyon were able to trace phylogenetic trees of HCV transmission to HIV-negative MSM [121].

Another striking feature of this sexually transmitted epidemic of HCV in MSM is the rate of re-infection, which has been recently calculated to be 15.2 per 100 patients-year among HIV-positive MSM in Amsterdam [122]. Analogous data have been described in a large German cohort, with a re-infection rate of 16.0 per 100 patients-year [123]. In England, the overall re-infection rate has been described to be 7.8 per 100 patients-year; among those who cleared such second infection, a relevant part acquired a third re-infection with a rate of 15.5 per 100 patients-year [124]. In these papers some fourth re-infections were also described. GT 1 HCV (especially 1a) is the most involved in all these re-infection episodes.

13.5.3 Diagnosis

The diagnosis of CHC is based on serology followed by molecular biology. Polymerase chain reaction assessment allows discriminating active disease from cleared infection or from cases of false-negative serology.

The diagnosis of AHC is hampered by the fact that the majority of cases with a recent HCV infection are asymptomatic. The window period between HCV infection and detectable anti-HCV antibodies is estimated to range from 34 to 70 days in studies among recipients of blood products [125] and IVDUs [126]. A delayed seroconversion was reported among HIV-infected MSM [127], with a median time to seroconversion of 91 days (but with responses appearing up to 158 days later). On the other hand, a paper by Vanhommering found that seroconversion window in a cohort of HIV-infected MSM with AHC was comparable to the window reported among HIV-negative subjects [128]. Nevertheless, in this study 17 out of 63 subjects (27%) did not develop anti-HCV antibodies 4 months after the estimated date of HCV infection, therefore the authors concluded that screening for AHC should be performed preferably using HCV RNA rather than anti-HCV serology.

Periodic testing of ALT concentration levels might contribute to improve the diagnosis rate, since this could be the first detectable sign of AHC. Although ALT levels might peak to 500 IU/mL or even more [129], ALT levels can normalize within the seroconversion window. As a result, diagnosis of AHC may be missed in patients with normal ALT levels and absence of anti-HCV antibodies. This further emphasizes the need for HCV RNA testing in patients at risk for AHC.

13.5.4 Treatment

Treatment is one of the most controversial issues in the management of AHC: an early treatment would achieve higher rates of sustained virological response (SVR), but the exact moment when to start is a difficult choice. Indeed, if therapy starts too early, there is the risk to treat also those subjects who would clear HCV spontaneously. Thus, an overtreatment might lead to an increase in toxicities and costs. On the other hand, a delayed treatment start favors the spread of infection into a high-risk population and allows liver disease progression. According to the AASLD guidelines, monitoring for spontaneous clearance is recommended for a minimum of 6 months unless the clinician and patient decide that a delay in treatment initiation is unacceptable. Again, if an immediate treatment during the acute infection period is planned, monitoring HCV RNA for at least 12–16 weeks before starting treatment is anyway recommended, in order to allow time for a possible spontaneous clearance.

Although high rates of SVR have been reported in a small number of patients with sofosbuvir-based combinations, the correct length of treatment with IFN-free regimens remains unknown. Three trials were performed with the fixed-dose combination of sofosbuvir plus ledipasvir in patients infected with GT 1. The SVR rates were: 93% (13/14) after 4 weeks of treatment in IVDUs [130], 77% (20/26) after 6 weeks of treatment in HIV-positive individuals [131], and 100% (20/20) after 6 weeks of treatment in HIV-negative, non-IVDUs [132].

The small sample size of these trials did not allow drawing any strong conclusions, thus, by analogy with CHC, the EASL guidelines recommend a treatment lasting for at least 8 weeks [133]. Patients with AHC should be treated with a combination of sofosbuvir plus ledipasvir (only for GT 1, 4, 5, and 6), or a combination of sofosbuvir and either velpatasvir or daclatasvir (for all genotypes), for 8 weeks without ribavirin. In case of HIV co-infection and/or a baseline HCV RNA level >6.0 $\log_{10}$ IU/ml, an extended treatment of 12 weeks might be needed. SVR should be assessed at 12 and 24 weeks post-treatment, because of the risk of late relapses. According to the AASLD guidelines [134], once the decision to initiate treatment is made, the therapeutic regimens are those recommended for CHC.

13.5.5 Prevention

No vaccine is available for the prevention of HCV. Although the risk for sexual transmission is low, the consistent use of condoms for sexual activity could be effective. People with CHC should discuss the risk of transmission with their sex partners: an open conversation regarding sexual and drug-use practices and methods to potentially reduce the risk of acquiring the infection (such as limiting the number of sex partners, use of barrier methods, avoiding or limiting identified high-risk sexual practices) should be discussed.

To date, both EASL and AASLD guidelines do not recommend the use of direct-acting agents as post-exposure prophylaxis.

13.6 Conclusions

Viral hepatitis infections include a wide range of different morbidities, varying between asymptomatic conditions and end-stage, life-threatening diseases. Several factors (virological, immunological, genetic, behavioral) are responsible for such a width of clinical manifestations. Although mostly limited to Western countries, in these years viral hepatitis scenario showed a rapid and significant change in terms of epidemiology, diagnosis, and treatment. Indeed, sexual diffusion might be considered a relevant, if not the main, way of transmission. Even if the numbers of novel infections due to sexual exposure are not comparable with those due to the traditional hematic spread for HBV and HCV, they represent nowadays the responsible of most of the new cases of acute and chronic diseases. On the contrary, the recent large epidemics of HAV have shown as sexual spread could be more efficient than the traditional ways of transmission.

Despite the continuing efforts in the development of new therapeutic strategies for HBV and HEV, only HCV has seen a dramatic change in treatment efficacy: the availability of direct-acting agents allowed to achieve extremely high rates of SVR in almost all categories of patients with few side effects. So far, economic cost is the only drawback of these new drugs.

Infection prevention remains a major issue. An effective vaccine is available for HAV, HEV, and HBV but the recent large epidemics of AHA in Europe, the USA, and Australia have demonstrated how the reduction of the immunity coverage at population level might lead to wide outbreaks in susceptible individuals. Thus, it is essential to maintain high vaccination coverage, as well as a continuous process of education and counseling for those at major risk of infection. To date, MSM represent the population mainly involved in sexually transmitted viral hepatitis infections and should be the target of new, tailored preventive strategies.

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Bacterial Vaginitis and Vaginosis

14

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14.1 "What was Old Is New Again" or "The Ancients Have Stolen Our Best Ideas"

The name Doderlein is very much linked to the topic at hand. In 1891, Albert Doderlein presented a paper to the German Gynecological Society entitled, "Concerning the vaginal secretions and vaginal bacteria." Although the focus at that time in history was the etiology of puerperal sepsis, he thought certain microorganism (later eponymously designated Doderlein bacillus) characterized healthy vaginal conditions contrasted to unhealthy conditions in which a mixture of microbial morphotypes was observed. Doderlein's organism could use glucose to produce lactic acid that suppressed staphylococci. These observations seem prescient in light of current understanding of vaginal microecology.

After Doderlein, invention of facilitating research technologies led to a great importance being placed on the indigenous microbiome of humans in relation to health and disease and the recognition that concepts of ecology could be applied to the microbiome. Improving culture techniques to include emphasis on obligate anaerobic bacteria, interest in infectious and non-infectious consequences of vaginal microbial content, and the ability through molecular taxonomy to characterize more completely the microbiome of various body sites has brought a better understanding of conditions that have not fit well with the early microbiology notion of a single disease caused by a single microorganism.

In 2008, with efficient metagenome sequencing methods available thanks both to sequencing technologies and the data pipelines necessary to deal with the so-called big data that results from such studies, the NIH initiated the Human Microbiome Project which brought clearer understanding to, among other things, the vaginal ecosystem. While this new wave of microbiome information had a breathtaking level of detail, some of the previous findings regarding indigenous microbiota remained intact. Looking back at some of Doderlein's observations we see bacteria inhabiting a particular site (niche), multiple types of organisms found together in that niche (communities), control of one micro-

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bial community by another (probiosis), and the coexistence of two communities simultaneously (Doderlein observed his bacillus occurred with yeast at a rate of 39%) which may suggest symbiosis or commensalism.

Clinical questions were common drivers of the quest for information about vaginal microbiology. In late 1800s it was debated whether the causative agents of puerperal sepsis were endogenous or exogenous microorganisms. In the mid-twentieth century, "non-specific vaginitis" was investigated and an etiology was sought. Later in the twentieth century the problem of endogenous surgical infections emerged and with the introduction of the term "BV" also came epidemiologic associations that gave it a more sinister reputation. The seeds for our understanding of the current role of microorganisms in symptomatic conditions of the lower female genital tract were clearly sown by important prior work that is worth remembering.

14.2 The Vaginal Ecosystem

Attention focused on *Lactobacillus* species in the vagina came from the long-known fact that the microbiome of the lower genital tract can involve a diversity of species even in the absence of symptoms. And intense focus on microbiology can lead to forgetting that rather than a mixture of microbes on an abiotic substrate as in the case of a culture plate, the microbiome exists on and interacts with a living human tissue, forming a dynamic ecosystem. Indeed the contemporary concept microbial ecology views the microbiome as "another organ of the body" rather than some alien population residing on an epithelium or inside a hollow viscus.

Concepts of ecology that have been made more prominent by the work or inspiration of the Human Microbiome Project (HMP) should be noted. While numerous reviews of the importance and impact of HMP have been published [1–3] we will focus on several terms that have particular relevance to the bacterial content of the female genital tract in health and disease. A useful term was introduced in a review [4] that referred to each individual human being as a "patch of habitat" which connects various ecosystem characteristics. These characteristics include vaginal microbiome diversity, community structure, drivers of community structure, spatial patterning, and temporal dynamics, realizing that each is interdependent with the others and many of the mechanistic questions remain.

Diversity With regard to diversity, between individual human hosts, the species richness may differ. However, the desire for generalizability may also lead to grouping individuals with similar degrees of species composition and richness. As illustrated by the report of Oakley et al. [5], a PCR-based comparison of BV and non-BV individuals revealed a threefold greater taxon diversity among BV cases compared to control individuals. Among many studies, an abundance of previously unrecognized phylotypes or taxa have been identified as potentially present among vaginal biota [6, 7]. Illustrating the aggressiveness with which scientists have been evaluating the diversity issue, a recent systematic review looked at 63 studies which summarized the findings of diversity, especially comparing women with and without BV [8]. While diversity as we now know it may be stunning in breadth compared to what had been elucidated from culture-based studies, the large number of microbial taxa found by molecular techniques is large but probably not limitless. The microenvironment, vaginal epithelium in this case, comprises particular physical and biochemical characteristics that make it suitable for some microbial species but not for others, promoting colonization for some organisms but not others.

Community Structure The concept of community applies to polymicrobial systems in which multiple species or taxa exist together at a particular place and time. Useful definitions [9] are important for this discussion, especially the term community structure which has been better understood in the last few years. Related to diversity, the community structure represents the variety of different microbial genomes present and the abundance of each individual numbers of that

community. Defining community structure through variable region deep sequencing of 16s rRNA has become the means of determining the relative abundance of each different genome in a specimen from an individual at a moment in time. The work the most instrumental in changing how we think about the vaginal microbiome was the work of Ravel, Forney, and their collaborators in which 400 almost samples from North American women of four ethnicities [10]. Five community structures described as community state types (CST) were identified. Four were characterized in which a particular *Lactobacillus* species (*iners*, *jensenii*, *gasseri*, and *crispatus*) dominated and a fifth (diversity) group with many anaerobic species and fewer lactobacilli. The findings also had distributions which coincided with Nugent scores used as a diagnostic aid in BV. A flood or additional reports from many groups have built on these key findings and have attempted to create more nuanced evaluation of CSTs in relation to clinical conditions.

Before leaving the topic of community types, it is important to mention that within a particular niche, the name of the microorganism is not as important as its biological function within the ecosystem [11]. Much attention and many articles have been written about lactobacilli as producers of lactic acid and hydrogen peroxide. However, some *Bifidobacterium* occurring in the vaginas of asymptomatic women seem to fill the same role as lactobacilli as they are able to produce acidic end products and some are hydrogen peroxide producers [12, 13].

Drivers of Community Structure The vaginal microenvironment not only provides a tissue substrate on which bacteria may reside, but also bathes these organisms in a complex milieu that has only been moderately well characterized. Components of interest include the tissue itself and vaginal contents including water, carbohydrate and proteins or amino acids that may provide nutritional support for the microbiota and physico-chemical properties such as hydrogen ion content and oxygen tension and thermoregulation. These properties of the vaginal epithelium are not only due to transudation of solutes into the lumen, but contributions from shedding of epithelial cells, secretions from the cervix, and inflammatory cells that may introduce antimicrobial substances. The microbiota itself may contribute to the environment by degradation of macromolecules to make nutrients available or release metabolites that affect the ability of "newcomer" organisms to become established. Together these factors seem to present a Gordian knot that cannot be solved. However, investigators have persistently sought to understand certain elements in isolation.

The role of tissue composition was a focus of microscopy-based studies of mouse stomach [14] in which it was found that secretory epithelium had a different microbial composition compared to non-secreting epithelium with a sharp line of demarcation between microbial communities. This implied differences in adherence sites between tissue types as well as differences in mucus contribution to the microbiota.

The most frequently cited factor in defining the vaginal microbiota is the nutritional support provided by vaginal glycogen, considered by most authors to be the key nutrient for lactobacilli in their presumed regulatory role in the microbiome. Glycogen concentrated in vaginal epithelial cells is purported to be fermented by the lactobacilli of the vagina to produce lactic acid which lowers pH and to restrict the range of microorganisms able to colonize this niche. While this scenario is attractive, Spear et al. [15] found some vaginal *Lactobacillus* species could not grow on glycogen. However, vaginal fluid contains amylases that degrade glycogen releasing mono-, di-, and tri-saccharides that can support *Lactobacillus* growth. Thus, the concept of glycogen production as a key carbon source for *Lactobacillus* remains intact by not necessarily through a direct, but rather and indirect linkage, and Nunn and Forney [16] suggest that other bacteria co-colonizing with the lactobacilli could provide the necessary glycogenoclastic activity. This may help explain why Mirmonsef et al. [17] reported significant correlation between vaginal free glycogen and *Lactobacillus* colonization. More recently,

Mitchell et al. [18] reported in a study of menopausal women that vaginal free glycogen did not relate to *Lactobacillus* content but serum estrone did. The estrogen issue will be addressed below.

The *Lactobacillus* story continues with the proposed mechanistic explanation that *Lactobacillus* regulates the vaginal biota through lactic acid production, limiting the range of organisms that can flourish at low pH. There have been questions as to whether *Lactobacillus* alone provides the vaginal acid content or whether it may be partly from epithelial cells as well. As far back as 1940, Louis Weinstein was addressing pH, estrogen, and "Doderlein" bacillus [19–22] and found that newborn females had low vaginal pH even before colonization occurred suggesting that transplacental estrogen acidified the vagina and not lactobacilli. This also raised the question of whether *Lactobacillus* colonization is the cause or response to a low vaginal pH. Boskey et al. [23] cultured lactobacilli commonly in vaginal cultures and found it lowered pH of the medium to levels similar to *Lactobacillus*-dominated vaginal pH, whereas similar cultures of BV-associated organisms produced a pH similar to that found in women with BV. Boskey [24] also exploited the fact that epithelial cells produce only D-lactate while *Lactobacillus* fermentation yields both D and L isomers. Based on the D isomer being greater than 50% of vaginal lactic acid, they concluded most vaginal acidity is of microbial origin. Finally, O'Hanlon et al. contend that the effect of lactic acid in the vagina is more potent than the literature suggests because when high carbon dioxide and low oxygen tensions are considered in women with *Lactobacillus*-dominated biota pH values range from 2.8 to 4.2 and lactate concentrations may reach 1% [25].

Another characteristic of lactobacilli that generated a large body of literature is based on the finding that strains able to produce hydrogen peroxide are more associated with probiosis than lactobacilli which do not produce this antibacterial substance. The facile assumption states hydrogen peroxide produced in situ should be protective against a variety of microorganisms, especially those lacking catalase. However, confusion arises as peroxide is generated in the presence of oxygen and lactic acid is generated through anaerobic processes. This seeming contradiction could be addressed if the healthy vagina is microaerophilic which has observational support [26]. Very recently, a systematic review of literature on oxygen tension in the female genital tract was published [27] and this should provide information that could suggest how much peroxide might be produced in vivo, a theoretic computation which remains to be done. The work of Strus [28] is enlightening, however, as it showed hydrogen peroxide produced in culture by vaginal lactobacilli ranging from microaerophilic conditions to vigorous aeration, produced levels that only modestly inhibited pathogenic bacteria leading to the conclusion that hydrogen peroxide plays "some but not a crucial role" in vaginal protection. Furthermore, Tachejian et al. [29] reported that cervico-vaginal fluid blocks the activity of hydrogen peroxide further reducing the significance of peroxide as a direct inhibitor of bacteria and concludes the purported role of hydrogen peroxide is "improbable."

A recent finding that may provide some enlightenment on this matter is a study in which interaction of hydrogen peroxide-producing *Lactobacillus* and non-producers were allowed to interact with cultured epithelial cells [30]. Bidirectional interactions were reported in which growth of the peroxide producers was stimulated by epithelial cell contact and epithelial cells in turn increased their production of muramidase and lactoferrin, both innate antimicrobial products of the mammalian cells. The implication may be that modest production of hydrogen peroxide could create sufficient oxidative stress on the host cells to produce factors that could along with other *Lactobacillus* factors such as lactate production produce a controlling effect on the vaginal microbiome.

The nutrient support of *Lactobacillus* may be augmented or modified by host factors that also add to the control over microbial community structure. An early proteomic study [31] identified 685 proteins from cervico-vaginal lavage specimens and reported that several previously unappreciated proteins with antimicrobial activity were present. Other proteomic evaluations have emerged which indicate the vaginal proteome is not a settled science. For example, a 2013 study [32] identified numerous immune

factors in cervico-vaginal fluids but the results were substantially influenced by different sample collection techniques. In addition, the vaginal proteome contains proteins of microbial as well as human proteins [33].

Not surprisingly the microbiota seems to affect the host even while the host immune factors may influence the bacterial communities. In a study involving four vaginal microbial patterns reflecting different levels of taxonomic diversity [34] greater diversity was associated with increases in vaginal samples of lactic dehydrogenase associated with cell death, proteolytic activity, psoriasin, calprotectin, histones, and inflammatory cytokines, while keratin, lysozyme, ubiquitin, and IgG decreased. In other studies, microbial products were found to influence expression of mucin genes [35] and antimicrobial peptides and other signaling or inhibitory substances including secreted leukocyte protease inhibitor (SLPI), human beta defensing, lactoferrin, IL8, IL1β, TNFα, lysozyme, cathelicidin, calprotectin, and immunoglobulins (IgA and IgG) [36–39]. The source of these factors may be expressed in epithelial cells of the cervix, inflammatory cells in the vagina, or cervix or may be part of transudate into the vagina. The presence of Toll-like receptors in genital tract tissues provides a likely mechanism of pattern recognition that can account for inflammatory and host defense factors elaborated in the presence of microbial communities that represent a danger to the host.

Spatial Patterning of Communities It has long been known that different body sites are colonized by different communities of bacteria. Perhaps less well appreciated is the possibility that different segments of the lower female genital tract may comprise different niches. An organism like *Streptococcus agalactiae* which may be found in the colon is sought in the lower vaginal segment as part of the prenatal care protocol but not in the cervix. Conversely, *Neisseria gonorrhea* and *Chlamydia trachomatis* require cervical sampling where columnar rather than squamous epithelium dominates. Culture-based evaluation of the vaginal microbial communities has proven insufficiently sensitive to reveal unbiased differences within the various segments of the vagina. However molecular methods have demonstrated that differences do exist. For example, a microbiome analysis of multiple samples from the same individual showed considerable disparity from cervix, fornix, and lower third of the vagina from eight healthy women [40]. The practical implication of this information is not clear at present but does suggest that different microenvironments may exist in the vagina and data from different sampling sites should be compared between individuals with caution. Indeed, the variation between microbiomes of individuals is substantial and when different vaginal segments are considered, complexity is increased.

Temporal Dynamics The female genital tract consists of a hormonally driven tissue structure with estrogenic hormones driving trophic and maturing effects on the epithelium. Even before the vagina was thought of as an ecosystem with its resident microbial communities, microscopic and culture-based observations indicated *Lactobacillus* colonization appeared abundantly after an infant become colonized, declined until puberty, became prominent through the reproductive years, and declined in menopause [41]. This phenomenon was of interest to our group and as a result we investigated the role of estrus cycle in rats (4–5 days duration) on vaginal colonization. As we reported, the results were profound with the vaginal bacterial counts recovered by lavage increasing and decreasing 10,000-fold from the estrogen dominated phase to the non-estrogenized phase of the cycle [42]. Oophorectomy yielded stable, low vaginal bacterial counts. Of further note, pH was near neutral throughout the estrus cycle and the dominant organisms were *Pasteurella* [43]. We did not find lactobacilli associated with this tissue. Thus, excursions in counts occurred to factors unrelated to pH, but the rapidly dynamic nature of the microbiota was clear.

Temporal dynamics of the human vaginal microbiome has repeatedly been confirmed with contemporary molecular techniques. For example, dynamics based on CST [44] evaluated five studies with secondary (not meta) analysis, and concluded that categorizing a microbiome by CST is useful in detecting major shifts. Healthy

women tended to remain in a CST for 2–3 weeks. Diversity states were also described as high risk and transitions tended to be toward or between high risk CSTs. Three *Lactobacillus* (*crispatus, jensenii, and gasseri*) dominated the low risk CSTs as was shown by the original work of Ravel and Forney and the high risk diversity CST was most associated with high Nugent scores resembling BV and an intermediate CST featured *Lactobacillus iners*. This organism produces little lactic acid and limited hydrogen peroxide [11].

Many studies have focused on the coincidence of BV symptoms and the diversity CST. However, CST has been an important feature of studies done during pregnancy in which women who delivered preterm had higher diversity CSTs compared to those going to term [45].

Practical Aspects of Microbiome Science The volume of data that has emerged from efforts to describe the microbiome and its function as part of the human host is immense and is not exhaustively summarized here. However, some important facts emerge from the foregoing discussion.

- The vaginal microbiome is more than a microbial culture sitting atop a tissue. Rather it is a network of microbial communities, the components of which interact with each other and with the host in ways we are still discovering.
- These microbial communities interact with the host locally and globally. Interactions can be synergistic or antagonistic and each interaction may be reflected in perturbations in community structure which can be documented to occur within hours to days.
- While methods to identify the microbiome at a moment in time exist, the variations over time and space relate to mechanisms that are more theoretical than empirically demonstrated in defining the vaginal niche.
- While vaginal microbiomes can be categorized in several broad CSTs, within each there is species diversity but relatively greater diversity in some, accordingly described as the diversity CST.
- Many microbiome studies have focused on differences between women with and without BV and indicators of BV tend to associate with the diversity CST. There is no bright line of demarcation between higher risk and lower risk CSTs and as a consequence CST do not categorize patients as abnormal or normal.
- Because some CSTs seem to be beneficial, efforts are underway to introduce probiotic bacteria into the vagina (probiotics) or create conditions that foster colonization by beneficial organisms (prebiotic).
- Microbial ecology suggests that bacterial communities show resilience so that after perturbation they may return to their original state which limit the effect that can be expected from probiotics or antibiotics.

14.3 Bacterial Vaginosis (BV)

While this section focuses on BV, in the clinical setting it is appropriate to consider all vaginal conditions infectious and non-infectious that may cause symptoms. Symptoms may point to BV, but trichomoniasis and candidiasis should be excluded along with atrophic conditions.

14.3.1 Epidemiology and Microbiology

Any attempt at unambiguous discussion of the epidemiology of BV is challenged by the fact that a substantial proportion of women with microbiome consistent with BV do not necessarily have clinical findings that reflect BV. It still engenders debate as to whether it is proper to call some microbiomes asymptomatic BV. Both clinical and microbiological indicators of BV are used to navigate through the literature on BV and it becomes important to know if prevalence data are based on symptomatic or asymptomatic conditions. In the 2015 CDC Sexually transmitted disease treatment guidelines [46] the description of BV is paraphrased:

- Polymicrobial,
- Clinical syndrome,
- Resulting from replacement of normal hydrogen peroxide-producing vaginal lactobacilli,

- Lactobacilli are replaced by high concentrations of anaerobic bacteria, *Gardnerella vaginalis*, *Ureaplasma*, *Mycoplasma*, and fastidious or uncultivated anaerobes.

Of particular interest is the association that BV has with risks of other conditions. Such relationships occur with risk of HIV acquisition and other sexually transmissible pathogens, risk of preterm birth when BV occurs during pregnancy, salpingitis following chlamydial or gonococcal infection, and post-operative infections following gynecologic surgery. Because of these concerns the correct identification of BV takes on additional importance.

Despite imprecision in numbers, there is consensus by the CDC expert panel that BV is the most prevalent cause of malodor and vaginal discharge and that most cases are asymptomatic with excursions in the vaginal microbiome and recurrences being common among women who have been treated. BV has been described as the most common vaginal disorder with current treatments yielding frequent recurrence possibly implying sexual transmissibility [47].

Historically, the work of Gardner and Dukes in 1955 identified an organism now known as *Gardnerella* which was thought to be an etiology for this non-parasite, non-fungal condition [48]. However the common finding of *Gardnerella vaginalis* in a high percentage of asymptomatic women and limited success in replicating vaginal symptom by infecting women with *Gardnerella* caused doubt as to the precise role of the organism. Several decades of work indicate that *Gardnerella* is an element of a microbiome that is referred to as dysbiotic. *Gardnerella*, however, is an important component and because of its heavy colonization of sloughing squamous vaginal epithelial cell contributes to microscopic diagnosis. The occurrence of *Gardnerella* in asymptomatic women explains why experts consider cultivation of this organism unhelpful in diagnosis.

While not generally considered a classical sexually transmitted disease, BV occurs mostly in sexually active women as well as with new or multiple partners [49]. Physicians most frequently observe BV when patients present with one or more symptoms and it is less likely that clinical value is gained in seeking to diagnose BV in asymptomatic women, though it has been part of research studies in the past [50].

Uncertainty surrounds questions of BV etiology and seems largely to hinge on manner in which the microbiome is established in a given individual, and how it is altered over time. In the broadest terms the tendency of the microbiome to be dominated by certain lactobacilli versus dominated by a diverse population favoring *Gardnerella*, anaerobic organisms and possibly mycoplasmas [51] is related to BV. But the reason for microbiome excursions is still being elucidated. We have evidence that vaginal microbiome is influenced from sex hormones [52], smoking [53], and newcomer bacteria as in sex partner microbiome [54], but other potential drivers such as cytokines and innate immune factors [55] remain theoretical.

14.3.2 Diagnosis

The Pubmed database when queried on the term bacterial vaginosis, produces 139–212 hits per year since year 2000. Despite the fact that there is a great deal of information and even controversy embedded in this literature, the method for diagnosis of BV is relatively well established on clinical and microscopic grounds. As noted before, the lack of a particular etiologic organism means that culture or nucleic acid tests are not particularly useful for diagnostic certainty. Amsel's criteria (Box 14.1) were established on the basis of

Box 14.1 AMSEL Criteria for BV Diagnosis. Three Criteria Must Be Positive (Modified from Mohammadzedah et al. [79])

a. Homogeneous, thin, white discharge that smoothly coats the vaginal walls;
b. Clue cells (e.g., vaginal epithelial cells studded with adherent coccobacilli) on microscopic examination;
c. pH of vaginal fluid >4.5; or
d. A fishy odor of vaginal discharge before or after addition of 10% KOH (i.e., the whiff test).

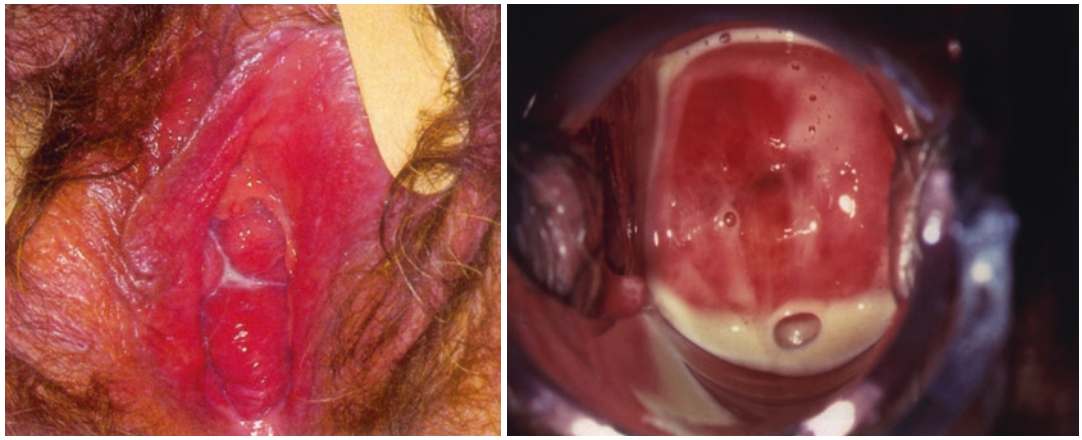

Fig. 14.1 Leucorrhea associated with bacterial vaginosis

clinical observations and are generally suggested by a patient's complaint of excessive discharge (Fig. 14.1) with or without notice of odor following intercourse or in association with menses. The CDC [45] as most standard texts describes observations which can be made from wet mount specimens of vaginal discharge. Microscopic evaluation of a wet mount of the discharge is also useful for identifying trichomoniasis or candidiasis as well. The addition of potassium hydroxide is used to digest epithelial cells to enhance visualization of fungal forms, but also liberates a fishy odor in specimens from BV cases, so is serving a dual function.

Another approach to BV diagnosis relies on the Gram stain appearance of vaginal smear material and provides a score the extremes of which indicate what is usually described as "normal" versus that indicating BV (Box 14.2 and Figs. 14.2 and 14.3). The large gram positive lactobacilli dominate at one extreme and the diverse flora with many gram negative forms and curved rod-shaped organisms are indicative of the other extreme yielding a score of 1–10 with scores of 7 or above considered as BV. It should be obvious that making a Gram smear and careful microscopic observation is a bit more labor-intensive than using the Amsel criteria and the involvement of an experienced microscopist in scoring is essential for making reliable calls.

Although *Gardnerella vaginalis* is not considered the etiologic agent of BV, its presence in high concentration does correlate sufficiently with Gram stain results that the CDC guidelines also lists a DNA hybridization test which indicates high levels of this organism (Affirm VP III, Becton Dickenson, Sparks MD). Sialidase is a product of *G. vaginalis* and a test (OSOM BV Blue, Sekisui Diagnostics, Framingham, MA) for this enzyme in vaginal samples may also be useful [46].

Box 14.2 Nugent Scoring Criteria (Modified from Money et al. [80])

Counts per 1000× field are scored as:

- 1+ is <1/field (none seen = 0+)
- 2+ is 1–5/field
- 3+ is 6–30/field
- 4+ is >30/field
- *Lactobacillus* (gram positive rods)
- Score 0–4 based on 4+ scores 0 and 0+ scores 4
- *Gardnerella/Bacteroides* (short gram-variable rods and cocci)
- Score 0–4 based on 0+ scores 0 and 4+ scores 4
- *Mobiluncus* (curved rods)
- Score 0–2 based on 0+ = 0 and 1+ or 2+ = 2 and 3+ or 4+ = 3
- Add scores from each category = Nugent score
- Interpretation: 0–3 "normal," 4–6 intermediate, 7–10 BV

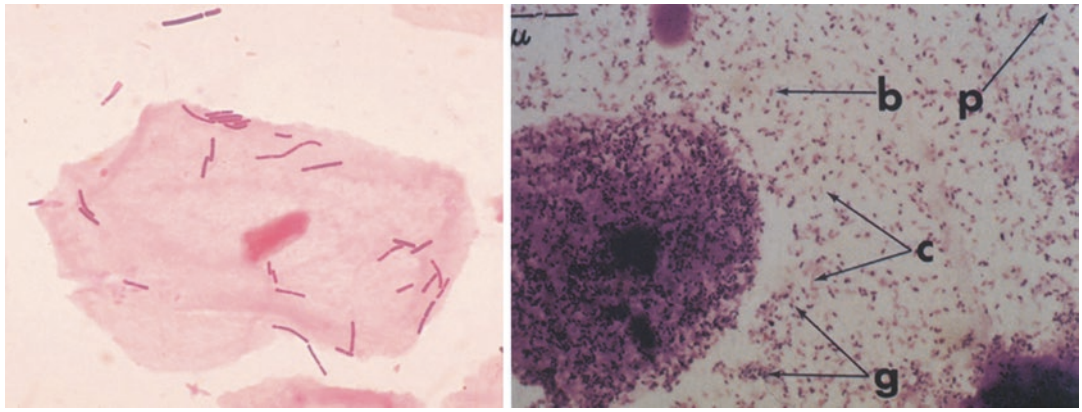

Fig. 14.2 Nugent score

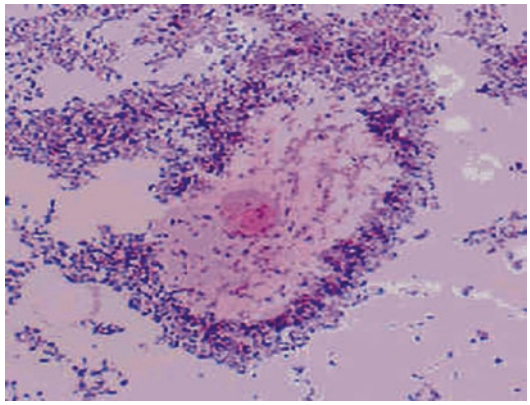

Fig. 14.3 Clue cell

Sha et al. studied cervico-vaginal lavage samples from a cohort of women with and without BV using Nugent scoring as the gold standard and found that qPCR tests for *Lactobacillus* (*jensenii* and *crispatus*), *Gardnerella* and *Mycoplasma hominis* alone or in combination could correlate reasonably well with the Nugent score, but head to head, just under 40% of samples having a Nugent score of 7–10 were positive for BV by the Amsel criteria [56].

14.3.3 Treatment

It is fortunate that despite the lack of certainty regarding diagnostic evaluation of BV, the therapeutic approach has been relatively stable for over a decade. The microbial milieu in BV differs from patient to patient and includes elevated levels of *G. vaginalis*, anaerobic bacteria, and possibly *Ureaplasma* and *Mycoplasma.* The antibiotics recommended for BV treatment include tinidazole and metronidazole which have activity against anaerobic bacteria. Clindamycin, also used for BV has a broader spectrum including anaerobic organisms such as *Bacteroides* and gram positive cocci. Of note, *Gardnerella vaginalis* is reported to be susceptible to nitroimidazoles and clindamycin [57]. Given the polymicrobial nature of the BV microbiome, it is possible to imagine that therapies may not need to eliminate all possible involved organisms, but it may be sufficient to disrupt balances between the microbial communities to resolve the symptoms.

Logic would suggest that if a characteristic of BV is diminution of *Lactobacilli*, particularly those able to produce hydrogen peroxide, that probiotic application of beneficial organisms might prove a useful therapy. This simple concept will have greater complexity in execution for reasons that involve microbial ecology and probiotic organisms. Because the community state types have different distributions among ethnic groups and even within and ethnic group, it would be challenging to predict what probiotic organism or organisms would be desirable in a particular population [58]. In addition, microecology teaches that two different species of bacteria could perform the same function in the biome but one may be better than the other at

becoming established. The clinical trials that have been conducted will not be reviewed here, but a recent summary [59] has noted that products have included oral and topical application of probiotics, involving various microbial strains alone or in combination, and high doses and repeated doses may be required and antibiotic treatment of BV may be required to pave the way for colonization.

14.3.4 Clinical Considerations

Diagnosis and treatment for BV are within relatively ready reach of clinicians, but one elusive aspect of BV remains challenging, namely recurrent BV. This may not be surprising as symptomatic cases arise and abate spontaneously and despite detailed characterization of the microbiome, the precise mechanism of microbial community shifts is unknown and return of the microbiome to a state consistent with BV after treatment continues to be a challenge for physicians. Patients may be advised that BV may return and the patient should be seen again to address the recurrence.

The CDC recommendations state that acceptable approach to a recurrence of BV is to retreat with the same or alternate approved treatment (Box 14.3). With multiple recurrences, however metronidazole vaginal gel may be used two times weekly for 4–6 months, though recurrence may still occur again after metronidazole suppressive therapy. Another approach which may be an option depending on availability is to employ intravaginal boric acid (600 mg) daily for 21 days followed by metronidazole gel two times weekly for 4–6 months according to Reichman et al. [60]. Another chronic suppression approach using 2 g metronidazole plus 150 mg fluconazole was cited by McClelland et al. [61] given monthly. Currently CDC recommendations do not include use of probiotic therapies, but since they are readily available over the counter, patients may choose to use them and ideally they will share that information with their provider.

Box 14.3 Therapeutic Options for Bacterial Vaginosis Treatment (Adapted from CDC Sexually Transmitted Disease Treatment Guidelines [46])

Recommended	Alternative
Metronidazole 500 mg orally twice a day for 7 days or Metronidazole 0.75% gel, one full applicator (5 g) Intravaginally at bedtime for 5 days or Clindamycin Crème 2% one full applicator (5 g) Intravaginally at bedtime for 7 days	Tinidazole 2 g orally once daily for 2 days or Tinidazole 1 g orally once daily for 5 days or Clindamycin 300 mg orally twice daily for 7 days or Clindamycin Ovules 100 mg intravaginally Once at bedtime for 3 days[a]

[a]Clindamycin ovules use an oleaginous base that might weaken latex or rubber products (e.g., condoms and vaginal contraceptive diaphragms). Use of such products within 72 h following treatment with clindamycin ovules is not recommended

Some additional clinical matters should be added for completeness. First, BV therapy involving nitroimidazole compounds should be accompanied by instruction on avoiding alcohol consumption during and for 72 h after completing the therapy. Second, vaginal clindamycin ovules contain an oily excipient that can compromise the integrity of rubber (condoms and diaphragms). Of additional importance is the need to consider diagnosis of various sexually transmitted diseases in women who are diagnosed with BV as there may be co-infection coupled with information indicating BV increases the risk of infection with other STDs.

BV occurring in a pregnant patient raises other matters. Symptomatic women should be treated and the standard therapies are acceptable for use in pregnancy [46]. The association between BV and preterm birth, membrane rupture, intra-amniotic infection, preterm labor, and postpartum endometritis adds additional weight to the recommendation; however, research on the pos-

sibility that antibiotics reduce these complications is mixed. A Cochrane review published in 2015 included 2100 women and not surprisingly data quality varied among studies and for various outcomes examined [62]. Of note was their conclusion that there was evidence that preterm birth was decreased among women with prior preterm birth receiving antibiotic during the second or third trimester if they had BV in the current pregnancy, but this did not hold for women who had prior preterm birth but without BV in the current pregnancy. While many other details will continue to emerge as research continues, it would seem the treatment of BV in pregnancy is supported.

14.4 Aerobic Vaginitis (AV)

Most symptomatic conditions involving the vagina and vulva especially if accompanied by noticeable vaginal discharge will be attributed to BV, candidiasis, or trichomoniasis and clinical approaches have been presented in this and other chapters. As described above, Nugent scoring yields two categories of reasonable confidence (Nugent score less than 4 is unlikely to be BV and a score of 7 or more is consistent with BV) but what is implied by the intermediate numbers between 4 and 7 is less clear.

A recently published comprehensive review of the condition known as AV, and which may include the condition described as desquamative inflammatory vaginitis (DIV) has suggested that Nugent scores between 4 and 7 may not simply imply movement toward or away from definitively diagnosed BV, but may include some individuals with AV [63] if evidence of inflammatory cells is concomitantly observed. This condition has a much shorter history in the literature than BV and not surprisingly is less frequently considered as a diagnosis and also has raised uncertainty and controversy. Such uncertainty will probably be associated with this diagnosis until a larger body of knowledge develops.

14.4.1 Epidemiology and Microbiology

The term "dysbiosis" has been applied regularly to BV, but is also used in describing AV both due to a paucity of lactobacilli, and to an abundance of enteric rods (*E. coli*) and staphylococcal and streptococcal forms (coagulase negative and positive *Staphylococcus* and group B *Streptococcus* and viridans streptococci). Historically, the finding of *Gardnerella vaginalis* in BV was inaccurately considered to be evidence that it was etiologic, but now is considered one feature of a microbiome that is associated with BV. Likewise, the microorganisms observed in AV cases may or may not be etiologic, so caution in over-interpreting descriptive results is warranted.

Dysbiosis in the case of BV or AV is not explained by specific known mechanisms, though the review of Donders et al. [63] has enumerated several findings that could be linked to microbiome shifts. The presence of lactobacilli may compete for nutrients, tissue binding sites or may produce inimical substances that control other organisms in the microenvironment of the lower genital tract. Significance has been attributed to hydrogen peroxide production by lactobacilli and while there are reasons to doubt a direct antimicrobial effect of the hydrogen peroxide, this substance could be a mediator of activities in both host and bacterial cells that alter innate host defense mechanisms in the vagina.

Inflammatory cytokines (IL1β, IL6, and IL8) have been reported to be elevated in cases of diminished *Lactobacillus* colonization [64–66]. Other micro-environmental drivers such as aerobic bacterial products including sialidase or toxins and inflammatory substances secondary to neutrophil granules are proposed as having a role. In addition, diminished cell maturation can result in vaginal cell content having a greater abundance of parabasal or basal cells which are also characteristic of vaginal findings in AV and could be a result of diminished estrogenic effect.

The above listing of elements associated with AV do not provide a cogent step-by-step explanation of pathogenesis, though continued research may help elucidate the steps involved and would also suggest ways of preventing this condition.

With regard to frequency of cases of vaginal symptoms, the occurrence of AV may be underestimated due to being under-recognized compared to other vaginal conditions including BV, candidiasis, and trichomoniasis. When it is sought, it appears to be possibly as common as BV, and as 12 papers reviewed by Donders [63] indicate, the rate of moderate to severe AV is estimated to be from 7 to 13% among non-pregnant European women which is similar to the rate of BV in this group.

14.4.2 Diagnosis

The Nugent score has been found useful in diagnosis of BV and in parallel to this system the microscopic appearance of the vaginal smear in women with AV has been proposed as a means of diagnosing and grading the severity of the condition. Severe AV has been equated with DIV. In common with BV, AV typically shows diminished *Lactobacillus* and elevated pH, but unlike BV, AV secretions are often thicker tinged with yellowish or greenish coloration. Microscopy of wet mount preparations will have leukocytes, which are usually absent in BV wet preparations. In addition leukocytes may have prominent granules (toxic leukocytes) and basal or parabasal cells are present. Degraded epithelial cells may also be present and can add complexity to microscopic evaluation of vaginal wet mounts. The characteristics have been assembled into a scoring protocol which is summarized in Box 14.4.

In contrast to Nugent scoring, a scoring rubric for AV (Fig. 14.4) is currently not widely used and according to Donders is not only based on careful microscopic evaluation of vaginal smears, but is also considered to be best viewed using phase contrast microscopy. There are inherent challenges in this approach. First, many physicians do not routinely employ microscopy as a point of care diagnostic aid. And microscopes if available in the primary care clinic are not likely to be equipped for phase contrast. Details sought both in Nugent scoring and AV scoring require a degree of experience that may not be generally taught in medical school or graduate medical education. As a practical matter, if attempts to employ diagnostic microscopy are not fruitful in clinical practice, this tool, despite its potential utility, will probably not be used regularly.

For clinicians who regularly see patients for vaginal symptoms and who do not regularly employ detailed microscopic evaluation, the clinical findings will be of paramount importance. Research is continuing on host and microbial factors that might be measured by biochemical assay methods (sialidase, leukocyte esterase, peroxidase, beta glucuronidase, microbial coagulase), PCR measurement of spe-

Box 14.4 Scoring Protocol for the Diagnosis of Aerobic Vaginitis (Adapted from Donders et al. [63])

Microscopic observation	Score
Lactobacillus grade	
"Normal microflora or slightly diminished lactobacilli"	0
"Diminished lactobacilli or fairly disturbed lactobacillus"	1
"Grossly abnormal lactobacillus"	2
Leukocytes and epithelial cells	
≤10/hpf leukocytes	0
>10/hpf leukocytes and <10 epithelial cells	1
>10 epithelial cells	2
Proportion of toxic leukocytes	
None/Sporadic	0
<50% of leukocytes	1
>50% of leukocytes	2
Background flora	
Unremarkable or cytolysis	0
Small coliform bacilli	1
Cocci or chains	2
Proportion of parabasal cells	
None or <1%	0
≤10%	1
>10%	2

Scoring: Sum <3 = no AV, sum 3–4 = "light AV," sum 5–6 = moderate AV, sum >6 severe AV

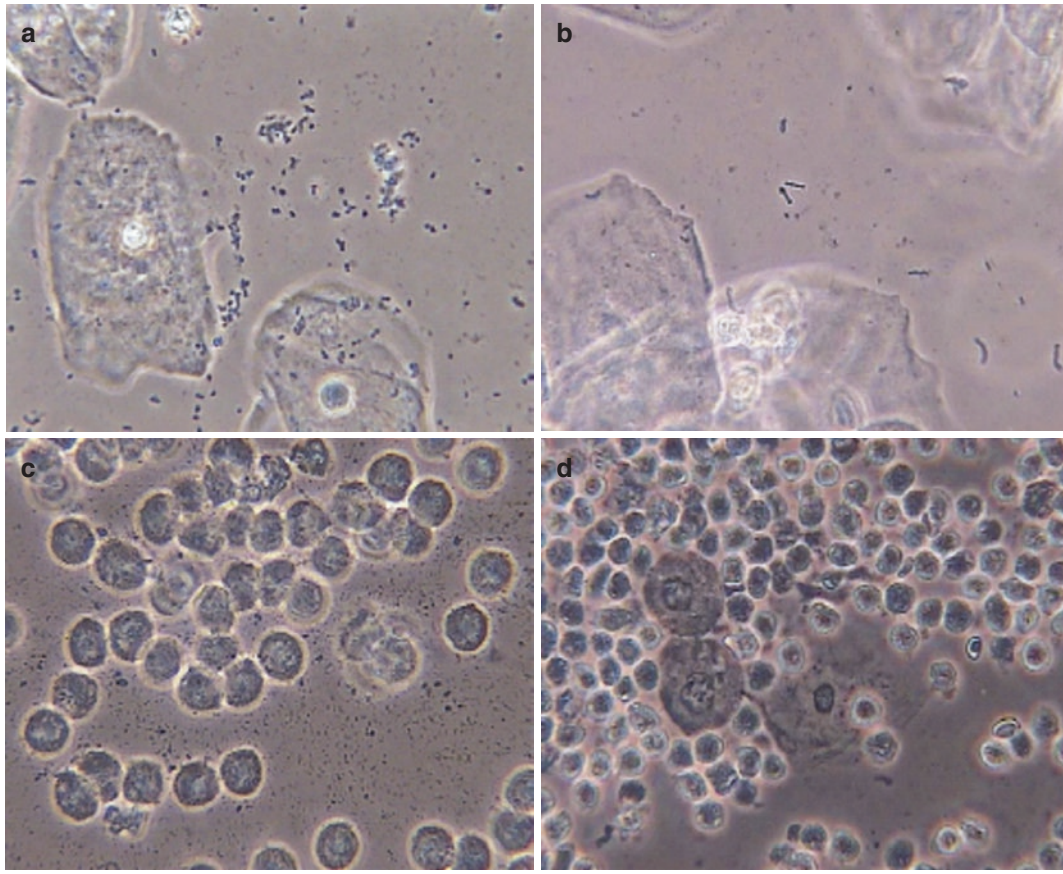

Fig. 14.4 Microscopic wet mount: AV score (from Donders [63]). (**a**) LBG I, (**b**) LBG IIa, (**c**) LBG IIb, (**d**) LBG III

cific organisms such as *E. coli* or microbiome characterization by sophisticated sequencing methods [64, 67–69] but these will not readily be appropriate for clinical diagnostic purposes until they have been made practical and have been demonstrated to have appropriate sensitivity and specificity.

14.4.3 Clinical Considerations

As mentioned before, the differential diagnosis of women presenting with vaginal symptoms includes all of the microbial and non-microbial causes of discharge, inflammation, odor, dyspareunia, and careful history. Within the constellation of symptoms, the presence of inflammation and adherent yellowish to yellow-green discharge are the main clinical findigs of AV. The introitus and vagina may be reddened and the patient may report a burning sensation and painful intercourse. Odor is not fishy but described as more of a decaying smell possibly related to abundant degradation of sloughing epithelial cells. It is unclear if AV and DIV are identical, although it has been claimed that DIV is the most severe manifestation of AV.

As mentioned earlier, estrogen support of the vaginal epithelium plays a role in regulating the vaginal microbiome and it is inferred from numerous studies going back even before molecular technologies were applied to microbiome characterization that estrogenized epithelium is associated with *Lactobacillus* colonization. While this may be an over simplification, it does suggest that the trophic condition of the vaginal epithelium should be considered in assessing the presence of AV or other vaginal symptoms. A

cardinal characteristic associated with AV is the presence of parabasal cells and this can imply lack of estrogen support. While Reichman et al. [70] did not find diminished serum estrogen in women with DIV (which may or may not be a form of AV), it is possible that estrogen may, for obscure reasons, be limited at the vaginal level despite adequate serum concentrations. This remains another area where additional research is needed.

Before leaving clinical considerations, it should be noted that AV has been associated with certain other concerning conditions. It has possible associations with sexually transmitted diseases as well as Pap smear abnormalities and preterm membrane rupture, preterm birth, and chorioamnionitis. The birth-related issues are not surprising considering the importance of inflammatory mediators in initiation of labor. Much remains to be elucidated about the connections between vaginal conditions and preterm birth, but the importance of birth before 37 weeks makes it essential to give attention to any inflammatory process in the pregnant patient even if the source of that inflammatory process is poorly defined or if the naming of the inflammatory process is disputed.

14.4.4 Therapeutic Options

The recent comprehensive review of AV by Donders et al. [63] exhaustively discussed therapeutic approaches to AV from the standpoint of inhibition of microorganisms, support for the estrogen status of the vaginal epithelium, and the control of the inflammation associated with this condition. The lack of a specific microbial species to target has led to the use of broad spectrum antibiotic or antiseptic products. Overarching issues, however, include concern for the development of resistance, especially if long term or repeated treatment is used. A theoretic goal of treatment would be to restore lactobacilli to the vagina after suppressing or eliminating the presumed offending organisms.

As details of the exact microbiology and physiology of AV are unclear, so clinical studies have been few and no best treatment has been established and few studies have explored AV specifically, especially apart from other causes of symptoms. Topical agents with wide spectrum have been used generally for multiple vaginal conditions, but dequalinium chloride and nifuratel each have shown symptom improvement but in the case of the latter, treatment included AV with *Candida* or *Trichomonas* [71, 72] and in one study [73] dequalinium chloride was combined with a non-absorbable macrolide (rifaximin).

Among antibiotic choices, clindamycin represents an interesting option as it has been used for its anaerobic spectrum of activity in BV, but also has activity against the aerobic gram positive cocci [74] which are included among the presumptive organisms of interest in AV. Sobel indicated that DIV is responsive to topical clindamycin [75]. Although gram negative facultative rods are generally not susceptible to clindamycin, a study showed anticytokine activity of clindamycin in mouse cells exposed to lipopolysaccharide (gram negative endotoxin). As inflammation is a key element in AV clindamycin may exert beneficial effects distinct from its antibiotic spectrum. Concern for survival or repopulation of lactobacilli in the vagina after clindamycin treatment is of interest, and a study of inhibitory concentrations of clindamycin on lactobacilli showed high concentrations of clindamycin as may be achieved with topical crème was inhibitory in vitro but lower concentrations in the range of 2–25 mg/mL did not harm *Lactobacillus* which suggests that use of systemic dosing might benefit survival of lactobacilli [76].

Kanamycin has also been studied in the context of AV [77] in which 100 mg doses intravaginally for 6 days and showed decreased burning symptoms and enteric gram negative bacteria along with sparing of lactobacilli at 2–3 weeks follow-up. Oral moxifloxacin was used by another group [78] who found 400 mg in six daily doses required two courses of therapy for best clinical results. However a different group found that a 12 day course of moxifloxacin was less efficacious than a 6 day course at 1 month follow-up [79].

Limited information is available in the literature to support the use of topical estrogen, or cortisone depending on microscopic findings (low maturation index versus abundant inflammatory cells). As mentioned previously, probiotics are also being considered and at times these may be presented with estrogen support. But whether it is antibiotic therapy, hormonal, anti-inflammatory, or probiotic approaches to AV, there is currently insufficient data to support a protocol for treatment. Well designed and executed clinical studies coupled with a repeatable approach to diagnosis will be required to establish an evidence base for AV treatment.

14.5 Conclusions

Vaginal infections (AV and BV) are an important public health issue, yet their pathogenesis remains controversial. New epidemiologic findings strongly favor the hypothesis that sometimes BV could be a sexually transmitted infection. Control of AV and BV has been advocated for decreasing the prevalence of these complications, but the precise etiology of BV remains unknown. As a result, current treatment regimens and prevention strategies are inadequate. Such a lack of understanding not only inhibits our ability to effectively manage these infections but also severely affects our ability to prevent its associated complications.

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The Clinical Spectrum of Human Immunodeficiency Virus Infection

15

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15.1 Introduction

Human immunodeficiency virus (HIV) can manifest in a wide spectrum of features: the infection can be totally asymptomatic despite the active replication of the virus, or result in acquired immunodeficiency syndrome (AIDS)-defining conditions [1].

HIV produces a numeric and functional deficit of CD4+ lymphocytes, resulting in loss of cell-mediated immunity. In half of the cases the infection is completely asymptomatic until the onset of AIDS, 6–10 years after acquiring the virus. The absence of symptoms during the latent phase of HIV infection is one of the main causes of delay in diagnosing new infections. The Center for Diseases Control (CDC) system historically classifies patients according to the CD4+ counts and clinical features (Table 15.1) [2]. Although, from an epidemiological and social perspective, the distinction between HIV infection and AIDS remains very important, the introduction of antiretroviral therapy (ART) has completely revolutionized the natural course and prognosis of new diagnoses, often allowing the survival even when these are at an advanced stage.

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15.2 Acute Retroviral Syndrome (ARS)

HIV primary infection is usually asymptomatic. However, in 50% of cases, it can be followed by an acute mononucleosis-like illness also called acute retroviral syndrome (ARS). Its onset ranges from 1 to 6 weeks after exposure and symptoms include sore throat, fever, sweats, lymphadenopathy, myalgia, arthralgia, rash, malaise, anorexia, nausea, vomiting, diarrhea, headache, photophobia, and mucocutaneous ulceration [3–5]. Less commonly, ARS manifests with an acute neurological onset (e.g., aseptic meningitis, myelopathy, radiculopathy) [6, 7]. Opportunistic infections (i.e., *P. jirovecii* pneumonia, cryptococcal meningitis, and Candida esophagitis) have also been reported, due to the temporary initial depression of the CD4+ count. The differential diagnosis of ARS includes a large number of other infectious diseases that share with it the sudden onset of fever, rash, and possible nervous system involvement. Epstein–Barr virus (EBV) and Cytomegalovirus (CMV) infection, secondary syphilis, measles, rubella and neurotropic viruses infection must be considered and excluded [3]. The diagnosis of ARS requires the execution of latest generation serological HIV tests (antibodies + p24 antigen) and HIV-RNA test [8, 9], as the research for specific antibodies can result in false negative results due to the delay in seroconversion. During acute HIV infection, viral

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Table 15.1 HIV infection staging by CD4+ count and selected clinical conditions

Stage	A Acute infection Asymptomatic infection	B Symptomatic infection Non-A non-C conditions	C AIDS-defining conditions
CD4+ count >500 cells/μL or CD4 percentage >29%	A1	B1	C1
CD4+ count 200–499 cells/μL or CD4 percentage 14–28%	A2	B2	C2
CD4+ count <200/μL or CD4+ percentage <14%	A3	B3	C3

RNA load reaches a peak of over 1,000,000 copies/mL in just 2 weeks, spontaneously declining to a set-point level of 100 copies/mL 4 weeks later [10]. The dynamics of HIV viremia and antibody seroconversion in primary HIV-1 infection have been studied by Fiebig EW [8] who classified it into six distinct laboratory stages based on the timing of the positivization of HIV RNA, p24 antigen, antibodies seroconversion, and Western blot results.

15.3 Progression of HIV Infection

The progression of HIV infection is extremely variable from patient to patient. In about 50% of the patients, it seems to evolve from primary infection to AIDS within 10 years and in 25% of them within 6 years [11]. It is not yet clear whether the different evolution of HIV-related disease depends on the immunological characteristics of the host, on the quantity of viral inoculum, or on the virulence of the pathogen itself [11, 12].

There are many indicators used to estimate the course of the infection. The CD4+ lymphocytes count is an excellent predictor of the development of symptomatic infection, but the initial levels of HIV RNA appear to be a stronger prognostic factor [13]. HIV DNA is a marker of HIV persistence, it can predict the disease progression or remission independently of HIV RNA and CD4 levels and it estimates the viral reservoir [14, 15]. A small percentage (5%) of patients remain asymptomatic with no immunological decline for many years even without ART: they are called long-term non-progressors (LTNP) [16]. Among these, elite controllers can perfectly control the replication of the virus, keeping the viral load at undetectable levels.

The impact of HIV on the health of the host follows different mechanisms that synergistically lead to AIDS. On the one hand, the progressive depletion of CD4 + lymphocytes causes an insufficient cell-mediated immune response. This ablation makes the host susceptible to opportunistic infections, increasing also the risk of cancer. On the other hand, the presence of chronic viral infection causes a systemic pro-inflammatory activation that results in non-AIDS-related pathology (e.g., cardiovascular disease). Although screening programs allow the diagnosis of HIV infection at an early stage, in which the patient is completely asymptomatic, even today, many new diagnoses are made among late presenters subjects.

The term "*AIDS-defining conditions*" has been used since the 1990s, to identify those pathological conditions indicative of the last stage of the infection (Table 15.2). These include those conditions that occur during severe immunosuppression (<200 CD4 + T-lymphocytes/μL):

- Opportunistic infections (OIs), such as mycobacterial disease, candidiasis, cryptococcosis or Cytomegalovirus disease.
- Oncological and hematological diseases, such as Kaposi's sarcoma, Burkitt's lymphoma, and invasive cervical carcinoma.
- Other conditions such as wasting syndrome.

Table 15.2 AIDS-defining conditions (CDC, 1990)

Bronchial, tracheal, pulmonary candidiasis
Esophageal candidiasis
Invasive carcinoma of the cervix of the uterus
Coccidioidomycosis, disseminated or extrapulmonary
Extrapulmonary cryptococcosis
Chronic intestinal cryptosporidiosis (>1 month)
Cytomegalovirus diseases except hepatic, splenic, and lymph node localization
CMV retinitis
HIV-related encephalopathy (AIDS dementia complex)
Herpes simplex: chronic ulcers lasting >1 month or bronchitis, pneumonia or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Chronic intestinal isosporiasis (>1 month)
Kaposi's sarcoma
Burkitt's name
Immunoblastic lymphoma
Primitive brain lymphoma
Mycobacterium avium complex or *M. kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any location (pulmonary or extrapulmonary)
Pneumocystis carinii pneumonia
Recurrent bacterial pneumonia (two or more episodes in a year)
Progressive multifocal leukoencephalitis
Recurrent salmonella septicemia
Cerebral toxoplasmosis
Wasting syndrome

This was defined by CDC as a body weight loss equal to or greater than 10% with associated fatigue, fever, and diarrhea unexplained by another cause. Today we know that its etiology is multifactorial, depending on reduced diet intake (due to dysphagia and odynophagia), intestinal malabsorption (due to chronic diarrhea), endocrine dysfunction, hyperproduction of inflammatory cytokines with accelerated metabolic degradation of nutrients.

Viral, bacterial, fungal, and protozoal OIs occur as a consequence of a deficient cellular immunity in late-stage of HIV infection. These are the most important causes of morbidity and lethality in patients with AIDS. The majority of OIs are due to the endogenous reactivation of previously acquired pathogens. OIs only exceptionally cause disease in the immunocompetent host, but during AIDS, they reactivate with disseminated and uncontrollable consequences.

15.4 Central Nervous System Complications

The central nervous system (CNS) is a reservoir for HIV, which is established very early during the course of HIV infection. HIV crosses the blood–brain barrier (BBB) transported by monocytes and migrating lymphocytes. HIV-infected activated perivascular macrophages and microglial cells can express neurotoxic molecules that cause an inflammatory activation and this can lead to an increased BBB permeability. Many brain cells such as astrocytes and microglia can be infected by HIV producing new viruses. Glial and endothelial activation causes chronic inflammatory and degenerative insult with consequent neuronal injury [17]. This can lead to a large group of syndromes, ranging from different behavioral disturbances (sleep disorders, depression, anxiety, psychosis) to HIV-associated neurocognitive disorder (HAND) that occurs in 15% of patients with AIDS and can be the first manifestation of the disease.

15.4.1 HIV-Associated Neurocognitive Disorder (HAND)

Host genetic predisposition, aging, antiretroviral neurotoxicity, cerebrovascular disease, metabolic disorders (insulin resistance), and co-infections (HCV infection, CNS opportunistic infections) are important co-factors contributing to the neurological damage of HIV.

HAND includes three sub-disorders: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), HIV-associated dementia (HAD).

The initial symptoms of HAND are usually subtle: decreased short-term memory, slowness of thinking, loss of concentration, minor psychomotor slowing, brisk reflexes, mild postural tremor [18].

Even if ART use improves clinical conditions, many patients develop cognitive impairment or depression also during anti-HIV effective treatment.

Cerebrospinal fluid (CSF) examination is indicated, and should be investigated for the pres-

ence of bacterial, fungal, and acid-fast bacteria presence, cryptococcal antigen, VDRL test, and cytology, as HIV-associated dementia complex is a diagnosis of exclusion. In addition, CSF polymerase chain reaction (PCR) for JC virus (JCV) and other neurotropic viruses can be useful to exclude viral disease.

Poorer penetration of antiretroviral drugs into the BBB and its active efflux systems are associated with continued HIV replication in the CNS and occasional detectable HIV CSF viral load [19]. Residual CSF viral replication despite undetectable plasma viral load, or a CSF HIV-RNA concentration ≥1 log higher than plasma viral load is called viral escape. This has been associated with HAND, depression, and acute manifestations such as viral encephalitis.

When choosing the correct ART regimen, it is important to consider the ability of antiretroviral drugs to penetrate the BBB, as these differ in their ability to reach the CNS. In particular, the ability of antiretroviral drugs to penetrate into CNS depends on the binding of plasma proteins and the molecular weight of the drugs.

The persistence of neurological symptoms, despite a good control of HIV plasma viral load may be a signal of residual viral replication in the CSF. In these cases, a genotyping test on the CSF sample may reveal different quasi-species of the virus (with different drug resistances profile) in the CNS compartment.

15.4.2 Progressive Multifocal Leukoencephalopathy (PML)

PML is a demyelinating disease of the brain caused by the polyomavirus JC (JCV), and it is usually a fatal neurological disease. JCV infects 90% of the normal adult population worldwide remaining quiescent in the kidneys. The presence of JCV in the urine occurs regardless of immune status, whereas JC viremia is usually only detected among immunosuppressed individuals [20]. Its reactivation induces a lytic infection of oligodendrocytes, causing multifocal demyelination of the central nervous system. This process produces white matter lesions, presenting subacutely as cognitive impairment, focal motor deficits that correspond to the location of the lesions, and sometimes seizures. Differential diagnosis includes AIDS dementia complex, CMV and other neurotropic viruses encephalitis, neuro-syphilis, and cerebral infarction [21].

CSF analysis may be normal or show signs of mild inflammation. Molecular PCR research allows the detection of JCV DNA in the CSF. Hyperintensity on T2-weighted MRI is the typical pattern of lesions caused by JCV in the CNS. There is no specific treatment for PML. Despite various attempts made with antivirals (cidofovir), interferon or mirtazapine, the prompt initiation of ART and the subsequent immunological recovery appears to be the only chance to stop or slow the course of PML, which otherwise remains fatal [22–24].

15.4.3 Toxoplasmosis

Toxoplasma gondii is a ubiquitous intracellular protozoan that causes zoonoses throughout the world by infecting herbivorous, omnivorous, and carnivorous animals, including also the human species in its life cycle. The sexual phase of this parasite occurs in felines, which shed oocysts in the environment through feces. Humans become infected by eating raw or undercooked meat containing tissue cysts, or by ingesting contaminated water and plants. Most infections occur before adulthood [25]. Bradyzoites released from tissue cysts or sporozoites released from oocysts turn to tachyzoites; these reach all the tissues spreading through lymphatics and blood [26]. Toxoplasma tachyzoites can infect every type of cell, predominantly the reticuloendothelial system, but also neurons, muscle cells, and hepatocytes. Parasitemia occurs only in the acute phase of the disease, and it lasts about 7 days. The infection may run completely asymptomatic in immunocompetent hosts; however, it can have severe consequences when congenital, or in the immunocompromised population [27]. After the primary infection, the immune response limits its

progression by eliminating the tachyzoites from the bloodstream, forcing them to turn into tissue cysts (latent infection).

Both primary infection and the reactivation of a latent one can cause serious and fatal diseases damaging different organs, when they occur in an immunocompromised host.

Toxoplasmic infection of the CNS typically occurs in patients with a CD4+ count of less than 200/μL. *T. gondii* can also cause pneumonia, chorioretinitis, hepatitis, and myositis. At the beginning of the epidemic, CNS toxoplasmic lesions were the most frequent brain lesions in patients with AIDS [28]. CNS disease may manifest as a widespread encephalopathy, meningoencephalopathy, or it may cause mass lesions (toxoplasmic abscesses). Fever, seizures, headache, confusion, agitation, hemiparesis, cranial nerve palsies, ataxia, dysmetry, aphasia, and sensory deficits are the most common symptoms. They usually have subacute onset and an insidious worsening [29]. Differential diagnosis of CNS toxoplasmosis includes lymphoma, aspergillosis, cryptococcosis, and mycobacteriosis. Serology may be useful for diagnosis. IgG is frequently positive and IgM are negative, because most of the CNS disease is caused by reactivation of a latent infection. CSF examination usually shows a moderate reduction in glucose levels, a modest mononuclear pleocytosis, and an increase in protein concentration. Molecular DNA research with PCR techniques on CSF samples is specific but not very sensitive, and false negative results are possible [30, 31].

Magnetic resonance imaging (MRI) of the brain is the most sensitive imaging study to detect toxoplasma lesions: they are typically multiple and located in the cortico-medullary junction, with a ring enhancement after administration of contrast material and peripheral edema (Fig. 15.1). However, the only way to make a definitive diagnosis is by histological examination of the brain biopsy, and therefore in most cases the treatment is started empirically. Therapy regimens include treatment with pyrimethamine and sulfadiazine or trimethoprim-sulfamethoxazole (TMP-SMX) combination.

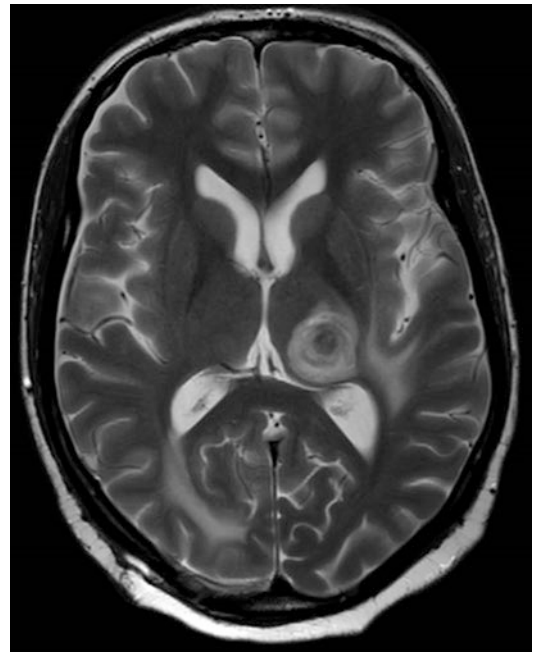

Fig. 15.1 Brain magnetic resonance in a HIV-positive patient with neurotoxoplasmosis

15.4.4 Cryptococcosis

Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated, saprobes fungi. They have been isolated in the environment (trees and soil contaminated by guano from birds) [32, 33]. Their incidence has dramatically increased with AIDS. Cryptococcus life cycle involves asexual and sexual forms and unicellular yeasts are the primary forms recovered from environment and human infections [34].

It appears that during the primary infection, the yeasts of Cryptococcus enter alveoli, where they are phagocytosed by alveolar macrophages. After the production of cytokines and the recruitment of other cells (such as Th1 lymphocytes) the yeasts are surrounded by granulomatous inflammation. In the immunocompetent host, Cryptococcus can be eliminated or remain dormant in the pulmonary lymph node complex. In the immunocompromised host (i.e., AIDS), primary infection is not limited and yeasts proliferate uncontrollably. Latent infections can also

reactivate with subsequent replication of the yeasts and spread to other organs through the bloodstream [35–37].

Cryptococcal meningitis is one of the most frequent OIs during AIDS, occurring when CD4+ count is less than 200/μL. Cryptococcus often causes meningitis, but can also occur as an encephalitis or brain mass lesions (granuloma or abscess). Symptoms include chronic headache, psychiatric disorders, hemiparesis, cranial nerve palsy (aphasia, acute deafness, diplopia), increased intracranial pressure, and acute onset of cerebellar symptoms too. In patients with severe immunosuppression, cryptococcal meningitis may have a non-specific evolution because of a reduced inflammatory response and an intense headache can be the only symptom [38, 39]. CSF examination shows a clear liquid, with increased proteins, reduced glucose, and monocytic pleocytosis. The microscopic examination of the CSF with India ink examination remains an excellent diagnostic method, but the culture and the finding of the polysaccharide antigen in the CSF are useful too. In 50% of cases CT and MRI scan of the brain can be completely normal during cryptococcal meningitis. No scan result is pathognomonic, but MRI usually shows numerous foci that are hyperintense on T2-weighted images in the basal ganglia or midbrain.

Skin abscesses, eye involvement, and bone lesions are other possible sites of infection in immunocompromised hosts, as a consequence of hematogenous dissemination [39, 40].

If the infection is not promptly treated, it is lethal. Paradoxical immune reconstitution inflammatory syndrome (IRIS) may arise after the initiation of ART in patients with severe ablation of cell-mediated response, with severe worsening of symptoms and life-threatening consequences [41, 42]. Randomized trials have shown that the early onset of ART in subjects with cryptococcal meningitis is not associated with a survival benefit [43]. Delaying ART initiation until 4–5 weeks of antifungal therapy is recommended [44] and in any case this should not be started before the end of the antifungal induction therapy.

Anti-cryptococcal therapy includes an induction phase with amphotericin B and flucytosine followed by a consolidation regimen with fluconazole. Secondary prophylaxis with fluconazole should be continued until CD4+ count is higher than 200/μL for 6 months.

15.5 Pulmonary Disease

HIV is the cause of both infectious and non-infectious pulmonary diseases. Although ART introduction has greatly reduced the incidence of OIs, diseases such as pneumocystosis and tuberculosis are still frequent.

Bacterial respiratory infections, frequently complicate HIV disease. The rate of bacterial pneumonia among patients with HIV is tenfold higher than that in HIV-negative population [45].

Encapsulated bacterial infections can manifest at every stage of the disease, having greater virulence when occurring during immunodeficiency. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most frequent community-acquired pathogens. Nocardia infection, although rare in the general population, is a common cause of disease in the immunocompromised host, and *Rhodococcus equi* is a gram-positive coccobacillus, often confused with normal bacterial flora on sputum samples, that can cause chronic and cavitating infection among AIDS patients [46, 47].

The differential diagnosis is not easy because of atypical radiological appearances, and the subtle course of symptoms. Diffuse interstitial infiltrates may be associated with common viral infections (influenza viruses) as well as with pneumocystosis, other fungal infections (e.g., *Histoplasma capsulatum*) or atypical tuberculosis manifestations. Cavitations, usually associated with tuberculosis in the general population, may be related to Nocardia, *Rhodococcus equi*, atypical mycobacteriosis, and aspergillosis.

15.5.1 *Pneumocystis jirovecii* Pneumonia (PCP)

Previously considered a protozoan, *Pneumocystis jiroveci* is today known to be a ubiquitous fungus, and the causative agent of interstitial plasma cell *Pneumocystis jiroveci* pneumonia (PCP) [48].

The first case of PCP in an HIV-positive patient dates back to 1981, and today PCP remains the most frequent opportunistic infection in the course of AIDS. Primary infection occurs due to inhalation of cysts during early childhood. This does not seem to lead necessarily to the development of PCP: the cysts' transformation into the trophic form and the adhesion of these to the alveolar epithelium are a necessary step for the pathogenesis of pneumonia [49].

The activation of alveolar macrophages and their phagocytic activity represent the most powerful defense against Pneumocystis, but in the absence of CD4+ cells, they are unable to control *P. jirovecii* infection [50]. The definition of "interstitial plasma cell pneumonia" is due to the large number of plasma cells observed in interalveolar septa. If in the early stages of the disease, PCP has purely interstitial characteristics, then alveolar involvement occurs too. The uncontrolled proliferation of Pneumocystis causes the formation of a foamy, eosinophilic exudate, resulting in the filling of alveoli, an impaired diffusing capacity, and alveolar-capillary block [51, 52]. In patients with AIDS, the onset of the disease is often insidious, with progressive appearance of fever, non-productive cough, and dyspnea, up to respiratory failure with distress and cyanosis [49, 53, 54].

Radiological images initially show typical bilateral interstitial infiltrates extending into the peri-hilar regions (Fig. 15.2); in the advanced phases the pattern can become confluent alveolar shadowing. Atypical manifestations include unilateral infiltrates, nodules, pneumatoceles, or even excavated lesions [49, 54].

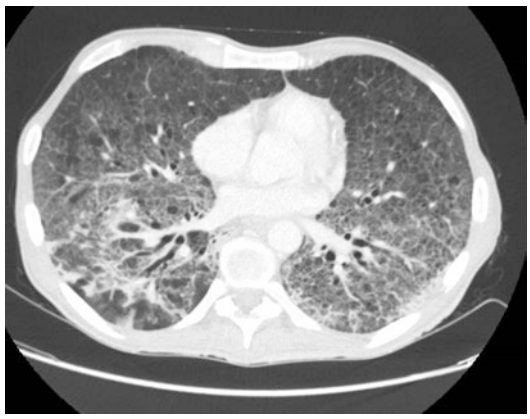

Fig. 15.2 Chest computed tomography in an HIV-positive patient with pulmonary PCP

Immunofluorescence and molecular PCR are the most efficient method of detecting Pneumocystis in a variety of different respiratory specimens: sputum and bronchoalveolar lavage (BAL) [54].

The TMP-SMX combination is the therapy of choice in the HIV patient (treatment must last for at least 21 days), whereas clindamycin and primaquine are the preferred alternative regimen. International guidelines recommend the initiation of ART within 2 weeks of starting treatment for PCP, as early HAART reduces the risk of progression to AIDS and death with no associated increased risk of IRIS.

PCP treatment should be followed by secondary prophylaxis with TMP-SMX or inhaled pentamidine, until CD4+ count is greater than 200/μl for over 3 months. Primary chemoprophylaxis is recommended in patients with a CD4+ count of less than 200/μL, oral thrush, or relevant concomitant immunosuppression and its discontinuation should not occur until the CD4 + count is greater than 200/μl for at least 3 months.

15.5.2 Tuberculosis

Mycobacterium tuberculosis infection is a frequent complication of AIDS. In HIV-positive patients, it may occur as both, a primary infection or a reactivation of latent tuberculosis, even when immunosuppression is not severe. The clinical features and the type of radiological images in patients with HIV-*Mycobacterium tuberculosis* co-infection depend on the degree of immunosuppression, ranging from classical tuberculosis (TB) patterns, to atypical characteristics both clinically and radiologically [55, 56].

In individuals with HIV infection, TB has predominantly pulmonary localization, but in subjects with CD4+ counts of less than 200/μL, extrapulmonary manifestations (bones, skin lesions, mesenteric tuberculous lymphadenitis, peritonitis, otitis, genitourinary TB) are more

frequent than the HIV-negative population (50% of AIDS cases) [57, 58]. Ten percent patients with tuberculosis and thirty-eight percent with extrapulmonary tuberculosis have miliary disease.

Among those with established immunosuppression, pulmonary involvement is more severe, miliary type often occurs, and unusual X-ray manifestations are also common, such as focal lesion on the lower and middle lobes or diffuse interstitial infiltrates. Cavitation is typically absent [59]. Subclinical onsets are frequent and some cases can only be identified by sputum culture. Acid-fast bacillus (AFB) stain on sputum specimen is less frequently positive than in classical cavitating forms and molecular PCR is necessary for diagnosis.

The timing of initiation of TB treatment in the HIV+ patient has long been debated due to the high frequency of IRIS following the beginning of ART, the combined toxic effects of therapies and drug–drug interactions.

Recent findings suggest that the early start of ART and anti-TB therapy reduce mortality in these patients. IRIS can occur among those who have already started anti-TB treatment (paradoxical IRIS) and among those in whom TB is not yet diagnosed, unmasking an active TB that was undiagnosed. In the first case, IRIS occurs with a paradoxical aggravation of clinical conditions: cough, fever, and the worsening of radiological images (Fig. 15.3). Eight to forty-three percent of patients with treated TB develop paradoxical IRIS and it is usually self-limited, lasting a median of 2 months, with a good response to prednisolone therapy [60, 61].

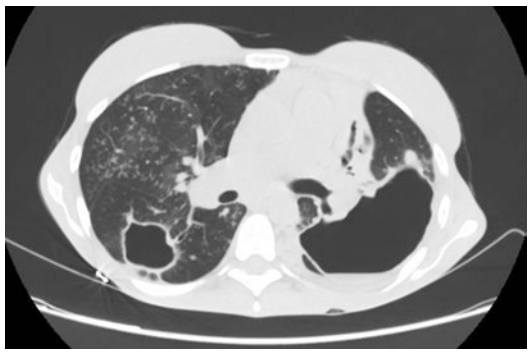

Fig. 15.3 Chest computed tomography in an HIV-positive patient with paradoxical TB IRIS

Clinical trials show that initiating ART 2 weeks after the start of TB treatment significantly increased survival among HIV-infected adults with CD4+ T-cell counts of 200/μL or lower [60]. An improved survival was also demonstrated with early ART initiation among HIV-infected patients with CD4+ count of less than 50/μL although this is associated with higher frequency of IRIS [62]. Modern international guidelines suggest starting ART within 2 weeks of initiating TB treatment if CD4+ count <50/μL, and deferring until the 8th–12th week of TB treatment if CD4+ count >50/μL.

15.5.3 *Mycobacterium avium* Complex (MAC)

MAC comprises two different organisms: *Mycobacterium avium* and *Mycobacterium intracellulare*. In AIDS patients, 90% of infections are caused by *Mycobacterium avium*. The reservoir of infection is in the environment (water, soil) and animals (birds, cattle, pigs) and the infection occurs through ingestion or inhalation, but it is not interhuman [63, 64]. In patients with AIDS, pulmonary and cutaneous involvement and lymphadenitis are common and the infection often leads to disseminated disease. Disseminated MAC is seen rarely in patients with greater than 100 CD4+ cells/μL.

Clinical features include high fever, cough, night sweats, weight loss, abdominal pain, and diarrhea. Many organs can be involved in the infection besides the lung (liver, spleen, intestines, lymph nodes, adrenal glands, bone marrow) and the symptoms depend on the infected system [65]. The diagnosis of MAC-disseminated disease needs molecular research or culture from blood or liver/splenic biopsy. The isolation of the mycobacterium in other organs such as lymph node, feces, sputum can only demonstrate a colonization or a localized infection, without excluding disseminated one [65].

The combination of a macrolide (azithromycin or clarithromycin) with ethambutol and

rifabutin is the first-line treatment according to international guidelines.

IRIS can be a serious complication in patients with AIDS and MAC infection, occurring weeks or even months after the start of ART. The lower the CD4+ count at the time of the initiation of antiviral therapy, the higher the risk of IRIS, whose clinical features include fever, painful lymphadenitis, and focal abscesses. According to guidelines, primary prophylaxis with azithromycin 600 mg once a week is recommended when CD4+ count is <50/μL.

15.6 Gastrointestinal and Hepatobiliary Diseases

Gastrointestinal and hepatobiliary diseases are a common cause of morbidity in HIV patients during both primary and advanced disease. The three most common causes of abdominal pathology during HIV infection are infectious, oncological, and drug induced.

During the advanced stages of infection, all tracts of the gastrointestinal tract can be affected. Dysphagia or odynophagia are common symptoms, resulting in the involvement of the upper GI traits during immunosuppression: candidiasis, CMV, varicella-zoster virus (VZV), herpes simplex virus (HSV) ulcers are common, but patients often present with reflux esophagitis and pill esophagitis too. Malignant causes include esophageal carcinoma, lymphoma, and Kaposi's sarcoma, that can also affect the stomach and any part of the gastrointestinal tract.

Acalculous cholecystitis is an AIDS-defining condition commonly associated with CMV, Cryptosporidium, and microsporidia infection and these pathogens can frequently cause cholangitis as well [66, 67].

15.6.1 Small Intestine and Colon Disease

The differential diagnosis for enterocolitis in a patient with AIDS includes bacterial, protozoal, and viral pathogens, as well as neoplastic or autoimmune inflammatory bowel diseases (IBD). Clinical features are non-specific and include bloating, nausea, cramping, profuse diarrhea that may result in malabsorption and weight loss. Colitis can cause lower abdominal pain and cramping, urgency, and tenesmus. Infections of the distal portion of the colon (proctitis) may have mild symptoms or cause rectal bleeding and diarrhea.

Salmonella spp., *Campylobacter jejuni, Escherichia coli,* and *Listeria monocytogenes* are the most frequent causes of infectious enteritis in immunocompromised patients. *Mycobacterium tuberculosis* and *Mycobacterium avium* infections are frequent especially during the last stages of the disease, due to dissemination through the bloodstream.

CMV, human herpesvirus 6, and adenovirus are frequent causes of viral colitis, producing mucosal inflammation with superficial ulceration. CMV enterocolitis leads to fever, abdominal pain, bloody diarrhea, and even intestinal perforation or toxic megacolon [68].

Cryptosporidium parvum is a protozoa that frequently causes intestinal disease in the immunocompromised host. The infection can involve all the gastrointestinal system, from the esophagus to the rectum and the biliary tract can be also involved. It causes diarrhea with an insidious onset, which progressively worsens simultaneously with the depletion of CD4+ lymphocytes. Clinical features include severe malabsorption and weight loss due to 10–20 watery stools per day, abdominal pain, vomiting, and electrolyte leakage.

Patients with AIDS presenting with diarrhea must be carefully examined. The duration of symptoms and the presence of blood in the stool must be investigated. Both sexual and travel history are important. The examination of stool samples with culture and molecular research for bacteria and mycobacteria is essential for diagnosis. Microscopic examination of feces can detect protozoal infections and the presence of mycobacteria.

Neisseria gonorrhoeae and *Treponema pallidum* are common cause of sexually transmitted proctitis, whereas serovar L2b of *Chlamydia*

trachomatis frequently causes rectal Lymphogranuloma venereum among HIV-positive men who have sex with men (MSM) [69, 70]. Among these, Shigellosis often has a sexually transmitted route with high infection rates due to direct oral-anal contact [71].

Amebic and *Giardia lamblia* infection may occur at any stage of HIV disease. Travel to areas of the world with low sanitary standards and oral-anal sexual practices are the main risk factors [72].

HIV-positive MSM and women are at greatly increased risk of human papillomavirus (HPV) associated anal cancer (Fig. 15.4). Meta-analysis shows that among HIV-infected MSM incidence rates of HPV-16 and HPV-18 are much higher than in the negative population, whereas HPV clearance is lower. The incidence of anal intraepithelial neoplasia (AIN) and the risk of transformation into anal cancer do not change despite the immunological recovery after ART initiation [73]. For this reason, modern guidelines recommend periodic screening with anal cytology and high resolution anoscopy for all HIV-positive MSM and women, for early detection and monitoring of AIN.

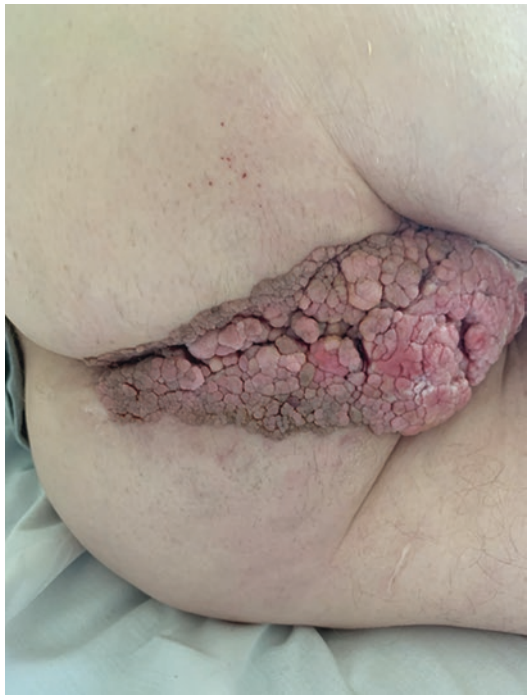

Fig. 15.4 Large perianal acuminate warts in AIDS patient. Biopsy demonstrated anal HPV-related cancer

15.6.2 Viral Hepatitis

Although there are multiple factors that can cause an increase in transaminase levels among those with HIV infection (ART, antibiotics, alcohol abuse, nonalcoholic fatty liver disease), hepatotropic viruses deserve a separate analysis, as these are often transmitted simultaneously with HIV and through the same route.

Hepatitis A virus (HAV) is a fecal-oral transmission virus, responsible for both asymptomatic infection and acute hepatitis. HAV hepatitis occurs predominantly in areas of low hygiene, but it has also been associated with sexual transmission through oroanal sex among MSM. All MSM should receive HAV vaccination, especially if HIV-positive.

Hepatitis B virus (HBV) shares both sexual and parenteral transmission routes with HIV. The host immune response against HBV and thus its natural history are strongly influenced by a coexisting HIV infection, whereas the immune control of HBV is reduced among co-infected ones. This leads to reduced rates of seroconversion of HBs antigen and higher HBV DNA levels during chronic hepatitis.

In untreated patients, co-infection leads to faster hepatic damage and cirrhosis and frequent monitoring of hepatic morphology with ultrasound scan is recommended.

Although antiretroviral drugs such as tenofovir and emtricitabine, commonly present in the backbone of ART regimens, are active on both viruses, HIV–HBV co-infection is associated with increased mortality even after the onset of ART and earlier and more aggressive hepatocarcinomas (HCC) are reported in this population. Early detection of HCC, through six-monthly ultrasound scan of the liver is recommended. Non-invasive assessment of liver fibrosis with fibroscan should be periodically performed. In patients with advanced cirrhosis esophagogastroduodenoscopy is recommended to exclude esophageal varices.

All persons with HIV/HBV co-infection should receive antiretroviral regimen that includes tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) because of their powerful activity against both, HIV reverse transcriptase and HBV polymerase.

HIV and hepatitis C virus (HCV) can also be transmitted through the same pathway and co-infection rate is high among injection drug users (IDU). Co-infection rate of 70% can be found in countries of Eastern Europe where the main transmission route of HIV is intravenous drug use, whereas in Central European countries, where HIV is transmitted through sexual intercourse, the co-infection rate of HCV is lower (10–15%). Data emerging from European cohorts have shown a reduction in new HCV infections among HIV+ population since the introduction of direct acting antiviral (DAA)-based HCV therapy, but acute infections and re-infections rates appear to be higher among MSM practicing chemsex (the use of drugs to facilitate sexual activity), due to needle-sharing and anal sex.

Like HIV, HCV infection can develop within different compartments as the liver, plasma, and peripheral blood mononuclear cells. The presence of both viruses may bidirectionally complicate the natural history of both infections and their treatment. Co-infected patients have higher HCV viral loads than the ones infected with HCV alone, and an underlying HIV infection decreases the chance of spontaneous hepatitis clearance because of the lack of immune response [74]. HIV–HCV infected patients seem to have a faster progression to liver fibrosis and HCV-related liver disease appears to increase the risk of hepatotoxicity of HAART [75].

Co-infection is associated with an increase in deaths from HCC and liver disease. In addition, HCV in HIV-infected patients was independently associated with an increased risk of progression to AIDS and AIDS-related death [76]. Every patient with HIC–HCV co-infection must be screened for HCC and considered for DAA regimens regardless of fibrosis stage. An undetectable HCV RNA viral load 12 weeks after the end of DAAs is called sustained virologic response and it is the primary aim of HCV treatment.

15.7 Other Clinical Manifestation of HIV

15.7.1 Heart Disease

HIV-related heart disease is not uncommon. Some opportunistic infections can affect the heart: mycobacteria are frequently associated with pericardial involvement and *Toxoplasma gondii* can be a cause of infectious myocarditis. Epstein–Barr virus, coxsackie viruses, and CMV can affect the heart, causing dilated cardiomyopathy.

Cardiomyopathy with left ventricular dysfunction, which may result in congestive heart failure and dilated cardiomyopathy are common among HIV patients, and the virus itself seems to be involved in the pathogenesis of cardiomyopathy, even if the cause of HIV-related cardiomyopathies is multifactorial [77–79].

15.7.2 Oral Manifestations

Many pathological manifestations of HIV can occur in the course of the infection, from the beginning to the most advanced stages: stomatitis and aphthous ulcers can be symptoms of acute retroviral syndrome during primary infection.

Several types of oral ulcers are associated with HIV infection, and the differential diagnosis includes herpetic lesions (HSV1, HSV2, CMV), syphilitic ulcers, and aphthous stomatitis.

Candida albicans, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* are the cause of pseudomembranous candidiasis (oral thrush) following AIDS immunity impairment. Candida white plates are adherent to the soft palate, tonsils, and buccal mucosa, involving the hypopharynx too. Treatment with antifungal mouthwash may be sufficient, but the involvement of the esophagus should be excluded with endoscopy.

The replication of EBV in the epithelium of keratinized oral cells can cause oral hairy leukoplakia. This appears to be a raised white lesion of the oral mucosa, usually on the lateral margin of the tongue and it is considered a precancerous

lesion, with a high risk of evolution into oral carcinoma.

Among the neoplasms, Kaposi's sarcoma lesions typically appear as purple papules in the palate, whereas non-Hodgkin's lymphomas may also arise in the mouth as a swelling or ulcers.

15.7.3 Cutaneous Manifestations

Both infectious and neoplastic pathologies can affect the skin in all stages of HIV-related disease.

During acute retroviral syndrome, a maculopapular rash can mimic rubella, measles, and other infectious diseases.

Seborrheic dermatitis is common, with a prevalence of 50% of cases, and the severity of symptoms may increase with the degree of immunosuppression.

Psoriasis is also common and it may have severe manifestations in the course of AIDS.

HSV1 and HSV2 infection is associated with frequent reactivations not only in the labial area, but also in the genital and perianal area (HSV2).

A dermatomical skin reactivation of VZV often occurs, and it may be an early symptom of immunosuppression in patients with unknown HIV infection.

Cutaneous manifestations of sexually transmitted infection, such as HPV condylomas, or Molluscum contagiosum poxvirus lesions, must be excluded in daily clinical practice. Syphilitic lesions may have atypical appearance among AIDS patients, presenting particularly aggressive evolution. Lues maligna is characterized by disseminated, papulonodular ulcerated cutaneous lesions. This is a particularly aggressive form of *Treponema pallidum* infection, which is almost always related to an underlying unknown HIV infection. Obliterative endarteritis appears to be the cause of its destructive cutaneous lesions [80].

Kaposi's sarcoma is a vascular neoplastic disorder that at the beginning of the epidemic used to involve 80% of new diagnoses. Its characteristic findings are vascular cutaneous red-purple nodules or plaques affecting the skin, mucous membranes, and viscera. It can occur at any stage of the disease, but is more frequent during AIDS. Human herpesvirus 8 (HHV-8), which is commonly sexually transmitted, is recognized as the cause of Kaposi's sarcoma. It mainly affects parts of the skin exposed to sunlight and the hard palate, while the lymph nodes, the gastrointestinal system, and the lungs are the most frequently affected organs. Histologically, its typical findings are proliferations of fusiform and endothelial cells with hemosiderin-rich macrophages. While localized skin disease appears to have spontaneous remission after initiation of ART, and subsequent immunological recovery, disseminated disease requires chemotherapy with doxorubicin, daunorubicin, or paclitaxel [81].

15.7.4 Ocular Manifestation

Almost half of patients with HIV infection have ocular problems in the advanced stages of the disease. Patients complaining of ocular symptoms or progressive visual loss, should undergo an ophthalmologic and retinal examination.

HIV itself can affect the retina causing generally a benign retinopathy, although some patients develop visual defects due to the formation of HIV-related microaneurysms and retinal hemorrhages.

Toxoplasma gondii, Pneumocystis jirovecii, VZV can affect the eye causing blindness among immunosuppressed individuals. Optic neuritis, iritis, and uveitis can occur in patients with secondary syphilis.

The reactivation of a latent CMV infection, due to the loss of CD4+ lymphocytes may be systemic, involving esophagus and gastrointestinal tract, adrenals, pancreas, brain, and lungs whereas ocular involvement is common too. CMV retinitis is one of the most serious ocular complication during AIDS. The onset is usually insidious, with painless, unilateral, progressive visual loss, blurring, and floaters. Although retinal involvement is usually unilateral, the disease can progress, affecting the contralateral eye. Fundoscopy findings include coalescing white exudates in a vascular pattern, with surrounding hemorrhage and edema [82].

15.8 Non-AIDS Disease

The spread and success of ART has led to an important reduction in the incidence of AIDS-related disease, especially in the high and middle income countries. Most patients on ART achieve durable and lifelong viral suppression and AIDS-related illnesses are no longer the main threat. Despite the lengthening of life expectancy, treated patients may not have completely restored health, because of an increased risk of several "non-AIDS" complications, including cardiovascular disease, cancer, kidney disease, liver disease, osteopenia/osteoporosis, and neurocognitive disease when compared with HIV-negative population [83, 84].

Many of these are a consequence of aging and chronic inflammation; others depend on the lifelong medication intake.

Chronic immune-activation and the resulting state of systemic tissue inflammation can accelerate the cell and organ senescence mechanisms. Several pathways are involved in the formation of this inflammatory environment, including ongoing HIV replication, other co-pathogens infections, damaged immunoregulatory system, and translocation of microbial products, leading to end-organ damage. Many markers of immune-activation are higher in antiretroviral-treated adults than in age-matched uninfected individuals [85] and elevations of these seem to be associated with an increased risk of several diseases: among these, interleukin-6 is the most studied marker and its levels are strongly associated with all-cause mortality [86].

HIV infection and antiretroviral treatment seem to be causally associated with early heart disease. Even if the risk of cardiovascular events is higher in the HIV untreated population, those on ART still have a high incidence of heart disease. Many antiretroviral drugs can have a negative effect on the cardiovascular system. Abacavir, commonly present in many ART regimens appears to increase cardiovascular risk [86], as well as cumulative exposure to protease inhibitors (PIs), such as darunavir and atazanavir, is associated with metabolic changes, including body fat redistribution, insulin resistance, diabetes mellitus, and hyperlipidemia [87]. Traditional risk factors, such as smoking, alcohol, hypertension, and recreational drugs use certainly contribute and strict lifestyles management has an important role in prevention, even more so than in the general population.

Suboptimal CD4+ T cell recovery after ART initiation has been also associated with many comorbidities (e.g., heart disease, cancer) and mortality [88, 89]. If the advanced immunodeficiency increases the risk of typical AIDS-defining cancer, such as Kaposi's sarcoma or Burkitt's lymphoma, HIV infection increases the risk of other types of neoplasia, including lung cancer, skin cancer, colorectal cancer, prostate cancer, and anal cancer [90].

High rates of chronic viral hepatitis and alcohol misuse can cause chronic hepatic damage, whereas lifelong exposure to antiretroviral therapy plays a fundamental role in liver disease.

The pathological effect of HIV on the kidney can have many different effects, including acute renal failure. Chronic kidney disease can be directly caused by HIV, with resulting HIV-associated thrombotic microangiopathies, immune-mediated renal diseases, and classic HIV-associated nephropathy (HIVAN), but exposure to drugs also plays a fundamental role as well.

HIVAN histological findings usually include focal and segmental glomerulosclerosis, glomerular injury, mesangial proliferation, tubular degeneration with microcysts formation, and capillary collapse, whereas ultrasonography usually shows large, echogenic kidneys. Nephrotic-range proteinuria, and renal insufficiency are often the initial findings, with mild elevation of serum creatinine levels, and low albumin concentration. Although renal damage occurs in every ethnicity, African patients seem to develop end-stage renal disease more frequently than others, a consequence of the APOL1 polymorphism.

Tenofovir disoproxil fumarate (TDF) can cause Fanconi syndrome and it is associated with proximal renal tubulopathy. Other anti-infective drugs known for their nephrotoxic effect include pentamidine, foscarnet, and aminoglycosides [91].

The causes of bone complications are many and multifactorial. The aging of the HIV-positive population, smoking, recreational drug use, and alcohol play a role, but HIV-1 itself appears to cause bone damage through several molecular mechanisms, including upregulation of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoclastic activity [92, 93]. The prevalence of osteoporosis in HIV-infected individuals is more than three times greater when compared with HIV-uninfected individuals [94] and TDF and PI regimens are both associated with bone pathology. In particular, TDF seems to be the most correlated with bone damage through altering the expression of genes involved in bone cell signaling, causing both osteoporosis and osteomalacia.

15.9 Immune Reconstitution Inflammatory Syndrome (IRIS)

While AIDS represents the most dangerous consequence of HIV infection, the recovery of the immune system following antiretroviral treatment could be an equally serious cause of illness [95].

The quick reduction in HIV-1 RNA viral load and the rapid increase in CD4+ lymphocyte count can be associated with a paradoxical worsening of clinical conditions in patients with diagnosed or unknown OIs or AIDS-associated malignant conditions.

Immunoreconstitution occurs as a consequence of ART initiation, following replenishment of immune circulating cells, regeneration of primary and secondary lymphoid tissues (lymph nodes, thymus, GUT-associated lymphatic tissue), and the restoration of pathogen-specific T, B, and NK cellular response.

IRIS features depend on the opportunistic pathogen, its specific immune responses and/or immune regulation, and the CD4+ count at the time of the start of ART.

IRIS includes two different components:

- an immune-restoration disease against OIs
- an associated autoimmune response.

Immune-restoration disease consists of immune responses against pathogen-specific exogenous antigens. The inflammation and the damage on the tissues that appear after ART initiation can be confused with the damage caused by the opportunistic pathogen, but they are the result of immune reconstitution. It is very important to discriminate between an associated AIDS infection and the effects of immune restoration. In the latter, the examination of an infected body fluid sample or tissue can reveal abundant lymphocytic infiltrate and granulomatous inflammation with a low pathogen count. Whereas, with immunodeficiency disease there may be an abundance of pathogens and unformed granulomata [61].

Immune-restoration disease usually presents during the first 3 months of ART, occurring in patients who have already received treatment for an opportunistic pathogen, with a paradoxical worsening of symptoms: paradoxical IRIS. Sometimes IRIS can also unmask OIs that were subclinical and hidden before the start of ART.

According to some studies 8–43% of patients with treated tuberculosis and 4–66% of patients with treated cryptococcal infection develop IRIS after the initiation of antiretroviral drugs [61].

If in most cases IRIS can be self-limiting, improving despite continuation of ART with excellent response to corticosteroid therapy, it can be also associated with severe complications and poor prognosis when occurring in the CNS (e.g., JCV infection, *M. tuberculosis* or *C. neoformans* meningitis) [41, 95, 96].

ART-induced immunoreconstitution can also complicate many autoimmune diseases and there are reported cases of Grave's disease occurrence in patients with severe CD4+ count reduction [97]. Many different autoimmune diseases are reported as a result of HIV infection and IRIS, probably due to a T-cell immune tolerance dysfunction [98, 99].

15.10 Antiretroviral Therapy (ART)

If the stigma linked to HIV continues to plague the lives of patients, ART has completely changed the natural history of the infection. In the devel-

oping world the AIDS-related mortality rate continues to be high, but in the high and middle income countries the pathologies associated with HIV have drastically changed, due to chronic inflammation, the aging of the patients, and the chronic drugs exposure. The main objective of ART is to reduce the morbidity and mortality related to the infection, improving the quality of life of the individual. Reaching an undetectable viral load within 3–6 months of the start of therapy is the main virological goal, consequently allowing an immunological recovery (restoration of the CD4 + count).

The structural characteristics of HIV require a pharmacological "attack" on multiple molecular targets to effectively control viral replication. The currently used drugs target enzymes and proteins that allow the virus to complete its replication cycle.

They actually are:

- Reverse transcriptase inhibitors (RTIs)
- Protease inhibitors (PIs)
- Integrase strand transfer inhibitors (INSTIs)
- CCR5 receptor antagonists.

Nucleoside analog RTIs (NRTIs) and non-nucleoside RTIs (NNRTIs) block the RNA-dependent DNA polymerase synthesizing viral complementary DNA (cDNA) from HIV RNA. RTIs were the first antiretroviral drugs to enter clinical use. They include drugs used in the past such as zidovudine, and others today widely prescribed in co-formulation such as TDF, emtricitabine (FTC), abacavir (ABC), lamivudine (3TC), doravirine (DOR) and efavirenz (EFV). Mitochondrial inhibition caused by this class, may occasionally lead to dysfunctions such as hyperlactatemia, lactic acidosis, hepatic steatosis, peripheral neuropathy, myopathy, and lipoatrophy [100, 101]. The main side effects of TDF are bone density loss and nephrotoxicity as it is associated with proximal renal tubulopathy. EFV has often been associated with psychiatric side effects such as insomnia, confusion, agitation, nightmares, and depression.

PIs inhibit HIV aspartyl protease, preventing this from cleaving Gag and Gag-Pol polyproteins. Their use has revolutionized the outcome of therapy thanks to their powerful action against the virus. To increase (or "*boost*") their plasma levels, currently used PIs, such as darunavir and atazanavir, are co-formulated with booster molecules: ritonavir and cobicistat inhibit PIs metabolism thereby increasing PIs systemic exposure. The major side effects of this class are metabolic disorders, such as dyslipidemias, increasing risk of insulin resistance, and diabetes. If co-formulation with boosters guarantees optimal antivirals bioavailability, it also represents a major problem of drug interactions when co-administered with other pharmacological classes, such as anti-TB drugs, statins, and immunosuppressants.

INSTIs are the newest antiretroviral drugs and they are recommended by international guidelines in first-line regimens. Raltegravir (RAL), elvitegravir (EVG), bictegravir (BIC) and dolutegravir (DTG) prevent integration of cDNA into the cellular genome and the strand transfer reaction by inhibiting viral integrase. In several comparisons between regimens containing PIs with boosters, INSTIs, were better tolerated and associated with fewer therapeutic interruptions [102, 103]. The most common side effects of this class are nausea, diarrhea, insomnia, and vivid dreams. Today, three single tablet regimes (STR) containing EVG or DTG are widely used allowing an easy daily intake and a high adherence. Clinical trials demonstrated the superior efficacy and reduced time to viral suppression of a DTG plus ABC/3TC regimen when compared with combination of NRTIs and NNRTIs [104]. Other trials demonstrated a higher virological response rate of DTG once daily than once-daily ritonavir-boosted DRV [103].

15.10.1 Choosing the Best ART Regimen

Modern first-line therapeutic regimens are based on an antiviral "backbone" (an association of two NRTIs) and a third drug belonging to the NNRTIs, PIs, or INSTIs groups. Currently used backbones are abacavir/lamivudine (ABC/3TC), tenofovir

alafenamide/emtricitabine (TAF/FTC), and tenofovir disoproxil fumarate/emtricitabine (TDF/FTC). When choosing the right therapeutic regimen, it is important to consider numerous factors such as renal function, comorbidities, drug–drug interactions and the immuno-virological set point: pre-therapy viral load, CD4+ count, viral genotyping.

While the regimens including abacavir can be used even on patients with renal insufficiency, they seem to be associated with increased cardiovascular risk in patients with pre-existing cardiac pathology [105, 106]. If the viral load at baseline is higher than 100,000 copies/mL the use of ABC/3TC is not recommended, as it is associated with higher virological failure regardless of the third drug, except when taken with DTG. Furthermore, before using abacavir, it is necessary to exclude the presence of the HLA B5701 mutation, as it is associated with an increased risk of severe allergic reaction. As previously stated, TDF is associated with proximal renal tubulopathy and its chronic use is shown to lead to bone density loss. TDF and its pro-drug tenofovir alafenamide (TAF) are not recommended when glomerular filtrate is <30 mL/min, although clinical trials have shown TAF to be less nephrotoxic when compared to TDF [107, 108].

Currently, there are six single tablet regimen (STR) allowing once daily intake (Table 15.3) [109–111]. Taking STR can help patients to adhere to the therapy despite different lifestyles, thus reducing the effects that non-adherence has on the immunovirological outcome [112].

If reducing the pill burden is an excellent strategy to facilitate patients' adherence to therapy, reducing the number of drugs to minimize chronic toxicity is one of the current challenges. Recently, a limited number of trials have studied ART initiation with dual therapy regimens. Finally, a randomized phase III trial demonstrated the non-inferiority of a dual regimen (dolutegravir with lamivudine) when compared for 48 weeks with a three drugs regimen in the initiation of antiretroviral therapy [113].

15.10.2 ART Initiation Time

The starting time of ART has long been debated with the purpose of finding the right balance between the benefits and toxicity of lifelong therapy. In the past, the rationale behind the initiation of therapy focused only on AIDS prevention and treatment: it used to be immediate for those patients with severe immunosuppression, who presented clear signs and symptoms of AIDS-defining conditions or for those who had a significant reduction in CD4+ count. Initiation was instead deferred in those who were asymptomatic, with a CD4+ count above a certain threshold who did not need immediate medical attention. Several studies have investigated the benefits and risks of an early start of ART over the years [114–117] and today we know that chronic HIV infection is strongly associated with non-AIDS pathology [118–121].

To determine the risks and benefits of the immediate initiation of ART, the START international study enrolled 4685 HIV-positive patients, comparing those who received immediate treatment (CD4+ count of more than 500 cells/μL), with those who received deferred initiation (when CD4+ count had reached 350 cells/μL threshold) demonstrating a significant benefit of immediate initiation regardless of CD4+ count [116, 122, 123].

Starting ART regardless of CD4+ counts brings enormous benefits also in terms of public health, preventing new infections: treatment as

Table 15.3 Current STR regimens

NNRTI STR	PI STR	INSTI STR
Rilpivirine/emtricitabine/tenofovir DF rilpivirine/emtricitabine/tenofovir AF doravirine/lamivudine/tenofovir DF	Darunavir/cobicistat/emtricitabine/tenofovir AF	Dolutegravir/abacavir/lamivudine Elvitegravir/cobicistat/emtricitabine/tenofovirDF Elvitegravir/cobicistat/emtricitabine/tenofovir AF Bictegravir/emtricitabine/tenofovir AF

prevention [124]. The PARTNER [125] and PARTNER-2 [126] studies have also shown the absence of transmission of the infection in homosexual and heterosexual serodiscordant couples engaging in condomless intercourse, where the HIV-positive partner has an undetectable viral load on ART. As far as acute infection is concerned, today we know that HIV implants the reservoir already in the very early stages after rapidly integrating and replicating into mononuclear cells (PBMC) and tissue sanctuaries [127]. The size of the reservoir predicts time to viral load rebound in the event of ART interruption [128] which suggests that the smaller the reservoir is, the easier it will be to obtain viral remission in the future [129]. As the HIV DNA set point is established at the beginning of the infection, initiating ART in the acute stages of this significantly reduces the amount of virus that integrates into cells and thus the size of the reservoir [48].

15.11 HIV Prophylaxis

The use of antiretroviral drugs to prevent HIV infection is a fundamental step in the fight against the spread of the virus. Different combinations of drugs can be used as pre-exposure prophylaxis (PrEP) and as post-exposure prophylaxis (PEP), resulting in an effective reduction of the risk.

15.11.1 Pre-Exposure Prophylaxis (PrEP)

PrEP represents a new and effective strategy in which antiretroviral drugs are started in HIV-negative persons before potential exposure to the virus, in order to prevent it.

Daily intake of a combination of tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) in a single tablet regimen has been shown to protect high risk individuals against HIV-1 infection [130, 131]. Sexual activity-dependent (on demand) PrEP with TDF-FTC was also associated with a relative reduction of 86% in the risk of HIV-1 infection [132].

It is important to identify individuals who could benefit from PrEP by investigating their sexual history and risk factors, including unprotected anal intercourse with multiple partners, previous sexually transmitted infections, previous use of post-exposure prophylaxis, HIV-positive sexual partners with detectable viral load and intravenous drug use. The practice of chemsex (use of crystal methamphetamine, mephedrone, and gammahydroxybutyrate in a sexual context) is a widespread phenomenon among MSM and it is connected with a high risk of HIV acquisition and other sexually transmitted infections and therefore is a strong indication for the use of PrEP.

Some cases of HIV infection despite adequate adherence to PrEP have been reported. In these, multiple resistance to drugs (MDR) HIV strains were transmitted [133, 134]. During the counseling patients should be advised of the possibility of infection with strains of the virus resistant to RTIs, although at the moment it seems extremely rare, as well as possible cases of failure of PrEP itself. Although it is an effective system of protection against HIV and HBV infection, PrEP does not protect against *Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae,* and other sexually transmitted pathogens. International guidelines recommend screening for HIV, syphilis, viral hepatitis, and monitoring of renal function before and during PrEP [135].

15.11.2 Post-Exposure Prophylaxis (PEP)

PEP with antiretroviral drugs is now an integral part of the measures available to reduce the risk of transmission of HIV, in which antiretroviral drugs are started in HIV-negative persons after an occupational or sexual exposure to prevent an infection. Several studies have detected viral vulnerabilities during entry into the mucous membranes in the earliest stages of infection, which, if effectively targeted, lead to the avoidance of systemic infection [136].

The risk of transmission of HIV following a single exposure is 0.3–0.5%. During occupational

exposure, the risk may depend on the presence or absence of blood on a cutting edge, or the depth of the lesion. For sexual exposures other factors can influence the risk, such as the presence of blood on the mucous membranes, the presence of lesions and ulcerations on the genitals, circumcision, the ejaculation during oral, vaginal and receptive anal intercourse. In particular, the probability of transmission correlates with the concentration of HIV in the blood or genitals secretions. If the source-patient is on ART, their HIV viral load has been less than 200 copies/mL for at least 6 months and if they are not affected by STD, the transmission of HIV is considered highly unlikely. The PARTNER [125] and PARTNER-2 [126] studies have demonstrated the absence of transmission of the infection among serodiscordant couples engaging in condomless intercourse where the HIV-positive partner has an undetectable viral load on ART.

PEP must be started within 72 h of exposure and it should last for 28 days. The best tolerated combinations, with lower frequency of side effects and interruptions, are TDF/FTC with an INSTI (RAL, EVG, DTG). In the case of exposure to a source-patient with uncontrolled HIV infection, therapeutic history should be considered (pharmacological history, viral resistances) to adapt the PEP regimen to the virus.

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Donovanosis, Chancroid, and Endemic Treponematoses: Clinical Features and Control

16

Aldo Morrone

16.1 Introduction

Chancroid, donovanosis, and endemic treponematoses such as yaws, bejel, and pinta are also known as tropical diseases because their causative agents can be found in warm climates, which favor their inter-human circulation. Until a few decades ago, these infections were considered rare diseases, particularly in Western countries. However, in recent years, they have spread from tropical areas of the world to more temperate zones, mainly due to the increased mobility of populations, the globalization of the behaviors associated with their transmission, and, not least, to an enhanced adaptability of their pathogens at higher latitudes.

In recent decades, the propensity to consider these diseases as rare has contributed to maintaining their status as neglected infections and to underestimating the importance of gaining more knowledge, particularly by clinicians from wealthy countries.

Still today, these infections afflict large populations around the world and several studies have demonstrated their biological synergisms with HIV disease and shown their role in increasing the transmission of this virus, particularly in heterosexual populations.

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Chancroid and donovanosis are sexually transmitted bacterial infections while the group of endemic treponematoses includes non-veneral infections of historical interest for sexually transmitted infections (STI) experts because they can share similar clinical pictures with classical STI and their causative agents are genetically similar to venereal treponemas (i.e., *T. pallidum*).

All these reasons more than justify dedicating an entire chapter to this group of infections to better exploit their epidemiological, clinical, and treatment features, particularly if one considers the difficulty in clinically distinguishing between these infections and other better documented STI.

16.2 Chancroid

Chancroid is a STI caused by *Haemophilus ducreyi* and results in painful, superficial ulcers, often with regional lymphadenopathy. This infection is also known as soft chancre, ulcus molle, Ducrey's disease, chancre blando, chancrelle, weicher schanker, and it is characterized by one or more genital ulcers, which are soft and painful, and regional lymphadenitis, which may develop also into buboes. The infection may easily be misidentified due to its rare occurrence in Western countries and difficulties in detecting the causative pathogen. Chancroid occurs in Asia, Africa, and the Caribbean, and can be important cofactor

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of HIV transmission. *H. ducreyi* is difficult to culture. Nucleic acid amplification tests can demonstrate the bacterium in suspected cases. Antibiotics are usually effective in curing chancroid.

16.2.1 Historical Aspects

Chancroid has been known to humans since time of ancient Greeks [1]. Some of important events on historical timeline of this STI are: in 1852 was first described by Leon Bassereau as ulcus molle differentiating it from the hard chancre of primary syphilis; in 1889 Augusto Ducrey, an Italian bacteriologist, identified the etiological agent by a series of autoinoculations, in vivo, and established *H. ducrey* as the etiological agent of chancroid; in 1900 Benzacon and colleagues isolated the bacterium [1].

16.2.2 Epidemiology

The number of cases of chancroid is decreasing overall with rare exceptions such as Malawi with 15% of Genital Ulcer Diseases (GUD) [2] and North India with 24% of GUD [3]. The substantial decrease in prevalence has followed the introduction of syndromic management for treating GUD by the WHO, and major social changes after 2000 [4]. Nevertheless, the global epidemiology of *H. ducreyi* is poorly documented due to difficulties in confirming a microbiological diagnosis [5, 6]. *H. ducreyi* has been demonstrated in asymptomatic individuals [7]. Male circumcision is associated with reduced risk of contracting chancroid [8].

16.2.3 Clinical Presentation

Chancroid is caused by the small gram-negative bacterium, characterized by ulceration and a painful inflammatory edema. The disease is accompanied by regional suppurative adenopathy.

The incubation period for chancroid is short. Three to seven days after sexual intercourse with an infected person, tender erythematous papules

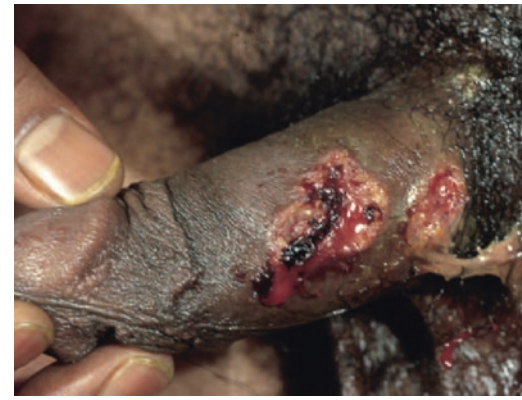

Fig. 16.1 Multiple, irregular necrotic ulcer with ragged margins over the penis shaft

can develop, most often on the prepuce and frenulum in men and on the vulva, cervix, and perianal area in women [9]. The lesion at the site of infection is, initially, an erythematous macula that rapidly evolves to pustule. Its rupture leads to the formation of a shallow ulcer, on an erythematous base. Multiple ulcerative lesions can be formed through autoinoculation (Fig. 16.1). The genital ulcer from chancroid is painful and tender. Symptoms usually occur 4–10 days after exposure. Autoinoculation from primary lesions on opposing skin may result in the so-called kissing ulcers. A frequent complication is represented by suppurative inguinal lymphadenitis, usually unilateral and painful, called bubo, which appears 1–2 weeks following the initial lesion. The disease becomes chronic if not treated, and healing leaves a scar at the site of the ulcer or at the suppurative inguinal adenitis sites. Other symptoms of chancroid include painful urination, vaginal discharge, rectal bleeding, pain with bowel movements, and dyspareunia.

According to the Center for Disease and Prevention (CDC), the diagnostic criteria for chancroid are [10]:

1. the patient has one or more painful genital ulcers;
2. the clinical presentation, appearance of genital ulcers, and, if present, regional lymphadenopathy are typical for chancroid;
3. the patient has no evidence of Treponema pallidum infection by dark field examination or

nucleic acid amplification test (NAAT) of ulcer exudate or by a serologic test for syphilis performed at least 7 days after onset of ulcers;
4. a NAAT for HSV or HSV culture performed on the ulcer exudate is negative.

Extra-anogenital skin ulcers due to *H. ducreyi* (or cutaneous chancroid) have been reported in children and adults [11, 12] and may represent a particular diagnostic challenge, as clinical suspicion may be low and the infection is not sexually transmitted. No adverse effects of chancroid on pregnancy outcome or on the fetus have been reported.

16.2.4 Diagnosis

Chancroid is distinguished by the characteristic rapid onset of shallow ulcers followed by suppurative inguinal adenitis. Pain is a diagnostic sign. A definitive diagnosis of chancroid requires the identification of *H. ducreyi* on special culture media. However, culture media for chancroid are not widely available. NAAT can be performed in clinical laboratories that have developed their own tests.

16.2.5 Differential Diagnosis

Chancroid has to be differentiated from numerous dermatoses: syphilis, pyoderma, herpes progenitalis, venereal lymphogranuloma, and cutaneous amoebiasis.

16.2.6 Culture

H. ducreyi is a very fastidious bacterium, and selective, enriched culture media are required for its isolation. Several different media have been used to isolate *H. ducreyi* from clinical specimens [13, 14]. As strains differ in their ability to grow on different media, a combination of at least two different media may be used for optimal recovery rates. Samples should be taken with a cotton-tipped swab from the base at the undermined edge of a lesion after cleansing by flushing with sterile saline. *H. ducreyi* will only survive a few hours on the swab, and bedside inoculation of culture plates followed by immediate incubation can be done to reduce loss of viable bacteria during transportation. A definitive diagnosis of chancroid requires the identification of *H. ducreyi* on culture media; however, the advent of more sensitive DNA amplification techniques has demonstrated that the sensitivity of culture of *H. ducreyi* reaches only 75% at best [15–17].

16.2.7 Nucleic Acid Amplification Techniques (NAATs)

NAATs are excellent for demonstrating *H. ducreyi* in clinical sample material. Specimens taken for culture may also be used for NAATs. The exudate from the ulcer should be collected by vigorous rubbing of the base of the lesion with a sterile cotton-tipped swab. Various different in-house PCR methods have been described, some of which have the advantage of simultaneously testing for other relevant pathogens, in particular *T. pallidum* and *HSV* [18–23].

16.2.8 Treatment

The World Health Organization (WHO) has proposed syndromic approaches for treatment of genital ulcers, to be used in settings where appropriate laboratory diagnosis is not available [24]. The antibiotics treatment should be based on local epidemiology and antibiotic susceptibility patterns. Several antibiotic regimens have been recommended for confirmed cases of chancroid:

- *First line*—Ceftriaxone as a single intramuscular injection of 250 mg or azithromycin, as a single 1 g oral dose.
  The response is generally good although failures, especially in HIV-positive individuals, have been reported.
- *Second line*—Ciprofloxacin 500 mg orally twice a day for 3 days, or erythromycin orally 500 mg four times a day for 7 days.
  Azithromycin and ceftriaxone offer the advantage of single-dose therapy.

Children can be treated with ceftriaxone. Ciprofloxacin is contraindicated for pregnant and lactating women as well as for children and adolescents less than 18 years where erythromycin or ceftriaxone regimens should be used. The multiple day regimens are recommended for HIV-positive patients rather than the single-dose treatments [25]. Rapid action sulphadiazine 1 g four times daily for 7–14 days, in any case until complete recovery. In the case of secondary infections with fuso-spirochetal microorganisms, penicillin or other antibiotics are indicated. Patients allergic to sulfa-drugs can be treated with tetracyclines, oxytetracyclines, and erythromycin, 500 mg four times daily until complete recovery. Repeated serological syphilis tests are indispensable.

16.3 Donovanosis

Donovanosis or "granuloma inguinale" is a bacterial infection of the genital region. It is chronic and progressive. Genital ulcers attributed to donovanosis were first described in India by McLeod, and the causative agent was identified by Donovan, who also described Donovan bodies [26].

Donovanosis is one of the causes of genital ulcer disease (GUD) with a low infection rate, characterized by beefy-red vascular lesions, which are usually painless and bleed readily to the touch. It is generally thought to be sexually transmitted, and lesions usually involve the external genitalia, although, interestingly the causative organism, *Calymmatobacterium granulomatis*, a gram-negative encapsulated bacterium, has been isolated from feces causing loco-regional lymphadenitis [27–29].

A proposal that the organism be reclassified as *Klebsiella granulomatis comb nov*, based on a phylogenetic similarity of 99% with *K. pneumoniae* and *K. rhinoscleromatis* has been put forward [30]. However, another study found lesser phylogenetic similarities of 95% and 94%, respectively, with the genera *Klebsiella* and *Enterobacter* and concluded *C. granulomatis* was a unique species [31]. In fact, the sexual transmission of this intracellular bacterium has been in question since 1962, despite other alternative ways of transmission being suggested at the time.

The *C. granulomatis* is a gram-negative coccobacillus. It is intracellular and encapsulated. It has been demonstrated to function as a facultative aerobe.

The latest European guidelines state that the incubation period should be about 50 days [26]. The region of the body most frequently affected is the area around the genitals, and in 80% of the cases the cutaneous lesions are present only in that area of the body, even though rare cases of disseminated donovanosis have been described [32–37]. The first successful culture of the *C. granulomatis* was in the mid-1990s by using peripheral blood monocytes and human epithelial cell lines [38].

16.3.1 Epidemiology

The transmission of donovanosis has been debated due to its apparent association with sexual contact despite reports of infection without sexual contact history. As of 1947, it has generally been accepted as a STI based on a literature review done at the time. There is usually a history of sexual exposure before the lesion develops. There are also high rates of infection among age groups with increased sexual activity. Donovanosis has been diagnosed in women with lesions primarily located on the cervix, and men who have sex with men have a higher incidence of anal lesions [26]. A piece of evidence that tends to point away from donovanosis as a sexually transmitted infection is the low rates of sex workers affected. Also, there have been some cases of fecal transmission and children infected in non-sexual ways. There are case reports of children infected by sitting on adult's laps and neonates infected during vaginal delivery. Some other risk factors are poor hygiene and low socioeconomic status. Also, the rate of transmission is thought to be low in general [38].

Donovanosis presents a curious geographical distribution. Most cases today are found in Papua

New Guinea, India, South Africa, Brazil, and Australia, and it seems to be endemic in some tropical and developing areas such as India, Papua New Guinea, Zimbabwe, parts of the Republic of South Africa, China, India, Brazil, and among the Aboriginal population of Australia, although the latter has been virtually eliminated [39–43]. The largest epidemic recorded was in Papua New Guinea, where 10,000 cases were identified from a population of less than 15,000 in Dutch South New Guinea between 1922 and 1952. Two significant foci were identified in the 1990s in the Durban/Natal/Kwazulu region of South Africa and in Texas, although it is not clear whether it was a new epidemic or a consequence of greatly underestimating the epidemic [32, 44]. In 1991, 1300 cases were clinically diagnosed at the Durban City Health STI Clinic and in the same geographical area. Further increases were reported in 1996 and 1997, with 2733 and 3153 cases, respectively [45]. After Rajam and Rangiah's historical work in the early 1950s, only a few large studies since the discovery of donovanosis have been undertaken in India, where the condition continues to be endemic both in the south and western parts of the country [45, 46]. There are limited published reports on donovanosis trends in developed countries, where the quality of surveillance data could undoubtedly be improved, particularly in this era of rapid communication and transport technologies.

16.3.2 Pathophysiology and Clinical Presentation

Donovanosis is a sexually transmitted infection that can rarely have non-sexual modes of transmission. It is classically associated with genital ulcers that demonstrate Donovan bodies on tissue smear samples [41].

Rajam et al. [47] had already described the four types of lesions caused by donovanosis in 1954 and recently O'Farrell et al. confirmed this classification with the latest guidelines:

1. Ulcerogranulomatous is the most common variant; non-tender, fleshy, exuberant, single or multiple, beefy-red ulcers that bleed readily when touched.
2. Hypertrophic or Verrucous type, an ulcer or growth with a raised irregular edge, sometimes with a walnut appearance.
3. Necrotic, usually a deep foul-smelling ulcer causing tissue destruction.
4. Sclerotic or cicatricial with fibrous/scar tissue.

The genital area most often affected in the majority of cases include: the distal region of the penis in men and the labia minora, fourchette, and perianum in women (Figs. 16.2, 16.3, and 16.4). Ulcers may occur on the cervix in women as well as the anus in those who practice anal intercourse [48]. In rare cases also the scrotum in man can be interested by lesions (Fig. 16.5). It is rare to find extragenital lesions. Cases of disseminated donovanosis have been described, with frequent hepatic and bone involvement. Lymph gland enlargement is uncommon. The ulcers tend to grow quickly during pregnancy and cases with atypical presentation are described in children [28]. The donovanosis ulcers bleed easily, consequently increasing the risk of HIV infection. All patients should undergo HIV testing.

The differential diagnosis with squamous cell carcinoma is important, in particular in the genital area where there are similar lesions to donovanosis. In case of doubt, a biopsy is necessary [49].

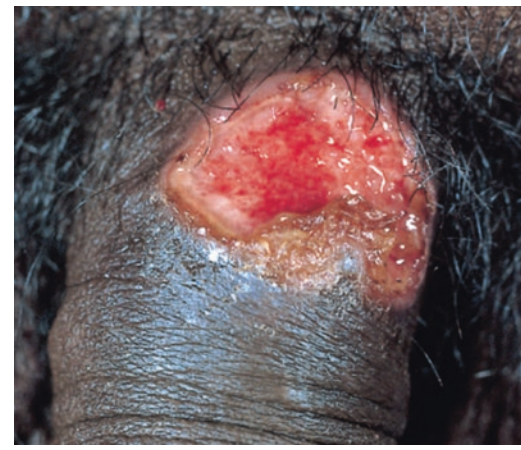

Fig. 16.2 Typical ulcerogranulomatous donovanosis ulcer on the basis of penis shaft

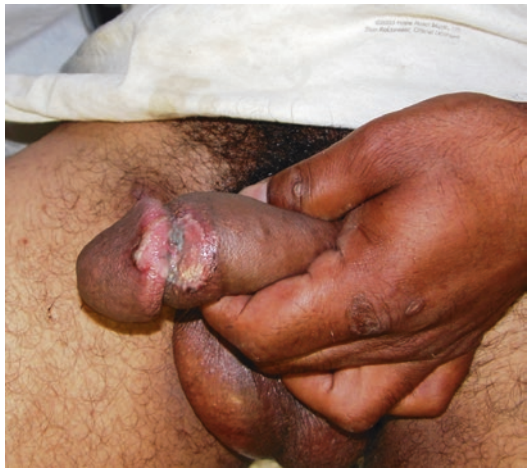

Fig. 16.3 Subpreputial ulcerogranulomatous lesion in patient with donovanosis (Courtesy Dr. Giuliani)

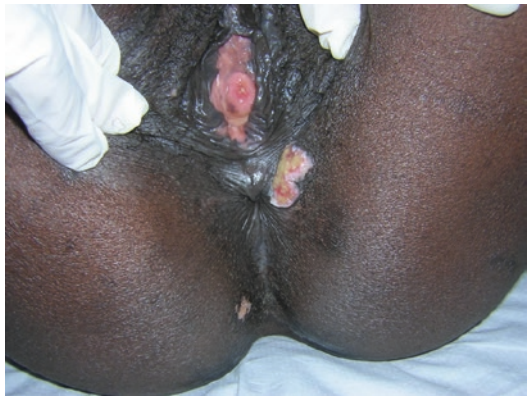

Fig. 16.4 Confluent donovanosis lesions in perianum area (Courtesy Dr. Latini)

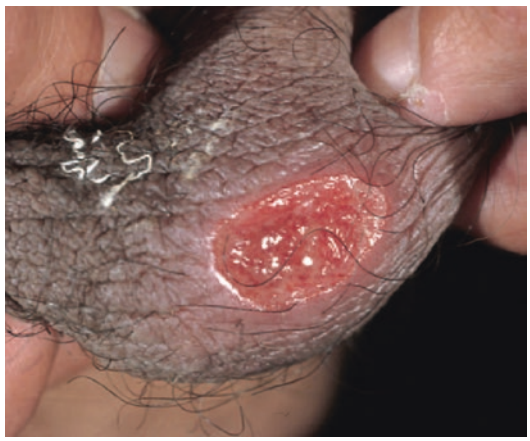

Fig. 16.5 Isolate donovanosis lesion on the scrotum

16.3.3 Histopathology

Histological evaluation is essential for the diagnosis of donovanosis. Donovan bodies are seen within large, mononuclear (Pund) cells as gram-negative intracytoplasmic cysts filled with deeply staining bodies. When these cyst structures rupture, they release infectious organisms. To obtain a sample for smear, first, clean the lesion with a dry cotton swab. Using saline before obtaining the swab may lead to an insufficient sample. The donovanosis swab should always be obtained before other testing to maximize tissue collections. An alternative method of tissue sampling is with either forceps or scalpel to obtain granulation tissue. The granulation tissue is then crushed between two slides to obtain a smear [41].

16.3.4 Diagnosis

Diagnosis can be made by an experienced physician in endemic areas but may be difficult in other areas of the world. In nonendemic areas, the diagnosis will require a high index of suspicion. The diagnosis is confirmed by identifying Donovan bodies in a tissue smear. PCR testing is possible but is not widely available. It is seen in the research setting and often used during eradication programs. There are serologic tests that can be used for population studies, but they are not accurate enough to diagnose an individual [38, 41].

16.3.5 Differential Diagnosis

The differential diagnosis for genital ulcers is broad and includes primary syphilis, secondary syphilis (condylomata lata), chancroid, lymphogranuloma venereum, genital herpes, neoplasm, amoebiasis, and several others. The possibility of a co-infection should always be considered since the risk factors for several of these diseases are similar. Lesions that have a more destructive appearance should be evaluated for carcinoma in addition to other causes. Also, pseudo-elephantiasis, which is a possible

complication of donovanosis, can mimic lymphogranuloma venereum. Additionally, women with cervical lesions should also undergo testing for carcinoma and tuberculosis. If the diagnosis of donovanosis is confirmed, the patient should undergo HIV testing due to being at increased risk of transmission with donovanosis lesions. Any patient found to have a sexually transmitted disease should be considered for HIV testing [41].

16.3.6 Treatment

Treatment should last until all lesions are healed. First-line treatment is azithromycin 1 g followed by 500 mg daily. Relapses can occur 6–18 months after seemingly successful treatment. Some alternative treatment regimens are doxycycline 100 mg twice per day, ciprofloxacin 750 mg twice per day, erythromycin 500 mg four times per day, and sulfamethoxazole/trimethoprim twice per day. Patients that are slow to respond can also be given gentamicin 500 mg every 8 h. Erythromycin is the medication of choice in pregnancy. There is no change in the recommendations for HIV-positive patients. The 2016 European Guidelines for donovanosis treatment state that antibiotics should continue for a minimum of 3 weeks and until symptom resolution. They also recommend azithromycin as a first-line treatment that can be given as 1 g initially then 500 mg daily or 1 g weekly. Children should be given azithromycin 20 mg/kg for a disease treatment course or prophylaxis for 3 days if exposed during birth. The first study to demonstrate the effectiveness of azithromycin was performed by Bowden et al. between June 1994 and March 1995 in Australia. Azithromycin was shown to be effective against donovanosis and has the added benefit of short, intermittent dosing, which may facilitate treating endemic populations. Medication alone may be the only treatment required. Surgery may be needed for extensive tissue destruction. Patients require consistent monitoring for disease resolution and possible recurrence [26].

16.3.7 Prognosis

The prognosis for uncomplicated donovanosis is positive with appropriate treatment. There is the possibility of relapse, which can occur even after symptoms appear to have resolved. Lack of improvement should prompt further investigation and testing for co-infection or alternative diagnoses. If left untreated, there can be significant scarring and tissue destruction. Malignant transformation is also possible [41].

16.3.8 Complications

Possible complications include neoplastic change, pseudo-elephantiasis, hematogenous spread, polyarthritis, osteomyelitis, vaginal bleeding, and stenosis of the urethra, vaginal, or anus. Dissemination into the abdominal cavity is a rare possible complication. Symptoms include fever, malaise, anemia, night sweats, weight loss, and sepsis. Also, many patients receiving care after having endured the disease process for a significant amount of time may have suffered emotionally. The lesions can be embarrassing and distressing to the patient. Always consider mental health disorders such as anxiety or depression and include suicide screening in these patients [41].

16.4 Nonvenereal Treponematoses

Nonvenereal treponematoses or endemic treponematosis has many loco-regional names, but is most commonly known in the West as yaws, bejel, and pinta. These related but distinct diseases have in common some characteristic aspects: they are all caused by spirochetes and can all be observed in rural regions of countries with very hot climates.

They have prominent cutaneous manifestations and relapsing courses, such as venereal syphilis.

The transmission of these diseases is nonvenereal and by direct contact. Children and

adolescents are more frequently affected. Lesions occur in extragenital localizations. They are chronic diseases and evolve in successive clinical stages. There are no congenital manifestations.

Treponematosis is seroresistant in advanced stages. Concerning geographic distribution, there are important differences: yaws is present in all the hot-humid tropical countries; pinta is endemic in the hot-humid areas between Mexico and Amazonia; endemic syphilis is usually present in small areas with arid climate in Africa and Asia, while it has been completely eradicated from Europe and Australia. *Treponema pallidum subsp pallidum* is responsible for venereal syphilis, whereas *T. pallidum subsp pertenue* causes yaws, *T. pallidum subspecies endemecium* causes bejel (endemic syphilis), and *T. pallidum subsp* carateum is responsible for pinta. Nonvenereal treponematoses are spread through lack of clothing, poor hygiene, crowded conditions, and poor access to health care. Children also serve as the primary reservoirs for these organisms, spreading infection via skin-to-skin and skin-to-mucous membrane contact, and possibly via fomites as well.

Changing patterns and prevalences of infection have made clinical diagnosis of endemic treponematoses difficult. Health care workers not familiar with the diseases may under or over report them [50, 51]. The serologic tests for these diseases are the same as those for venereal syphilis. The rapid plasma reagin (R.P.R.) and the venereal disease research laboratory tests use a nonspecific cardiolipin antigen that is cross-reactive among all the various treponemes. The fluorescent treponemal antibody absorption test, *T. pallidum* immobilization test, and *T. pallidum* hemagglutination assay are specific for treponemal antigens, but can remain positive for life even in an adequately treated patient [52, 53]. Although dark field microscopy and radiology can aid in diagnosis, economics and logistics make serologic tests far more practical for diseases occurring in remote locales [54]. Before the antibiotic era, endemic treponematoses were treated with arsenicals, bismuth, heat, and topical applications of rust and citrus juices [55]. Since its introduction in clinical practice, penicillin had been the mainstay of treatment, and until recently, the organisms had shown no signs of developing resistance.

16.4.1 Yaws

16.4.1.1 Epidemiology

The most prevalent nonvenereal treponematosis is Yaws, caused by *Treponema pallidum sub pertenue*, and also known as pian in French, framboesia (raspberry) in German, bouba in Spanish, and paru in Malay. It occurs in tropical regions with heavy rainfall and annual temperatures at or above 27 °C (80 °F), and most commonly affects children younger than 15 years. It spreads to extragenital skin via contact with open lesions, excoriations, or bites [56].

In the 1950s and 1960s, the World Health Organization (WHO) and the United Nations' Children's Fund (UNICEF) launched massive treatment campaigns against the estimated 50–150 million cases of active yaws in Africa, Asia, Central and South America, and the Pacific Islands [57, 58].

The initial apparent success of these campaigns led to a false perception that nonvenereal treponematoses were under control and nearly eradicated, resulting in a subsequent lack of vigilance that permitted a multifocal resurgence to precampaign rates of infection.

16.4.1.2 Histopathology

The primary form shows acanthosis and papillomatosis. The epidermis is edematous with neutrophil exocytosis. The derma presents a plasma-cellular infiltrate of leukocytes, lymphocytes, histiocytes, and fibroblasts. The secondary form has substantially equal alterations. The third form is characterized by ulcer lesions similar to the tertiary syphilis. The early bone lesions show an inflammatory reaction. The articular nodules have a central necrotic zone, an intermediate tissue granulation zone, and a peripheral fibrotic layer.

16.4.1.3 Clinical Presentation

Primary Stage

Primary yaws begins 2–4 weeks after inoculation. The "mother yaw," known in local dialects as buba madre (Spanish), frambesia (German), paru (Malay), or mamanpian (French), is a sentinel lesion marking the site where T pertenue penetrated the skin through a scratch or bite, and for that reason is usually found on the buttocks or lower extremities [59, 60]. The mother yaw is a pruritic but non-tender ulcer, which ultimately forms a honey-brown crust and radially expands to 2–5 cm in diameter, occasionally fusing with smaller satellite lesions. The crust frequently sloughs off, revealing a soft, red, moist bed reminiscent of a raspberry, its exudate teeming with infectious treponemes. The mother yaw lasts anywhere from several weeks to months, and may persist into the second stage of the disease. On rare occasions, no primary lesion is identified [61]. The primary lesion may undergo condylomatous changes and typically regresses into a depigmented, pitted scar with dark margins [62]. Regional lymphadenopathy, fever, and arthralgias may be present.

Secondary Stage

Several weeks to months after the mother yaw appears, secondary yaws begins. The secondary stage is far more pervasive and associated with considerably more morbidity.

Multiple cutaneous lesions behave like smaller versions of the primary and are therefore called "daughter yaw," pianomas, or framboesias. They spread diffusely, ulcerate, secrete infectious treponemes, and much like venereal syphilis, they favor locations near orifices such as the mouth and nose. Occasional central resolution results in circinate or annular scaly lesions resembling fungal infection and therefore called "tinea yaws." Condyloma lata also appear in a seasonally variable distribution that is more diffuse in wet weather and more confined to intertriginous areas in dry. Morbilliform eruptions may also be seen.

Hyperkeratotic plaques form on the palms and soles, fissuring into painful secondary infections responsible for a characteristic "crablike gait." A paronychia called "pianic onychia" results from hyperkeratotic macules and papules within the nail folds [63].

Periostitis and osteitis result in marked pain in the hand, forearm, leg, and foot. Early bone changes are usually visible by plain radiography, and periosteal thickening is often palpable [64, 65].

Polydactylitis can cause a radishlike swelling of the proximal two phalanges.

Arthralgias, generalized lymphadenopathy, headaches, and malaise are common, as are asymptomatic cerebrospinal fluid changes. All symptoms are generally reversible, subsiding in weeks to months. Recurrences are possible for up to 5 years, followed by either eradication of the organisms or an indeterminate latent period [60].

Tertiary Stage

Five to ten years after inoculation, approximately 10% of infected individuals will develop tertiary yaws, a devastating and deforming process [61].

Diffuse subcutaneous gummatous nodules suppurate and break down into large, serpiginous ulcers with massive necrotic tissue destruction; this is followed by debilitating scarring and contracture. Hyperkeratotic plaques and keratoderma frequently recur on the palms and soles [66]. Leukodermic manifestations on the body, in particular of the hands, face, and legs can be observed (Figs. 16.6, 16.7, and 16.8).

Destructive osteitis can result in saddle nose deformity, a rhinopharyngitis obliterans known as gangosa, or bowing of the tibia (saber shins) [67].

Bone gummas are common at periarticular sites, and hypertrophic periostitis can lead to a characteristic unilateral or bilateral exostosis of the paranasal maxilla called gondou [67].

Although it is generally believed that nonvenereal treponemes do not affect the central nervous system or viscera, reports of multiple cases of optic atrophy suggest otherwise [68, 69]. Roman and Roman [70], looking at associations between myeloneuropathies and late yaws, have

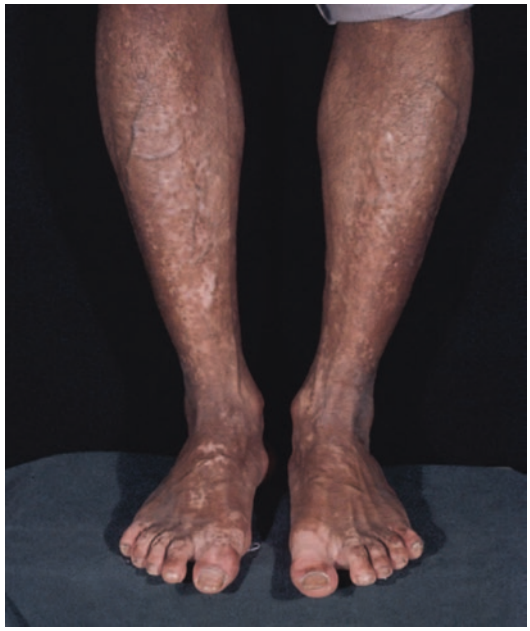

Fig. 16.6 Yaws: leukoderma of tibia and feet due to *T. pertenue* action

Fig. 16.7 Leukodermic manifestations on the face of a patient from Central Africa

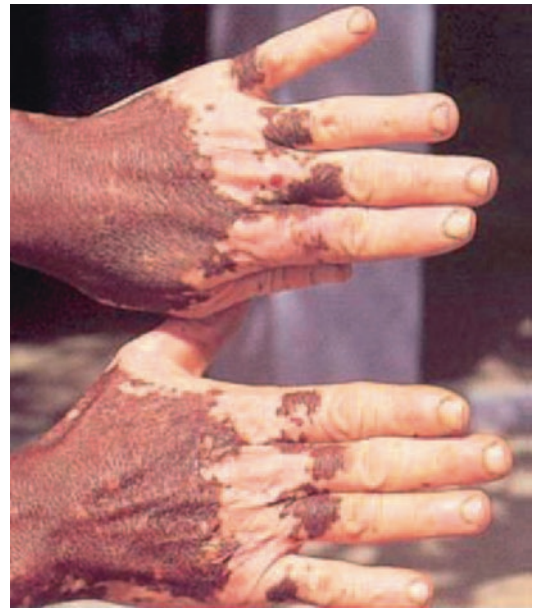

Fig. 16.8 Leukoderma due to a *T. pertenue* in correspondence of the hands

proposed that all potential sequelae of venereal syphilis are possible, including neurologic pathology.

16.4.1.4 Differential Diagnosis

Similar to all the treponematoses, yaws are serologically indistinguishable from venereal syphilis, and only clinical evaluation can differentiate the two [71, 72]. In 1986, a study reviewed the 25 cases of yaws seen at a London hospital over 4 years and found 20 of them questionable [72]. As noted in this article, similar cutaneous manifestations might be caused by eczema, psoriasis, idiopathic keratoderma, infected bites, excoriated chronic scabies, tungiasis, sarcoidosis, verrucae, and vitamin deficiencies. Leprosy, leishmaniasis, ecthyma, and deep mycoses (such as blastomycosis) must also be considered [72, 73].

16.4.1.5 Treatment

Identical to endemic syphilis. People with yaws generally are treated with penicillin G, given in various doses depending on the stage of the disease and the age of the patient. In case of allergy

to penicillin treatment, tetracycline hydrochloride or erythromycin can be used as alternatives, as these have also proven to be effective.

16.4.2 Bejel (Endemic Syphilis)

16.4.2.1 Epidemiology

Bejel is the name for endemic syphilis given by the Arab bedouins of Syria and Iraq, but it is also known as belesh, firjal, loath, or bishel by Saudi bedouins, and as njovera in Zimbabwe, and dichuchwa in Botswana [74, 75]. Bejel, caused by *T. pallidum subsp* endemecium, tends to run in families and preferentially affect children 2–15 years of age. The disease is encountered in dry, arid climates. Although once prevalent in northern Europe, northern Asia, eastern Mediterranean, and southern Africa, bejel is now found predominantly in the Arabian peninsula and the Sahel region [52, 76]. Although isolated geographically, bejel's resurgence is far from negligible [77–79].

16.4.2.2 Histopathology

Primary lesions show epidermis atrophy with perivascular plasma cells and lymphocytes infiltrate. Treponema pallidum is present. In the secondary endemic syphilis, the derma contains dense perivascular plasmocytes infiltrates. Numerous spirochetes are present in the late condyloma. Late endemic syphilis (third stage) is characterized by granulomatous infiltrates of lymphocytes, histiocytes and plasmacytes, fibroblasts, epithelial cells, giant foreign body cells. Nodules of the articulations present the same aspect of the yaws case.

16.4.2.3 Clinical Features

Primary Stage

Primary bejel occurs approximately 2–4 weeks after inoculation. The initial lesions are painless, tiny papules or ulcers that usually go undetected because of their small size and location within the oral and nasopharyngeal mucosa. Transmission is believed to occur through oral contact: kissing, sharing food, or fomites such as cups and kitchen utensils. A study showing treponemes to be present on a drinking flask supports this theory [74, 80]. Primary ulcers have been seen surrounding the nipples of mothers nursing infected children.

Secondary Stage

Clinically recognizable bejel typically occurs 3–6 months post-inoculation.

Shallow, painless mucous patches develop within the oral cavity and nasopharynx, often descending to cause laryngitis. Regional lymphadenopathy is common, as is angular stomatitis [74]. Condyloma lata appear in intertriginous areas such as the armpit and groin. Ten to fifteen percent of those afflicted develop diffuse, nonpruritic, maculopapular, papulosquamous, or annular cutaneous lesions and hypochromatic signs (Figs. 16.9a, b and 16.10).

Osteitis and periosteitis lead to severe bone pain. Symptoms subside in 6–9 months, after which the organism is either eradicated or enters a latent stage that is generally much shorter than that of yaws [63].

Tertiary Stage

Tertiary bejel can occur as early as 6 months or as late as several years after initial symptoms resolve. As in tertiary yaws, gummatous nodules develop within the skin, most eventually regressing to form depigmented, no contracted scars with dark margins.

Mucosal gummata can ulcerate and lead to massive tissue destruction through necrosis, secondary infection, and scarring. Swallowing and articulation difficulties may result, and complete nasal obliteration ("gangosa") may ensue [81].

Arthralgias and bone pain from periosteitis are quite common, but seldom produce the marked changes seen in tertiary yaws, although mild diaphyseal bowing, blastic or lytic changes, and bony gummata may be seen on plain radiography [82, 83].

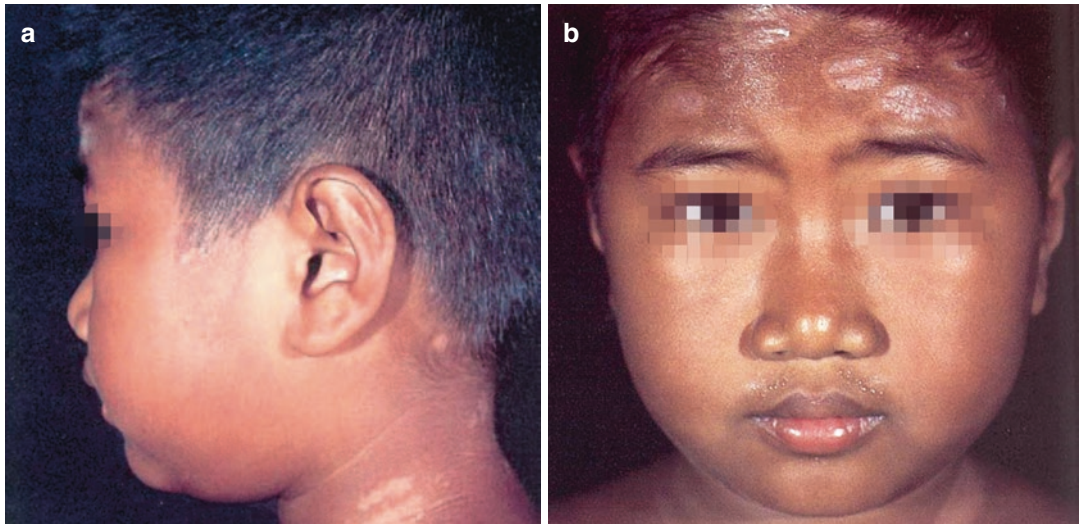

Fig. 16.9 (**a**, **b**) Endemic syphilis: secondary lesions, located on the forehead region and neck (hypochromatic) in a child from Eritrea

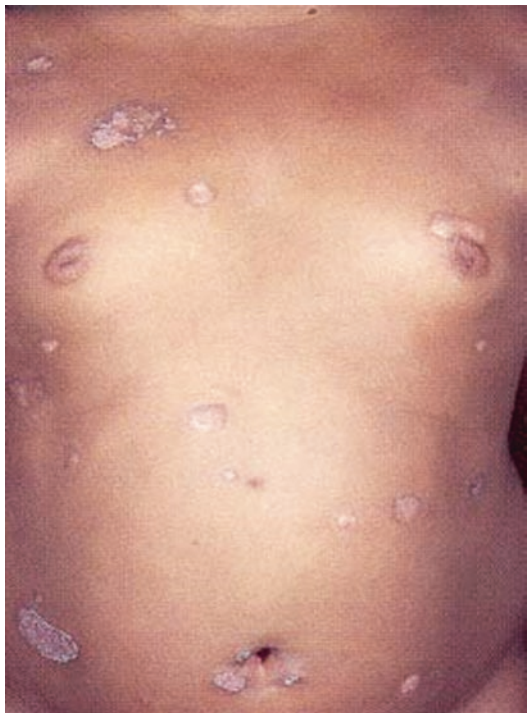

Fig. 16.10 Endemic syphilis: secondary lesions in an Ethiopian child

16.4.2.4 Treatment

The therapy of bejel, such as the other endemic treponematoses, is based on the use of penicillin. In adult 2,400,000 units of penicillin G procaine, or benzathine penicillin; in children under 12 it is recommended the use of half of adult dose. In the general campaign for the eradication of endemic treponematosis the WHO recommends 1,200,000 units for adults and 600,000 for children under 12. In general campaigns it is not possible to perform more than one injection. Penicillin hypersensitive patients can be treated with appropriate doses of tetracyclines and erythromycin.

16.4.2.5 Differential Diagnosis

As with yaws, the main difficulty lies with distinguishing bejel from venereal syphilis. Older studies have shown that in an Arabian cosmopolitan center, venereal disease is actually much more likely, whereas among nomadic Bedouins, the opposite is true [82, 84]. Aside from venereal syphilis, bejel's oral lesions can be confused with aphthosis, monilial perleche, vitamin deficiency, and herpetic stomatitis. Rhinopharyngitis mutilans may also be caused by tuberculosis, leprosy, rhinoscleroma, rhinosporidiosis, and mucocutaneous leishmaniasis (such as espundia) [74].

16.4.3 Pinta

16.4.3.1 Epidemiology

The mildest of the nonvenereal treponematoses, pinta is caused by *T. pallidum subsp* carateum, unique among spirochetes in causing only

cutaneous disease. Also known as mal de pinto, enfermedad azul, carate, and cute, pinta is localized to the western hemisphere and, like its cousins yaws and bejel, largely affects children younger than 15 years [85, 86]. Direct skin-to-skin contact is the most likely mode of transmission, and primary lesions in infants occur often at points of continuous maternal contact [85, 86]. Once prevalent in Mexico, Central America, South America, and the Caribbean, the disease was reported among the Carib and Aztec Indians by the early conquistadors [50, 87]. The disease has sustained a marked decline over the past century, but remains endemic in remote regions of Mexico (in the states of Oaxaca, Guerrero, Michoacan, and Chiapas), and Central and South America (such as the western Amazon region of Brazil) [75, 88–90]. A 1982 survey of a remote Panamanian village found a 20% prevalence of clinical pinta and a 52% seropositivity for treponemes [89]. There is remarkably little recent data on the prevalence of pinta. *T. carateum* has been successfully transmitted to chimpanzees since 1968, but there has been a persistent perception that for pinta, animal reservoirs are improbable [91–93].

16.4.3.2 Histopathology

The early pinta presents a moderate hyperkeratosis, acanthosis, and exocytosis of lymphocytes and neutrophils. In the older lesions we can notice quantitative alterations of the melanin content in the cells of the basal layer. In the late pinta it is possible to observe irregular acanthosis atrophy of the epidermis. In the hyperchromic lesions melanophores may be observed in the epidermis and derma; in the achromic patches it is possible to notice a melanin deficiency in the cells of the basal layer. The epidermis in the early pinta appears rich in treponemata, while they are scarce or absent in the late form. Enlarged lymph nodes show a chronic aspecific inflammation [94–96].

16.4.3.3 Clinical Presentation

Primary Stage

One to eight weeks after inoculation, this treponematosis begins with a small number (one to three) of papules or erythematous macules that expand and coalesce into a scaling plaque surrounded by a red halo [51]. Like primary yaws, this sentinel lesion is most commonly found on the exposed lower extremities and is teeming with treponemes. Unlike primary yaws, this lesion is nonpruritic and does not ulcerate, but rather irregularly expands to greater than 10 cm with occasional central depigmented resolution. Regional lymphadenopathy is common, but serologic tests for treponemes may still be negative during primary pinta [87]. This lesion may resolve with macular dyschromia or may persist into the secondary stage [50, 86].

Secondary Stage

Three to five years post-inoculation, a generalized cutaneous eruption occurs, with scattered sparse or numerous smaller versions of the initial lesion called pintids [87]. These erythematous, scaling papules expand, coalesce into psoriasiform plaques, and are teeming with infectious spirochetes. Pintids change from their initial red color into a variable brown, slate blue, black, or gray hue. Often forming circular arrangements within larger lesions, pintids wax and wane for 2–4 years, leading to extensive depigmentation intermixed with hyperpigmentation [50, 97]. Constitutional symptoms are not seen.

Tertiary Stage

Three to ten years after the pintids have subsided, generalized pigmentary abnormalities develop. These lesions often form in a symmetrical pattern over bony prominences and range from totally achromic (resembling vitiligo) to mottled brown, gray blue, or black (Fig. 16.11). These lesions are generally not considered infectious, although large numbers of treponemes have been observed histopathologically in biopsies of late hypopigmented skin [61, 87]. Periarticular cutaneous atrophy and extensor-surface hyperkeratotic plaques are also common. No attenuated form of pinta has been reported.

16.4.3.4 Treatment

Pinta is treated with benzathine penicillin G (Bicillin), given as a single injection. After penicillin therapy, lesions become non-infectious within 24 h. Primary and secondary lesions usually heal slowly within 6–12 months.

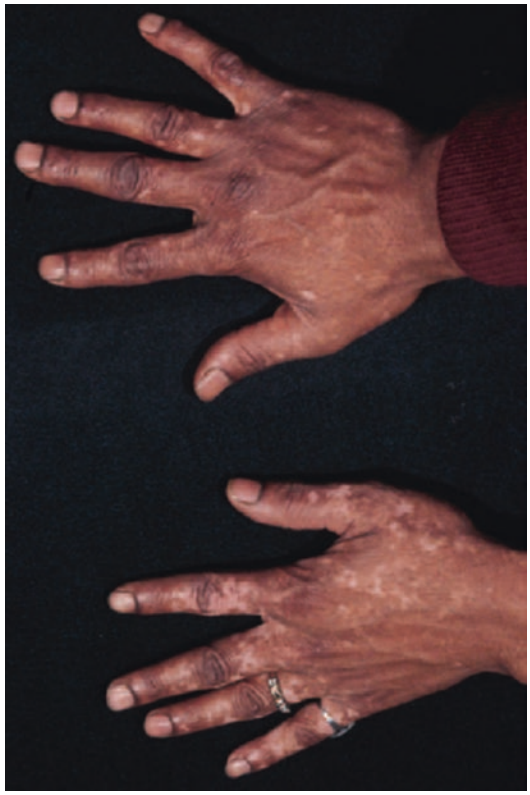

Fig. 16.11 Pinta: dyschromatic late lesions in a patient from Senegal

16.4.3.5 Differential Diagnosis

The lesion of primary and secondary pinta can resemble those of yaws, bejel, or venereal syphilis, as well as eczema, psoriasis, erythema dyschromicum perstans, atrophic lichen planus, lupus erythematosus, vitamin deficiency, tinea corporis, tinea versicolor, or even leprosy. Late pigmentary changes can appear nearly identical to vitiligo.

16.4.3.6 Control Measures

Ideally, an eradicable disease such as smallpox is briefly contagious, obviously symptomatic, and induces lifelong immunity. Nonvenereal treponematoses can be contagious for decades, are often subclinical, and do not induce lifelong immunity. The mass treatment campaigns of the 1950s and 1960s, however, showed the power of organized effort to subdue these diseases. One hundred fifty-two million were screened and 46 million were treated [88]. The endemic disease was eradicated in many regions such as Bosnia [80]. The dramatic success of the campaigns undermined the need to establish integrated, active control measures in local health services to ensure sustainable remission. Reservoirs thus persisted and have expanded in poorly clothed, crowded communities with insufficient hygiene and inadequate health care [77]. Epidemiologic data past the 1980s are scant, as many developing nations have ceased collecting such information to avoid the stigma of appearing backward [52]. The WHO has recently estimated that 2.5 million people worldwide are infected with endemic treponemes, with some 460,000 being actively infectious [98]. Antal et al. recently suggested that the nonvenereal treponematoses serve as telltale bellwethers for an emerging nation's health system. If an easily treatable disease like yaws, bejel, or pinta cannot be properly screened for and controlled, then that country's health system has few hopes of managing tuberculosis, leprosy, malaria, schistosomiasis, or HIV [52].

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17 Lymphogranuloma Venereum as Re-emerged Sexually Transmitted Infection

Martí Vall-Mayans

17.1 Epidemiology

Lymphogranuloma venereum (LGV) is an invasive ulcerative sexually transmitted infection (STI) caused by the biovars L1, L2, or L3 of *Chlamydia trachomatis*. It is considered an endemic disease in some tropical regions where it may be a cause of genital ulcer and inguinal bubo. In contrast to the biovars A-K of *C. trachomatis* which remain confined to the mucosa, L biovars disseminate via underlying connective tissue and spread to regional lymph nodes [1].

With the exception of a few imported cases reported, LGV has not historically been identified in Western countries. However, since 2003—when clinicians identified a cluster of such infections in the Netherlands [2]—LGV has re-emerged in North America, Australia, and Europe among men who have sex with men (MSM) [3] and 15 years later it is becoming endemic in this population. MSM with LGV have presented with severe proctitis, the majority were co-infected with HIV and other STI such as hepatitis C, and reported high-risk sexual behaviors such as substance use, sex parties, anonymous sex, rectal douching, use of sex toys, fisting, and unprotected anal intercourse [3]. In 2015, 23 European countries provided LGV surveillance data. Thirteen of these 23 countries reported a total of 1787 cases, while the remaining ten countries reported zero cases. Compared with 2014, the number of cases reported in 2015 increased by 26%, with increases of 25% or more being reporting in the Czech Republic, France, Portugal, and the United Kingdom. Decreases in reported cases were observed in Denmark, Norway, Ireland, and Italy [4] (Fig. 17.1). Reported cases likely underrepresent the true burden of infection because LGV was not a notifiable disease in many countries and some of them had no access to LGV diagnostics. Also, asymptomatic reservoirs (e.g., urethra) have not been fully characterized and the majority of cases with rectal *C. trachomatis* were not tested for LGV in the absence of symptoms. In Amsterdam, Madrid, or London 11–27% of anorectal LGV cases were found to be asymptomatic [3].

Studies done among MSM showed that LGV in Europe was caused in the majority of cases by the biovar L2b, also known as the Amsterdam biovar, which shows a high degree of clonal relatedness as found by multi-locus sequence typing in contrast to the strains circulating in the USA, which show more molecular diversity [5]. From this it has been speculated that the LGV epidemic among MSM in Europe caused by the L2b variant prevailed already in the USA around the 1980s and may have been imported to Europe.

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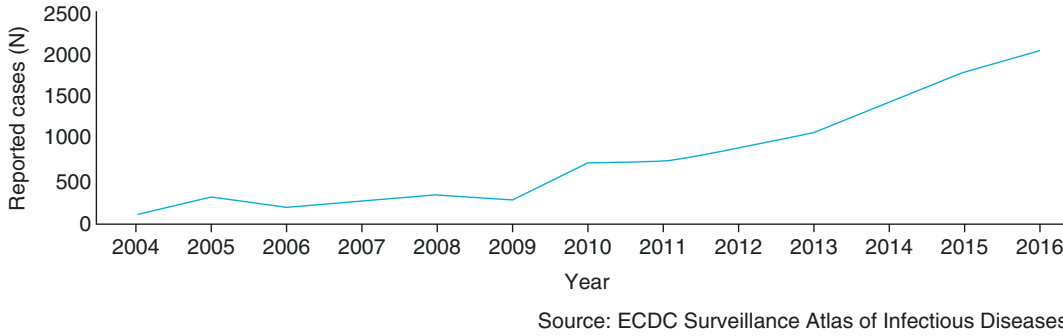

Fig. 17.1 Number of confirmed LGV cases in the five EU/EEA Member States with the largest number of cases in 2015 (2006–2015)

However, cocirculation of variants other than L2b has been described: L2 and a new variant originating from a recombination of L2 and D *C. trachomatis* causing severe proctitis designated L2c [6, 7]. The extent of transmission of these variants within the community needs further investigation and reinforces the need for screening and genotyping of LGV strains. As of 2018 sporadic female cases with LGV have been reported [6, 8], but it is not expected that in the short term LGV will spread to the heterosexual population on a wide scale since the current epidemic is focused on MSM.

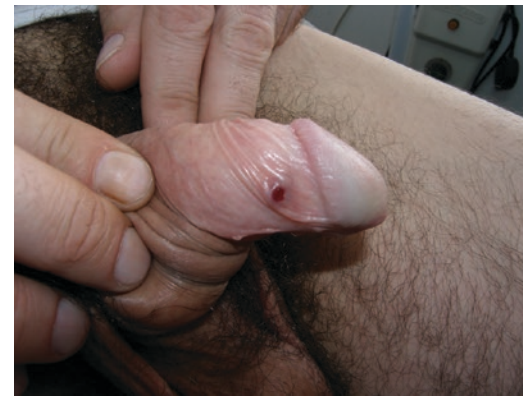

Fig. 17.2 Primary stage penile lymphogranuloma venereum ulcer

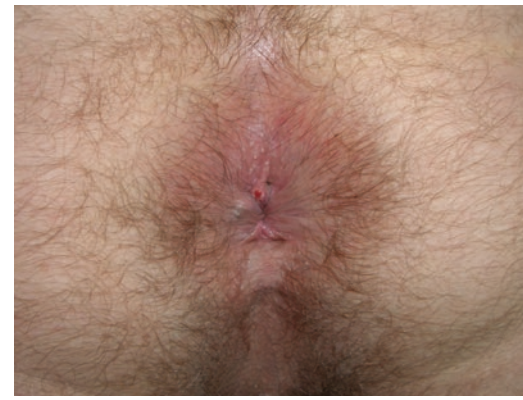

Fig. 17.3 Primary stage perianal lymphogranuloma venereum ulcer

17.2 Clinical Presentation

LGV can cause a variety of clinical syndromes, of which the classical inguinal syndrome (usually after inoculation of the genitalia) and the anorectal syndrome (usually after inoculation via the rectum) are the most common. Following an incubation period of 1–4 weeks LGV symptoms are classically divided into three stages: local infection (primary stage), regional dissemination (secondary stage), and progressive tissue damage (tertiary stage) [1].

17.2.1 Primary Stage

Although often unnoticed by patients, about 3–30 days after inoculation localized inflammation may manifest at the site of exposure. Classically, in the anogenital region this lesion is a transient papule or pustule but more often can be an ulcer (Figs. 17.2 and 17.3). So far in the current rectal epidemic affecting MSM in Western countries genital LGV is rarely described

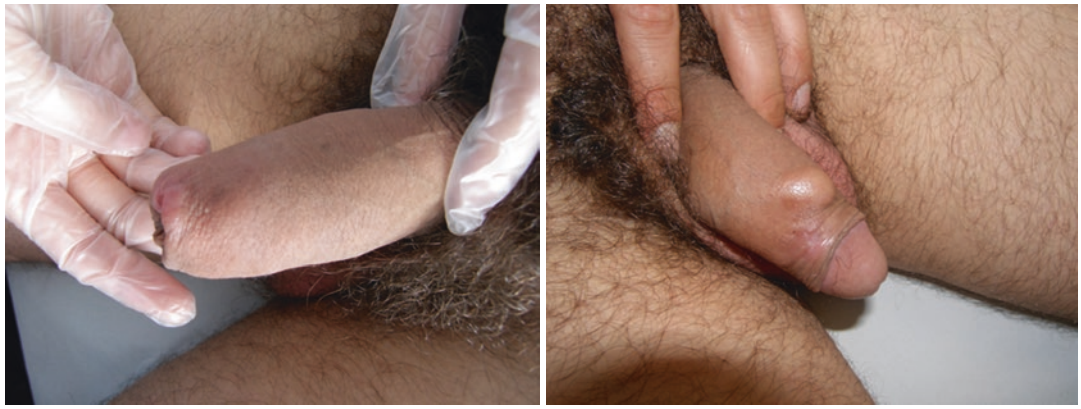

Fig. 17.4 LGV bubonulus before and after antibiotic treatment

and might account for approximately 5% of cases. The differential diagnoses for the primary genital lesions include herpes, syphilis, other bacterial and fungal infections, contact dermatitis, fixed drug eruption, trauma, and Behçet syndrome. Bubonulus is a lymphangial nodule, a rare presentation of primary LGV resulting from lymphangitis of the dorsal penis (Fig. 17.4). Bubonuli may rupture, form draining sinuses, and leave sequalae.

The rarity of genital cases may be related to preferential rectal tropism of the L2b biovar, sexual practices (rectal douching, fisting), or host-specific factors (HIV). However, as the epidemic evolves the clinical presentation may evolve too. In France, in the span of 6 years (2010–2015), 56 extrarectal LGV cases (50 genital and six pharyngeal) were confirmed through genotyping, cases most commonly presenting with inguinal adenopathy alone and adenopathy with genital ulcer [9]. In that study extrarectal LGV seemed to share a common epidemiological background with rectal disease in terms of affected population and genovar distribution. Nevertheless urogenital LGV infections could be much more common than previously believed. Recently it has been reported in The Netherlands a 2.1% urethral LGV prevalence among patients with anorectal LGV and 6.8% urethral LGV prevalence among contacts of patients with anorectal LGV [10]. LGV might infect urethral epithelium only temporarily too short to become symptomatic and detected or clinically suspected. These findings indicate a probable undiagnosed reservoir especially of urethral infections, of which the majority remains asymptomatic. The existence of these reservoirs went undetected because guidelines only recommended LGV screening for rectal samples [11].

The majority of case series and case reports describe clinical manifestations of LGV among MSM, and most of these individuals have been HIV infected. Proctitis and proctocolitis are the most commonly reported clinical manifestations, with findings that resemble, and may be confused with, inflammatory bowel disease [12]. Proctitis due to direct rectal inoculation (this can occur either directly from a men with urethral infection or indirectly from rectal-to-rectal transmission via fomite spread at sex parties or during group sex) result in symptoms of rectal pain, anorectal bleeding, mucoid or hemopurulent rectal discharge, tenesmus, and constipation mainly [13] (Table 17.1) (Fig. 17.5). The anorectal syndrome is not associated with inguinal lymphadenopathy, unless perianal sores are also present, because the locoregional drainage is internal, in the abdominal cavity. Lymphadenopathy can be identified through imaging (e.g., computed tomography or magnetic resonance imaging) of the pelvic region. Besides inflammatory bowel disease the differential diagnoses for proctitis include lymphoma, anorectal carcinoma, and other STIs (e.g., gonorrhea, chlamydia serovars A to K, herpes, syphilis). If left untreated, the anorectal syndrome can lead to permanent anal strictures.

Table 17.1 Anorectal symptoms in men who received a diagnosis of lymphogranuloma venereum, no. and (%) United Kingdom, 2006 [13]

Symptom (s)	Patients Number (%)
Anorectal only	
No. of patients	228
Rectal discharge	179 (79)
Rectal pain	157 (69)
Rectal bleeding	133 (58)
Tenesmus	65 (29)
Constipation	56 (25)
≥3 Local symptoms[a]	116 (51)
Systemic symptoms[b]	68 (30)

[a]Indicates three of the previously mentioned anorectal symptoms
[b]Systemic symptoms include fever, weight loss, and malaise

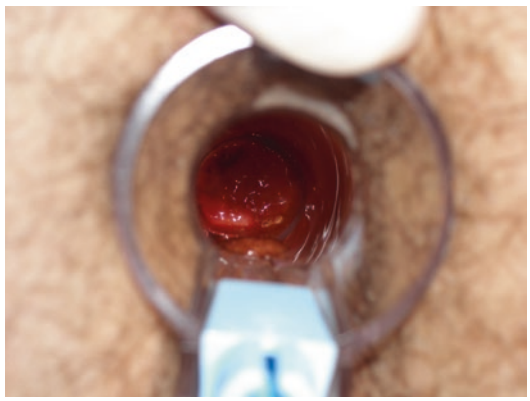

Fig. 17.5 Anorectal LGV with discharge and mucosal inflammation

17.2.2 Secondary Stage

About 2–6 weeks after the primary lesion appears, the infection spreads beyond the mucous membranes into the underlying connective tissue and can be accompanied by constitutional symptoms (e.g., fever, chills, malaise, myalgia, arthralgia). Symptoms will depend on the site of inoculation. With penile, urethral, or vulvar inoculation, the main presentation is an inguinal syndrome. Inguinal LGV infections have been rare in the current epidemic among MSM. In such cases, LGV induces often unilateral, painful, firm, inguinal, or femoral lymphadenopathy known as buboes

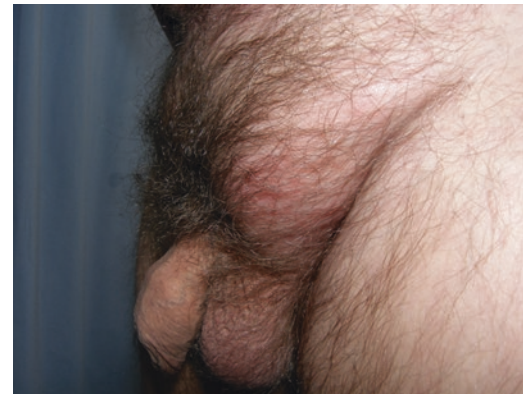

Fig. 17.6 Second-stage inguinal LGV with bubo

(Fig. 17.6). Concurrent inguinal and femoral lymphadenopathy can create the groove sign, which is present in around 15% of cases. These lymph nodes can suppurate, ulcerate, and possibly lead to discharge through cutaneous fistulas. Inguinal LGV may require prolonged courses of antibiotic treatment [14]. Lower abdominal or low-back pain due to involvement of the pelvic and retroperitoneal lymph nodes may be symptoms related with second-stage LGV proctitis. The differential diagnosis for localized inguinal or pelvic lymphadenopathy includes herpes, syphilis, gonorrhea, lower-limb infections, lymphoma, and pelvic malignancy [15]. Systemic spread of LGV occasionally results in sexually acquired reactive arthritis (SARA) [16].

17.2.3 Tertiary Stage

If untreated, LGV can lead to irreversible tissue destruction and scarring. The third stage of the disease is often called the "anogenitorectal syndrome" and is more often present in women. Chronic progressive lymphangitis leads to chronic edema and sclerosing fibrosis, resulting in strictures and fistulas of the involved region, which can ultimately lead to elephantiasis, esthiomene (the chronic ulcerative disease of the external female genitalia) [17], and the frozen pelvic syndrome.

17.3 Diagnosis

Historically, LGV diagnosis was based on clinical presentation coupled with non-standardized serologic findings (elevated microimmunofluorescence or complement fixation titers). With the re-emergence of LGV in Western countries molecular methods were developed focusing on confirming LGV-associated biovars through sequencing of the outer membrane protein A (*ompA*) gene, or through the use of real-time polymerase chain reaction to identify an L2b-specific deletion in the polymorphic membrane protein H (*pmpH*) gene, or by combining *C. trachomatis* detection and genotyping with reverse hybridization assay. Since these methods are rarely available in clinical practice, nowadays most laboratories use nucleic acid amplification tests (NAAT) to detect LGV. The diagnosis of LGV is confirmed by the detection of biovar-specific *C. trachomatis* DNA in (1) ulcer material from primary anogenital lesions, (2) rectal specimens (in suspected cases of anorectal LGV); anorectal swabs are preferably collected from the mucosal lining under proctoscopic vision, alternatively a blind anorectal swab can suffice, or (3) bubo aspirates (in suspected cases of inguinal LGV) [11]. A presumptive LGV diagnosis in a patient with a clinical syndrome suggestive of LGV is supported in the presence of a high antibody titer (especially IgA anti-MOMP antibodies) using Chlamydia genus-specific serological assays.

Guidelines recommend clinicians consider LGV in the differential diagnosis when sexually active patients present with inguinal or femoral lymphadenopathy or buboes or proctitis, particularly when patients are sexually active, HIV-positive MSM. In most circumstances, the diagnosis of LGV is typically based on epidemiological and clinical findings, confirmation of *C. trachomatis* infection by routinely available NAAT (which are positive in both LGV and non-LGV chlamydial infections), and the exclusion of other potential etiologies of proctocolitis, lymphadenopathy, or genital ulcers [12]. In Europe LGV cases are classified according to the 2012 European case definition for LGV (https://ecdc.europa.eu/en/infectious-diseases-public-health/surveillance-and-disease-data/eu-case-definitions). Only confirmed cases are reported (Table 17.2).

Table 17.2 Classification of LGV cases according to the 2012 EU case definition for LGV (https://ecdc.europa.eu/en/infectious-diseases-public-health/surveillance-and-disease-data/eu-case-definitions)

Clinical criteria
At least one of the following five
– Urethritis
– Genital ulcer
– Inguinal lymphadenopathy
– Cervicitis
– Proctitis
Laboratory criteria
At least one of the following two
– Isolation of *Chlamydia trachomatis* from a specimen of the anogenital tract or from the conjunctiva
– Detection of *Chlamydia trachomatis* nucleic acid in a clinical specimen
and
– Identification of serovar (genovar) L1, L2, or L3
Epidemiological criteria
An epidemiological link by human-to-human transmission (sexual contact or vertical transmission)
Case classification
A. Probable case: Any person meeting the clinical criteria and with an epidemiological link
B. Confirmed case: Any person meeting the laboratory criteria

17.4 Treatment

More than half a century of clinical experience supports the use of doxycycline, 100 mg twice daily for 21 days, as the treatment of choice for LGV. This recommendation is based on reported treatment efficacy in numerous case series, coupled with a favorable pharmacokinetic profile, minimal toxicity, and convenient dosing. The alternative treatment option is erythromycin 500 mg four-times daily for 21 days. Disadvantages are gastrointestinal side effects, which occur more often than during

doxycycline therapy [11, 12]. Azithromycin in single- or multiple-dose regimens has also been proposed but evidence is lacking to recommend this drug currently [18]. Recent findings suggest 7 days of doxycycline is effective in achieving cure of rectal LGV in the majority of MSM [19]. There is a case for a randomized controlled trial of LGV treatment including a 7-day regimen of doxycycline.

In addition to antimicrobial therapy, local management of buboes (by aspiration through intact skin, or incision and drainage) may also be considered to prevent the development of ulcerations or fistulous tracts. As LGV is sexually transmitted it is essential that partner notification is initiated when the diagnosis is made. Sexual contacts within the last 3 months should be offered testing for Chlamydia/LGV and empiric treatment with antibiotic therapy commenced until Chlamydia/LGV has been excluded in the partner [11]. Patients diagnosed with LGV should be counseled regarding prevention of other STIs including HIV and hepatitis C.

17.5 Conclusion

The LGV epidemic among MSM in many countries of the Western world is not under control 15 years after its resurgence in 2003. Given this, it is important that clinicians dealing with urogenital and rectal pathology (e.g., STI clinicians, GPs, emergency medicine and gastroenterology and urology specialists) are aware of both the clinical course, the presentation of this infection, and the diagnosis as outlined in current guidelines. However, many clinical and epidemiological questions remain unanswered and the exact modes of transmission are still not elucidated. Unprotected receptive anal intercourse as a key risk factor supports the hypothesis that rectal infection causing proctitis is due to direct inoculation. Although it is not known how this epidemic will develop in the future, current data underline the importance of more intensified and active LGV testing with the support of modern laboratory techniques. This is important in order to prevent complications in the individual patient and also to avoid further expansion in the community. Clearly, the reservoir of this infection has yet to be defined accurately.

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Part V

Modern Challenges in STI Area

Giovanni Rezza

The fifth part is devoted to some of the challenges that have been brought to the attention of STI researchers in recent years. Current challenges in the field of sexually transmitted infections (STI) need to be identified in order to plan future research activities and to establish public health priorities. HPV remains one of the most threatening agents, with its potential carcinogenesis. Identifying pathways through which this oncogenic virus induces the development of cancer in different sites of the genital tract is a key to understanding how infections due to oncogenic viruses may be controlled. This issue is well addressed by Massimo Tommasino from the International Agency Research on Cancer (IARC). Although there have been many studies that have focused on the risk of cervical cancers, the natural history of HPV and its role in anal cancer still needs to be better defined. This is masterfully done in the article of Gabriella Donà and Massimo Giuliani, who are based in a large STI clinic and who have great experience in cohort studies conducted on men who have sex with men (MSM). Last but not least, HPV vaccination strategies, including changes in screening policies for cervical cancer, are also discussed in another chapter of this part by Giovanni Rezza and Cristina Giambi. However, even though HPV represents the most common, and to some extent the most severe STI, other biological agents merit special consideration. First of all, *Neisseria gonorrhea*, with its high potential for antibiotic-resistance. In this regard, sporadic cases and outbreaks of multiresistant isolates have been reported in several areas around the world, as reported by Paola Stefanelli in a comprehensive and updated article. Finally, special attention has been given to immunological issues regarding *Treponema Pallidum* infection. In fact, the historical plague of syphilis is not yet over, and—as discussed by Lorenzo Giacani—a better understanding of immune response to this germ is crucial in order to develop appropriate prevention and control strategies.

18 Biological Pathways of HPV-Induced Carcinogenesis

Maria Gabriella Donà and Massimo Tommasino

> Before I came here I was confused about this subject.
> Having listened to your lecture, I am still confused
> – but at a higher level.
>
> Enrico Fermi

18.1 Introduction

Human papillomaviruses (HPVs) include a very large number of viruses, which have a strict tropism for the stratified squamous epithelium. To date, more than 200 types have been identified that can infect either the skin (cutaneous types) or the mucosal surface of the anogenital and upper aerodigestive tract (mucosal types) [1, 2]. HPVs are classified into more than 10 different genera. The known mucosal HPVs, all belonging to the alpha genus, can be divided into low-risk (LR) and high-risk (HR) types; LR types cause only benign lesions, such as anogenital warts (condylomata), whereas HR types are involved in the development of neoplastic lesions. HPVs are involved in the etiology of 4.5% of all human cancers [3]. HR-HPVs cause almost 100% of cervical cancer cases and about 90% of anal carcinomas [4], in addition to a subset of vulvar, vaginal, penile, and head and neck cancers [3]. Between 7% and 88% of oropharyngeal squamous cell carcinomas (SCCs) are attributable to HPV infection, depending on the geographical region, with a higher HPV-driven fraction in Northern Europe and North America, and a lower fraction in India and other countries, where the population is exposed to other oral carcinogens [5]. The other head and neck sites are marginally affected by HPV infection. In fact, a causal role of the HR-HPVs has been established only for a small percentage of oral cavity and laryngeal cancers [6, 7].

Twelve HPV types have been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC), because their role in the development of cervical cancer has been incontrovertibly established, biologically and epidemiologically [8]. These are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. In addition, HPV 66 and 68, which have been classified as probably carcinogenic (HPV 68) or possibly carcinogenic (HPV 66), have been included in the group of HPVs with a significant role in the development of cervical neoplasia.

Apart from the recognized role of mucosal HPVs in the etiology of anogenital and oropha-

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ryngeal cancers, compelling evidence suggests a role for beta HPVs in the development of cutaneous squamous cell carcinoma (cSCC) [9]. A subset of beta HPVs, namely HPV 5, 8, 9, 12, 14, 15, 19–25, 36, 38, and 47, have a causal role in cSCC arising mainly in ultraviolet (UV)-exposed areas of individuals affected by *epidermodysplasia verruciformis*, a rare genetic disorder characterized by an increased susceptibility to HPV infection [8, 10]. In addition, beta HPVs seem to play a role in skin carcinogenesis in immunocompetent subjects.

18.2 Mucosal HPV Carcinogenesis: The Model of Cervical Carcinogenesis

HPV-driven carcinogenesis in cervical cancer has been investigated in depth. Historically, the first association of HPV infection with a human cancer was established for cervical cancer [11]. The pivotal role of E6 and E7 viral proteins in the carcinogenic process driven by infection with HR-HPVs has been widely and incontrovertibly demonstrated (see below). Indeed, their expression is always retained in HPV-positive cancer cells.

In cervical infection, HPV targets cells of the epithelium at the transformation zone, i.e., the area of the uterine cervix between the squamous stratified epithelium of the ectocervix and the columnar epithelium of the endocervix. Importantly, specific cells at the squamocolumnar junction (junctional cells), which appear to have an embryonic origin and a unique gene expression profile, appear to be the preferential target of HPV infection, and could be responsible for HPV-associated pre-cancer and cancer of the uterine cervix [12].

Well-recognized pre-neoplastic lesions precede the invasive cancer (Fig. 18.1), which is called cervical intraepithelial neoplasia (CIN) and is classified into low-grade (CIN1) and high-grade (CIN2–3) lesions. CIN1 and CIN2 lesions, which may harbor productive HPV infections (i.e., viral particles are produced), may still regress within 1–2 years. CIN3 is considered to be the real cancer precursor lesion, although even CIN3 does not necessarily progress to invasive cancer. The incidence rate of CIN3 lesions is higher than that of cervical cancer, suggesting that CIN3 does not always lead to cancer, and whether this happens may depend on the HPV genotype causing the lesion.

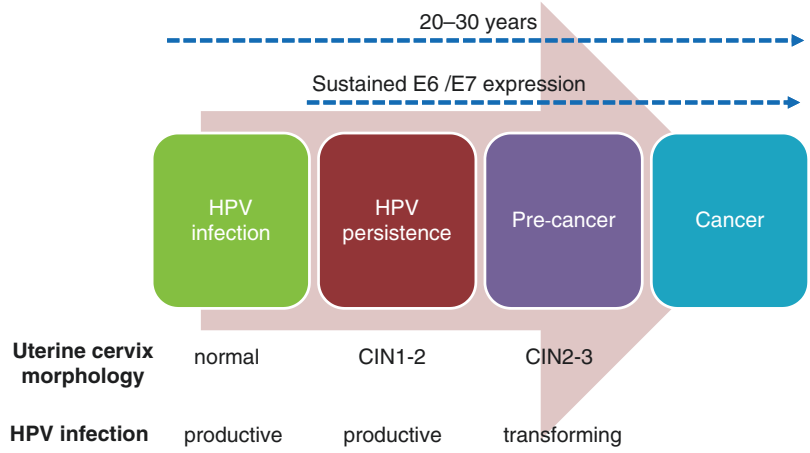

Fig. 18.1 Schematic representation of HPV-associated cervical carcinogenesis. Cancer development starting from HPV infection implies establishment of a persistent infection and thus prolonged expression of the E6 and E7 viral oncoproteins. In the presence of a persistent infection, morphological alterations of the epithelium of the uterine cervix may develop, which may be classified as cervical intraepithelial neoplasia (CIN) of grade 1, 2, or 3. In low-grade lesions (CIN1), the infection is productive, i.e., viral particles are produced and released. In transforming infections, virions are no longer produced, and, in the long term, high-grade lesions (CIN2–3) and ultimately cancer may develop

The pathway that leads from infection to cancer has a long duration and includes several key steps. The first step is the establishment of a persistent infection [13]. Genital HPV infections are mostly transient, because they are effectively cleared by the host immune system in 6–18 months. These transient infections are clinically irrelevant; they do not lead to any clinical manifestations. In contrast, development of pre-cancer and cancer is tightly linked to persistent infections. Whether cancer develops depends on host characteristics, such as genetic factors (e.g., HLA haplotype) and the status of the immune system. Individuals with a defective immune system, such as HIV-positive subjects, have a higher risk of persistent infections and HPV-associated neoplastic lesions. Viral factors also play a relevant role. The HPV genotype may confer a different risk of persistence and lesion progression. HPV16 is associated with a higher risk of persistent infection compared with the other HR-HPV types. Accordingly, it is the genotype with the highest oncogenic potential, and has a broader tissue tropism and a stronger disease association. HPV16 is responsible for 50% of all cervical cancer cases worldwide, in addition to being associated with more than 80% of HPV-driven oropharyngeal carcinomas [3]. Also, the lineage and sub-lineage of a specific type (i.e., intratypic variants) appear to influence the oncogenicity of specific HPV types, as observed for HPV16 [14, 15].

A persistent infection is accompanied by a prolonged and sustained expression of the E6 and E7 viral oncoproteins, which deeply alter cell proliferation pathways (see below). Once a persistent infection is established, a transformed phenotype may be acquired, with viral activity leading to genomic instability, somatic mutations, and viral genome integration. After genetic (and epigenetic) changes have occurred in the infected cells to a significant extent, invasion may occur.

Although the model of cervical carcinogenesis is generally accepted as universal, the carcinogenic mechanisms may be different at other anatomical sites. Patterns of viral gene expression and viral protein functions might be site-specific and may vary in different target cells. The natural history of the infection may also be different. However, for other HPV-related cancers, a basic understanding of carcinogenic mechanisms is still lacking.

18.2.1 Evasion of Immune Surveillance

HPV-induced immune suppression may partially account for the establishment of a persistent infection and may thus play a role in HPV-driven oncogenesis. HPV has evolved specific mechanisms to evade host immune defenses [16]. Some of these mechanisms are intrinsically linked to the viral life cycle. The high-level expression of viral proteins and the production of viral particles take place only in the upper layers of the stratified squamous epithelium, which is less accessible to cells of the immune system. This may cause a delayed or inefficient immune response. In addition, HPVs have developed the ability to directly modulate several components of the host immune system, in order to prevent recognition of the viral antigens by the host immune system. Various immune suppressor strategies can be ascribed to E7 oncoprotein, which can interfere with both innate and adaptive immune responses.

HPV16 and, to a lesser extent, HPV18 oncoproteins have been shown to compromise the functionality of the Toll-like receptor 9 (TLR9) pathway, through a significant decrease in the TLR9 messenger RNA (mRNA) level [17, 18]. TLR9 plays a key role in the recognition and elimination of bacterial and viral pathogens. The expression and activity of immuno-modulating molecules, such as interleukin 8 (IL-8) [19] and type I interferons (IFNs), are also profoundly perturbed. HPV16 E7 has been shown to inhibit multiple IFNα-inducible genes [20]. HPV16 E7 also impairs IFNγ-induced transactivation activity of IFN regulatory factor 1 (IRF-1), which is in turn involved in controlling IFNβ gene expression [21, 22].

Several components of the pathway responsible for antigen processing and presentation on the cell surface by major histocompatibility complex (MHC) class I molecules are also affected,

so that the chance that HPV-positive cells are recognized and eliminated by T-cells is significantly decreased [23, 24]. Expression of MHC class I molecules is also directly down-regulated by E7 [25].

HR-HPVs are also capable of influencing adaptive immune responses. HPVs alter the distribution and function of the Langerhans cells, professional antigen-presenting cells of the epidermis, where the viral infection occurs [26]. Cervical pre-cancerous and cancerous lesions are characterized by depletion of these cells. Viral oncoproteins may induce a decreased infiltration of these cells into the HPV-infected epithelium by reducing the expression of pro-inflammatory cytokines and chemotactic factors. By reducing the presence of Langerhans cells in the epidermis, HR-HPVs limit the activation of an effective CD8+ cytotoxic T lymphocyte (CTL) response to viral antigens, hampering the recognition and elimination of infected cells.

Taken together, all these data show that HR-HPVs actively and directly inactivate the host immune system, and this significantly contributes to the ability of HPV to evade immune surveillance and establish a persistent infection.

18.2.2 Integration of the Viral Genome

Historically, integration of the viral genome into the host genome has been considered a key step in cervical carcinogenesis. Upon integration, the expression of several viral genes is lost, whereas E6 and E7 expression is always retained, and is in fact observed in all HPV-positive cancer cells. Lesions induced by LR-HPVs never contain integrated viral DNA. In cervical carcinogenesis HPV integration seems to increase with disease progression. Viral integration is observed in most (about 85%), but not all, cervical SCCs [27]. In addition, the prevalence of an integrated viral genome varies with the genotype present in the cancer. Almost all HPV18-positive cancers harbor integrated viral DNA. Differently, HPV16-associated cervical carcinomas do not always contain an integrated viral genome [28]. These observations suggest that HPV integration in the host genome is not a required event for the acquisition of the malignant phenotype. This concept seems to be confirmed by the fact that HPV-associated oropharyngeal cancers contain integrated viral DNA at a low frequency (9–30% of cases) [29, 30]. In terms of the number of integration sites, a very large range has been reported (up to 599, according to a recent study) [31].

One of the major consequences of viral genome integration is the loss of E2 expression. Upon integration, the E2 open reading frame (ORF) is often disrupted or deleted [32, 33]. Disruption of this regulatory gene results in loss of viral oncogene transcriptional repression, and thus increases the expression of E6 and E7 viral oncoproteins. Their up-regulation confers a growth advantage to cells harboring an integrated viral genome versus cells containing episomal DNA. In addition, increased stability of E6-E7 mRNAs upon integration has been reported [34]. Despite these observations, recent advances in the knowledge of HPV integration, thanks to next-generation sequencing, do not entirely support the view that integration-associated E2 disruption is responsible for oncogene over-expression. In fact, integrated viral DNA does not necessarily correspond to high expression levels of E6 and E7 [35]. Altered expression of viral oncoproteins may also depend on deregulated host gene expression as a consequence of the viral integration.

In the human genome, it appears that HPV integration can take place in any region. However, regions of minimal homology between the viral and host genome and areas of genomic instability seem to be preferred. Integration frequently occurs in proximity to common fragile sites [27, 30, 36]. Integration events may also occur close to cellular oncogenes (e.g., *c-Myc*). Importantly, the expression of host genes close to or within the integration sites may be altered. In some instances, significantly increased levels of host gene expression have been reported [28], whereas in other cases, levels of host gene expression are significantly reduced [31] or completely lost [37].

In summary, HPV-dependent carcinogenesis is a multistep process that relies on several events,

in which an essential step is the over-expression of the viral oncogenes. Whether deregulated expression of E6 and E7 depends on integration of HPV DNA or other mechanisms (e.g., epigenetic modifications of the viral DNA) does not seem to be important, as long as this provides the HPV-positive cells with a selective advantage.

18.2.3 Genomic Instability

Genomic instability plays a key role in HPV-induced carcinogenesis, in particular in the progression from pre-malignant to malignant lesions. Although the carcinogenic process is initiated by E6 and E7, the accumulation of genetic (and epigenetic) changes is essential for the development of invasive cancer. Viral integration may contribute to this instability, although some authors believe that viral integration depends on chromosomal instability and not vice versa [27]. This concept appears to be supported by the observation that certain aberrations, such as aneuploidy, may be observed in pre-malignant lesions that lack viral integration. Disruption of chromosomal stability may ultimately promote integration of viral DNA into the host genome.

Both viral oncoproteins are known to cause chromosomal instability, which is driven by DNA damage, centrosome number abnormalities, and defective chromosome segregation [38]. HPV-positive cancer cells frequently have structural chromosomal changes, such as translocations, deletions, and amplifications [39]. Copy number alterations may affect the expression of oncogenes and tumor suppressor genes, thus contributing to the carcinogenic process. Common alterations in cervical carcinomas include loss of chromosome 3p and 11q and gain of 3q [39]. As also observed for other HPV-induced effects, chromosomal alterations are induced to a varying extent by different HPV genotypes. For instance, the number and type of alterations are different in HPV16-associated cervical lesions compared with those caused by HPV18.

Numerous studies have provided evidence of the ability of E7 to induce abnormal centrosome duplication [40]. HR-HPV-positive lesions typically contain abnormal centrosome numbers [41, 42]. Therefore, chromosomes do not symmetrically segregate during cell division, and aberrant mitotic figures are induced. Abnormal centrosome synthesis appears to depend on deregulation of cyclin-dependent kinase 2 (Cdk2) (see below), as well as on the ability of HPV16 E7 to associate with the gamma-tubulin component of centrosomes [43]. E7 protein of LR-HPVs does not have the capacity to induce abnormal centrosome synthesis, which is a unique property of the HR-HPV protein [40, 44]. Therefore, it seems clear that this ability is greatly relevant in transformation driven by E7 of HR-HPVs, because it confers an intrinsic element of genomic instability.

Both E6 and E7 have been shown to hamper DNA damage response, mainly via inhibition of p53-mediated pathways. Degradation of p53 by E6 and inactivation of p21 by E7 (see below) abrogate p53 activation in response to DNA damage. Importantly, E7 can induce aberrant mitotic entry in the presence of DNA damage, leading to genomic instability and contributing to the accumulation of genetic alterations.

18.3 The Viral Oncoproteins E6 and E7 in HR-HPV-Driven Carcinogenesis

As already emphasized, the oncogenic potential of the HR-HPVs is ascribed to the activity of the viral oncoproteins, mainly E6 and E7, with a minor role for E5. E6 and E7 of HR-HPVs have unique functional properties compared with the corresponding proteins of LR-HPVs. Importantly, expression of E6 and E7 is always retained in HPV-positive cancer cells [45, 46], whereas the expression of other early proteins, including E5, may be lost [47]. This suggests that E5 plays a role in the early stages of HPV-mediated carcinogenesis, altering the processing of epidermal growth factor (EGF), and promoting immune evasion through down-regulation of human leukocyte antigen class I (HLA I) molecules [48].

Both E6 and E7 possess transforming potential in several experimental settings. Oncogenic activity of E6 and E7 has been extensively shown

both in tissue culture and in transgenic mouse model systems. E6 and E7 can transform established murine cell lines, and their continuous expression is required for the maintenance of the transformed phenotype [49–51]. These viral oncoproteins are necessary and sufficient to immortalize primary human keratinocytes [52, 53], as well as tonsil epithelial cells [54]. When expression of E6 and E7 is hampered, cancer cells cannot further proliferate, as a result of an irreversible proliferative arrest (senescence).

HPV-associated cancers are characterized by the over-expression of these viral oncoproteins, which may derive from the loss of the viral pathways that regulate E6 and E7 expression, as mentioned above.

E6 and E7 are very small proteins that do not possess any enzymatic activity. They are both zinc-binding proteins, due to the presence of four and two CXXC motifs, respectively (Fig. 18.2). These oncoproteins are characterized by a flexible structure, which can be modified according to the interacting partners [55, 56]. The transforming capacity of E6 and E7 relies on their ability to bind to and deregulate or inactivate a very large number of cellular proteins and, consequently, to subvert key cellular pathways (Fig. 18.3). New targets of these viral oncoproteins are regularly reported [57, 58]. Nonetheless, the most relevant interactions are classically recognized to be that of E6 with p53 and that of E7 with the "pocket proteins" pRb, p107, and p130, which modulate cell proliferation, differentiation, apoptosis, and DNA damage response [59].

18.3.1 E6: A Brief Portrait

E6 targets numerous cellular proteins and thus affects a plethora of cellular pathways with key roles in apoptosis, genome integrity, cell polarity, adhesion, and differentiation. The major target of E6 is p53 tumor suppressor (Fig. 18.4). E6 induces degradation of this protein via association with the E6-associated protein (E6AP), a cellular ubiquitin-protein ligase [60, 61]. E6 does not directly bind to p53 but forms a trimeric complex with p53 via E6AP binding, which thus induces polyubiquitination of p53 [62]. As a consequence of p53 degradation, p53-dependent pathways, such as apoptosis and senescence, are inactivated. This step is essential to counteract the pro-senescent and pro-apoptotic effects of E7 (see below). Notably, E6 protein of LR-HPVs can inhibit p53 transcriptional activity but does not target p53 for degradation.

Inhibition of p53 activity is only one of the E6-mediated functions that play a key role in

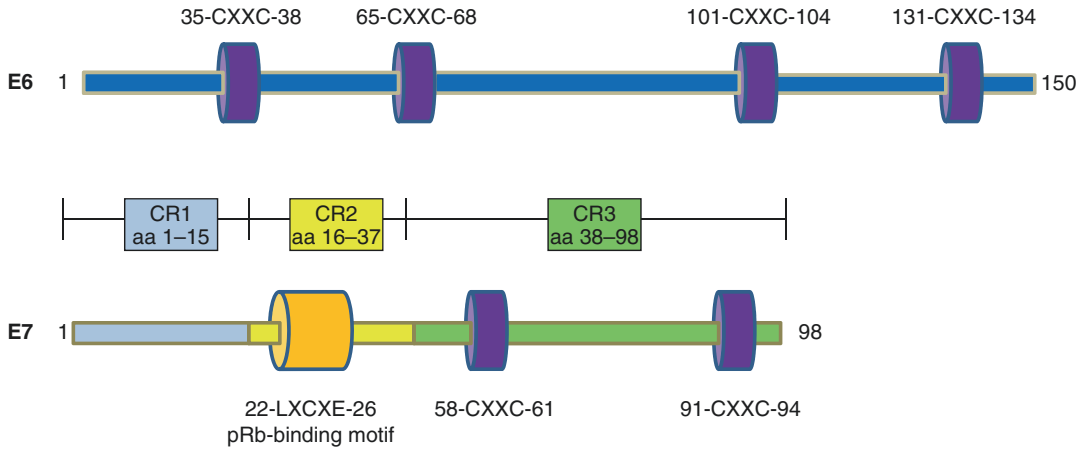

Fig. 18.2 Schematic representation of E6 and E7 viral oncoproteins. The positions of the CXXC motifs (four in E6 and two in E7), required for zinc finger formation and consequent zinc binding, are indicated. The LXCXE motif of E7, involved in the binding of pRb tumor suppressor, is also shown. The conserved regions (CR) of HPV16 E7 are shown schematically, as well as the amino acid (aa) positions included in each domain

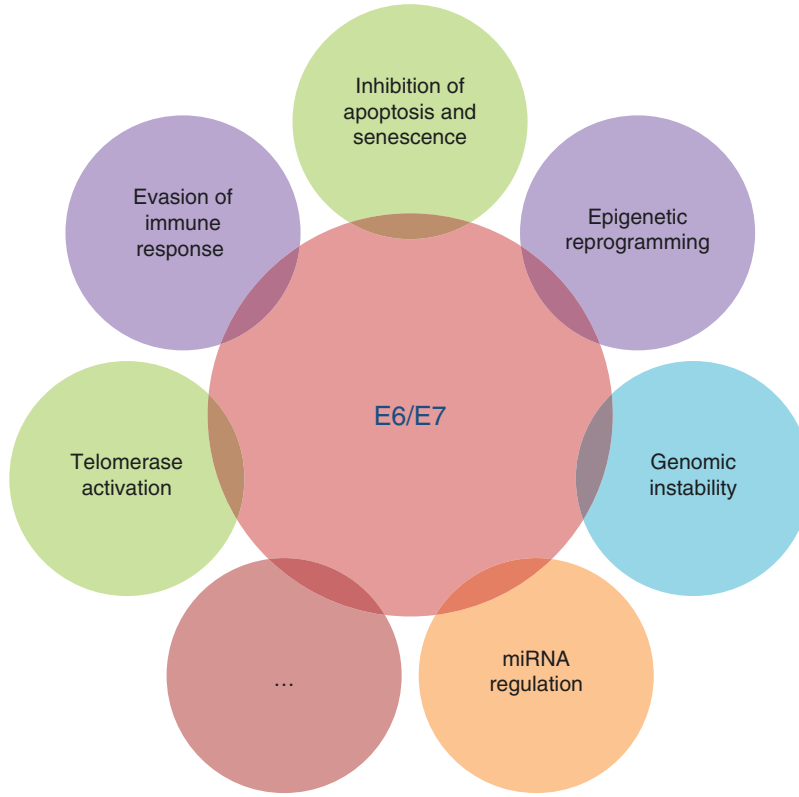

Fig. 18.3 Cellular pathways and activities inhibited or deregulated by E6 and E7 viral oncoproteins, which are the main drivers of HPV-mediated carcinogenesis

HPV-induced transformation. In addition to p53, E6 promotes the proteolytic degradation of several other cellular proteins, which are targeted through the PDZ binding motif, which is not present in E6 protein of LR-HPVs and can thus be considered a specific trait of oncogenic potential [63]. Among PDZ domain-containing proteins targeted by E6, several tumor suppressors have been recognized (e.g., Dlg, Scribble, and MAGI-1).

The transforming ability of E6 tightly correlates with its capacity to activate the catalytic unit of human telomerase reverse transcriptase (hTERT) [64]. E6 protein of LR-HPVs is unable to activate telomerase. In normal cells, this enzyme no longer functions, so that chromosome ends (telomeres), which shorten at each DNA replication, reach a critical length, and replicative senescence is induced. E6AP plays a central role in induction of hTERT expression by E6. The E6/E6AP complex promotes the ubiquitination and thus degradation of an inhibitor of hTERT transcription [65]. In addition to this mechanism, hTERT expression is further regulated by E6 through epigenetic modifications of its promoter and post-transcriptional modification. This multilevel regulation of telomerase strongly indicates that restoration of its activity is essential in HPV-induced transformation.

18.3.2 E7: A Brief Portrait

E7 is a multifunctional protein, highly conserved among HR-HPVs, which modulates the activity and/or stability of tens of cellular proteins, including cell cycle regulators, transcription factors, and metabolic enzymes. Novel targets of E7 are regularly described, even though the biological relevance of these interactions is not always clear.

E7 influences G1–S checkpoint transition, cellular differentiation, apoptosis, host immune response, and cell metabolism. E7 can abrogate several signals of growth arrest, such as those provided by cytokines with cytostatic effects on

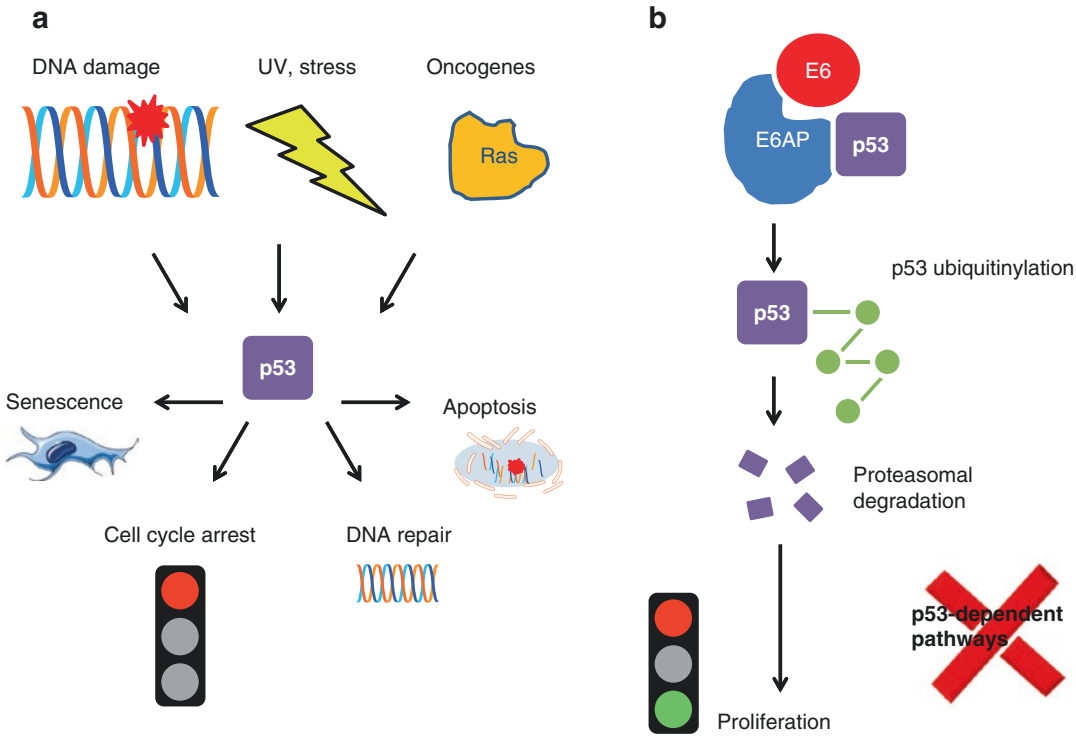

Fig. 18.4 Schematic representation of p53-dependent cellular pathways (panel A) and HPV E6-induced degradation of p53 mediated by the formation of a trimeric complex with E6-associated protein (E6AP) (panel B). After this interaction, p53 is ubiquitinated and thus degraded by the cellular proteasome. As a consequence, p53-dependent pathways, such as apoptosis and DNA damage repair, are deregulated

epithelial cells. The E7 oncoprotein of HR-HPVs can abolish growth suppression induced by tumor necrosis factor alpha (TNF-α) [66] and transforming growth factor beta (TGF-β) [67]. Moreover, E7 can induce escape of quiescent cells from G0 phase or premature entry of proliferating cells into S phase [68, 69].

HR-HPV-induced malignant transformation is intimately linked to the interference of E7 with cell cycle control pathways. Several cellular proteins involved in the control of cell proliferation are the main targets of E7. The most relevant and well-characterized interactions are those established with the members of the retinoblastoma (Rb) family of "pocket proteins": pRb [70], p107, and p130 [71].

18.3.2.1 E7 and pRb

Association of E7 with pRb is essential for E7-induced transformation. Interestingly, the oncogenic potential of E7 does not depend on the binding affinity for this target. It appears, instead, that its oncogenic properties tightly correlate with the ability to promote pRb degradation, which is mostly effective in neutralizing pRb-dependent pathways. E7 protein of HPV1, which only causes benign skin lesions and does not possess any transforming ability, binds to pRb with almost the same efficiency as HR-HPV E7 but does not induce pRb degradation [72, 73]. The ability of E7 to promote pRb degradation strongly correlates with its capacity to circumvent p16-induced cell cycle arrest [73].

pRb has primarily a growth suppression function, mainly accomplished through the interaction with the E2F family of transcription factors. These control the expression of proliferation-associated genes necessary for DNA replication and cell cycle progression. When the hypophosphorylated form of pRb binds to E2F (mainly E2F1–E2F3), the expression of E2F-responsive

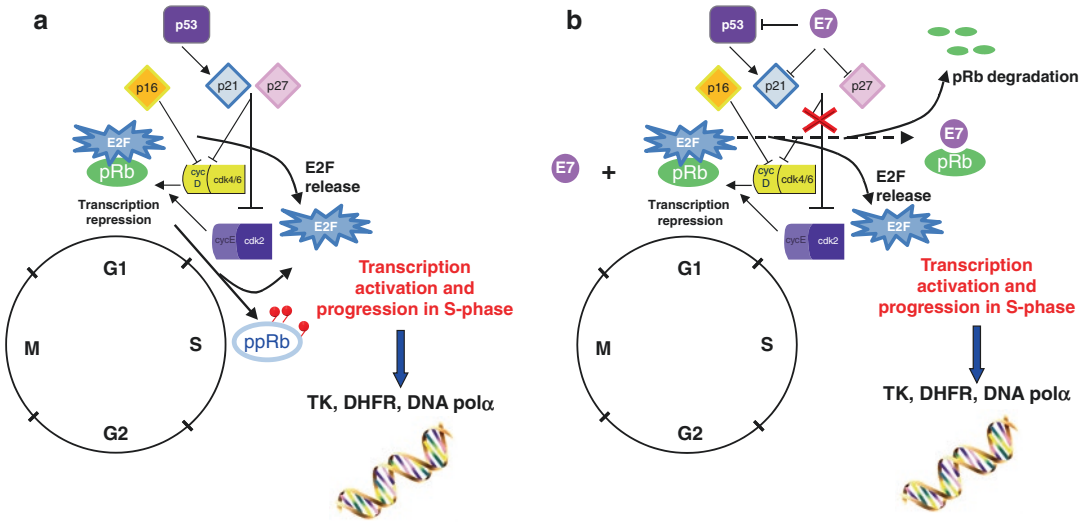

Fig. 18.5 Schematic representation of the pRb-dependent pathway of regulation of E2F activity (panel A) and HPV E7 interference with pRb function (panel B). A. In normal keratinocytes, hypophosphorylated pRb binds to the E2F transcription factor, and the expression of genes necessary for cell cycle progression is repressed (tyrosine kinase, TK; dihydrofolate reductase DHFR; DNA polymerase, DNA pol). Phosphorylation of pRb by cyclin (Cyc)/cyclin-dependent kinases (Cdks) complexes induces release of E2F and transcription of E2F-responsive genes. B. In HPV-positive cells, binding of E7 to the hypophosphorylated form of pRb promotes E2F release and S-phase entry. Cell proliferation is further induced by E7 blocking the activity of the Cdk inhibitors p21 and p27

genes is inhibited (Fig. 18.5). In late G1 phase, phosphorylation of Rb by cyclin-dependent kinases (mainly the CycD/Cdk4–6 and CycE/Cdk2 complexes) induces the release of the E2F transcription factor.

The association of E7 with pRb is mediated by an LXCXE motif, shared by many cellular proteins that interact with Rb and other viral oncoproteins that functionally inactivate pRb, such as Ad5 E1A and SV40 T-Ag [74–77]. E7 preferentially binds to the hypophosphorylated form of pRb [75, 78], thus preventing its association with E2F and inducing release of E2F from already established complexes [79]. As a consequence of the increase in the cellular levels of free E2F, progression to S phase is promoted. It is worth noting that the ability of E7 to promote the expression of E2F-responsive genes is not linked exclusively to impairment of pRb activity but is also achieved through direct interaction with the E2F and E2F-DNA complexes even in the absence of pRb [80].

18.3.2.2 E7 and p16

Interestingly, E7-mediated impairment of pRb function has typically been considered a cause of up-regulation of $p16^{Ink4a}$ (p16), an inhibitor of Cdk4 and Cdk6. HPV-associated neoplastic lesions over-express p16, which is considered an excellent surrogate marker of transforming HPV infections, in both cervical [81] and head and neck pathology [82]. The latest World Health Organization (WHO) classification of head and neck cancers indicated p16 immunohistochemical staining as a stand-alone test for the identification of oropharyngeal cancers attributable to HPV infections [83].

Recent data indicate that aberrant expression of p16 is not an indirect consequence of E7-induced pRb inactivation but a result of the ability of E7 to directly trigger p16 expression. The model of E7-mediated p16 up-regulation has been revised, and p16 now appears to be a major carcinogenic determinant. E7-induced replicative stress appears to activate a p16-mediated cellular

defense response, namely oncogene-induced senescence. By triggering the expression of H3 histone demethylases KDM6A and KDM6B, E7 promotes H3 demethylation, and this epigenetic reprogramming leads to de-repression of genes silenced by polycomb proteins, including the locus ARF-INK4A that encodes for p16 [84, 85]. However, inhibition of Cdk4/6 mediated by p16, and consequent induction of Rb-dependent senescence, is overcome due to E7-induced pRb degradation. Importantly, E7-expressing cells appear to be addicted to p16, so that p16 may be regarded as pro-oncogenic in RB-defective cells [85, 86].

18.3.2.3 Other Cellular Targets of E7

The oncogenic activity of E7 does not rely exclusively on the interaction with its traditional target pRb. Several other proteins, mainly involved in control of cell cycle progression, have been described as targets of E7, and their modulation may play a role in explaining the transforming potential of E7. E7 can still induce hyperplasia and dysplasia in pRb-null tissues of transgenic mice, suggesting that pRb-independent mechanisms are also responsible for the oncogenic potential of E7 [87].

HPV16 E7 has the specific ability to promote the activation of Cdk2 in the CycA/Cdk2 and CycE/Cdk2 complexes [88, 89]. This activity may further enhance pRb inactivation, because Cdk2, as already mentioned, is involved in pRb phosphorylation. A further abolishment of pRb function occurs through E7-mediated blockage of Cdk inhibitors (CKIs) $p21^{CIP1/WAF1}$ (p21) and $p27^{KIP1}$ (p27) [90–92]. Notably, p21 is a transcriptional target of p53 and is involved in response to DNA damage, senescence, and differentiation. Therefore, its inactivation seems to contribute to the subversion of cell cycle control and to bypass p53-dependent anti-proliferative pathways, which normally lead to p21 transcriptional activation, as already mentioned. Because of its critical role in G1 arrest upon DNA damage, perturbation of p21 activity may promote genetic instability during HPV-associated cancer progression.

E7 also has the ability to subvert the function and localization of p27 [92]; HPV16 E7 causes cytoplasmic retention of this CKI. Cytoplasmic p27 appears to be a positive modulator of migration and tumor invasion [93, 94]. Therefore, perturbation of p27 activity may have relevant consequences in terms of tumor invasiveness.

A considerable amount of evidence supports the idea that Cdc25A is an important mediator in E7-induced proliferation and tumorigenesis. Unlike HPV16 E7, E7 protein of the LR-HPV11 does not have the ability to modulate this target [95]. Cdc25A is a tyrosine phosphatase that controls the activity of the CycE/Cdk2 and CycA/Cdk2 complexes, which are activated upon removal of inhibitory phosphorylation. E7 promotes up-regulation of Cdc25A, and this effect probably involves epigenetic mechanisms (see below). Importantly, E7-induced defects in Cdc25A activity may result in genomic instability. Cdc25A is normally degraded or inactivated in response to DNA-damaging agents in order to stop cell cycle progression, either to repair DNA or to initiate apoptosis. Deregulation of this phosphatase may thus contribute to perturbing the cell response to DNA damage.

As described above, subversion of the functions of p53 is mainly dependent on the E6 oncoprotein. Nonetheless, it has also been reported that E7 has the capacity to interfere with p53 activity. E7 can overcome p53-induced growth arrest in response to cell damage [96, 97], and this might depend on the capacity of E7 to induce aberrant expression of CycA and CycE, degradation of pRb, and inactivation of p21, as previously described. Interestingly, in E7-positive cells, p53 is not efficiently targeted for proteasomal degradation and is thus stabilized [98, 99]. This could thus lead to the apoptotic death of E7-expressing cells [100]. However, this possible consequence is bypassed due to E6-mediated degradation of p53 and Bak [101]. Therefore, the synergistic and complementary activity of E6 and E7 ensures the survival of HPV-infected cells.

18.3.3 E6, E7, and Epigenetic Changes

An increasing body of data has shown that HPV oncoproteins profoundly alter the epigenome, affecting both the expression and the activity of

enzymes involved in histone post-translational modifications, DNA methylation, and chromatin remodeling (reviewed in [102]). Importantly, virus-induced epigenetic alterations may play a role in both tumor initiation and progression.

By altering the activity of histone-modifying enzymes, HPV oncoproteins affect the physical state and transcriptional competence of chromatin. For instance, HR-HPVs E7 proteins are capable of binding the histone deacetylases 1 and 2 (HDAC-1 and HDAC-2) [103, 104]. Most interestingly, E7 mutants defective in HDAC binding are deficient in transforming activity, suggesting a close link between the oncogenic potential of E7 and its association with HDAC. HDACs promote nucleosome condensation by removing acetyl groups from the histone N-terminal tails. Consequently, the access of transcription factors to the DNA is impaired. Modulation of HDAC activity by E7 appears to be associated with an increase in E2F-mediated transcription. Notably, the modulation of HDACs exerted by E7 might contribute to HPV evasion of immune surveillance, and thus may favor a persistent infection, a key step in HPV-induced carcinogenesis, as emphasized above. In fact, through recruitment of HDAC, E7 appears to regulate the transcription of immune-modulating molecules, such as IRF-1, which plays a central role in IFN signaling and immune surveillance [21] and is silenced by E7. E7-induced modulation of HDACs could also play a key role in down-regulating the expression of MHC class I molecules on the surface of HPV-positive cells [105], and this may further contribute to immune system evasion. HPV oncoproteins also affect other histone-modifying enzymes. The activity of histone acetyltransferases (HATs) is modified in HPV-positive cells. Both E6 and E7 can alter HAT function [102].

Host DNA methylation is profoundly affected by HR-HPVs. As previously discussed, HPV16-E7 induces the expression of histone demethylases KDM6A and KDM6B [84]. HPV-mediated tumorigenesis is also associated with hypermethylation of the host genome. Interestingly, methylation levels in cervical carcinogenesis increase with the severity of cervical disease [106]. Both E6 and E7 promote the expression of DNA methyltransferases, particularly DNMT1, leading to aberrant methylation patterns in the host genome [107, 108]. E7 also binds to DNMT1 and stimulates its activity. Alterations in host DNA methylation may lead to silencing or activation of various target genes involved in HPV-driven tumorigenesis. Tumor suppressor genes may be silenced through DNA methylation of CpG islands in their promoters. In contrast, oncogenes may be activated. In addition, expression of non-coding microRNA (miRNA) is also affected through HPV-induced aberrant methylation [102]. Notably, host immune responses may be deregulated through methylation, and thus suppression, of immune-related genes. This appears to be a common strategy of oncogenic viruses to evade the host immune system (reviewed in [109]). For instance, HR-HPVs down-regulate the expression of the type I interferon IFNκ, an antiviral molecule that is expressed in keratinocytes, inducing the hypermethylation of its promoter [110].

It is worth noting that the viral genome may also be modified in terms of methylation. This may occur in the long control region (LCR), and in the late ORFs encoding for the L1 and L2 capsid proteins, although with an unclear biological significance. Importantly, methylation of E2 binding sites in the LCR, reducing E2 binding, may induce increased expression of E6 and E7 and may thus be a mechanism of up-regulation of oncogene expression in the absence of viral integration [111].

18.4 Conclusions

Carcinogenesis associated with infection by mucosal HR-HPVs is a multistep process, which may take up to 30 years. HPV-driven tumorigenesis is primarily based on the prolonged overexpression of E6 and E7 viral oncoproteins upon establishment of a persistent infection. E6 and E7 subvert cellular pathways with a key role in cell cycle control, and promote proliferation, genomic instability, and genetic and epigenetic events. The accumulation of changes in the host genome further leads to deregulated expression and/or function of host oncogenes and tumor suppres-

sors. Over time, these events lead to the irreversible acquisition of a malignant phenotype. The monoclonal expansion of the HPV-infected cell(s) with the greatest advantage will ultimately lead from pre-cancer to cancer.

Many epidemiological and biological studies have provided several lines of evidence for the involvement of beta HPVs, together with UV radiation, in the development of cSCC [112]. However, the carcinogenic mechanism of beta HPVs appears to differ from the well-established strategy of the mucosal HR-HPVs [113]. Whereas in HR-HPV-driven cancer E6 and E7 expression is essential for the initiation of the oncogenic process and the maintenance of the malignant phenotype, in cSCC the viral oncoproteins act at an early stage of carcinogenesis, facilitating the accumulation of the UV-induced DNA damage. In accordance with this model, a recent study showed that beta HPV38 E6 and E7 expression is dispensable after the establishment of UV-induced cSCC in an animal model, whereas loss of viral gene expression before initiation of UV irradiation prevents the development of skin lesions [114].

Taken together, these data indicate that the contribution of HPV to the development of cancer in humans relies on complex mechanisms, and that different genotypes are involved in human carcinogenesis to different extents, as main drivers (mucosal HR-HPVs) or cooperating passengers (beta HPVs). These different models might depend on the differential exposure of the target cells to site-specific environmental factors. It would be interesting in future studies to evaluate whether the model proposed for the beta HPV types and UV radiation in human carcinogenesis could also be extended to other HPV types and environmental risk factors at different anatomical sites.

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19 Antimicrobial Resistance in *Neisseria gonorrhoeae*: A New Challenge

Paola Stefanelli and Anna Carannante

19.1 Global Resistance Epidemiology

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* constitutes an increasingly health hazard. The first antimicrobial-resistant gonococcal strain was identified in Southeast Asia in the mid-1990s [1, 2]. In the USA, the first report was in the Hawaii [3–5], as imported strain from Asia. Resistant gonococci spread to West Coast States, mostly among men who have sex with men (MSM) [6–8].

Wi et al. 2017, in the World Health Organization (WHO) Global Gonococcal Antimicrobial Surveillance Programme (GASP) Report described the spread of resistance to penicillin, tetracycline and ciprofloxacin; the increase of resistance to azithromycin and the emergence of decreased susceptibility and resistance to extended-spectrum cephalosporins (ESCs) worldwide (Fig. 19.1). Gonococcal susceptibility to ciprofloxacin was evaluated in 72 countries, to azithromycin in 58 countries and to ESCs in 77 countries, showing 97%, 81% and 61%, of resistant strains, respectively [9].

In USA, data from Gonococcal Isolate Surveillance Project (GIPS) showed a percentage of azithromycin resistant strains almost low from 2000 to 2013, with a rapid increase from 0.6% in 2013 to 2.5% in 2014 [10]. The percentage of cefixime resistant gonococci increased up to 0.8% in 2014; the ciprofloxacin resistant strains decreased from 14.8% in 2007 to 9.6% in 2009, to increase again more recently [10]. Moreover, respectively, the 16.2% and 25.3% of gonococci resistant to penicillin and tetracycline, or in combination with other resistance phenotypes, were also reported in 2014 [10].

In Europe, the European Gonococcal Antimicrobial Surveillance Program (Euro-GASP), coordinated by the European Centre for Disease Prevention and Control (ECDC), collects data from 24 EU/EEA Member States, including Italy [11, 12]. A total of 2134 isolates, in 2015, were collected and tested, covering 3% of the gonorrhoea cases reported by routine surveillance [12]. The increased rate of reported cases is also worrying due to the threat of *N. gonorrhoeae* AMR. The latest resistance data from the Euro-GASP suggests stable levels of resistance to cefixime and no significant increase in resistance to ceftriaxone. Resistance to azithromycin, however, appears to be increasing and the development of resistance to ESCs is feared to be only a matter of time (Fig. 19.2) [12].

In 2015, 1.7% of gonorrhoea cases were due to cefixime resistant strains (Fig. 19.3) in nine European countries. The resistance to cefixime remained quite stable compared to 2014 (2.0%)

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Fig. 19.1 Drug-resistant *Neisseria gonorrhoeae* (Reproduced from CDC, https://www.cdc.gov/drugresistance/biggest_threats.html, Accessed 11 Jan 2018)

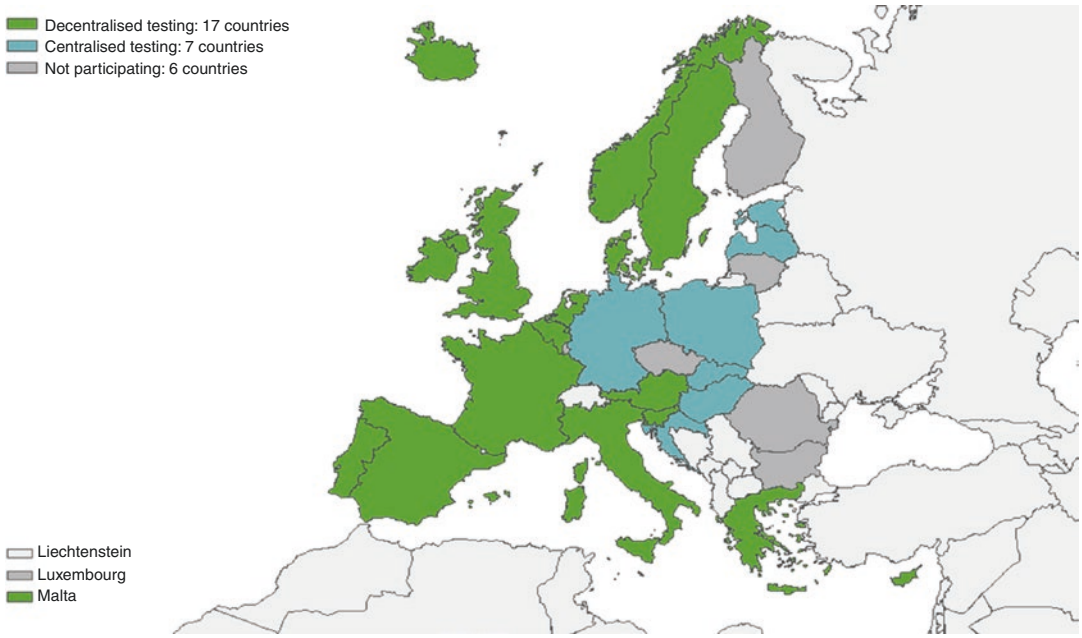

Fig. 19.2 Euro-GASP network, 2015 (Reproduced from ECDC, https://ecdc.europa.eu/sites/portal/files/documents/gonococcal-antimicrobial-susceptibility-surveillance-Europe-2015.pdf, Accessed 11 Jan 2018)

and an increase was observed in Greece (from 5% in 2014 to 11% in 2015) and a decrease in Norway (from 5.5% in 2014 to 0.9% in 2015) [12]. Furthermore, among MSM, a decrease of cefixime resistance was observed in 2015 (0.5%) compared to 2010 (7.3%) [12].

In the years 2014 and 2015, ceftriaxone-resistant strains have been also reported in Europe. In particular, five strains, in 2014, in Greece (n = 3), Germany (n = 1) and Norway (n = 1) and one, in 2015, in Greece isolated from the urethra of a heterosexual male; the ceftriaxone resistant gonococcal strains belonged to Genogroup (G) 1407 [12–14], known as an international clone of resistant gonococci.

The mean percentage of resistance to azithromycin, in 2015, was 7.1% in Europe, (Fig. 19.3), passing from 0% (Cyprus, Estonia, Iceland,

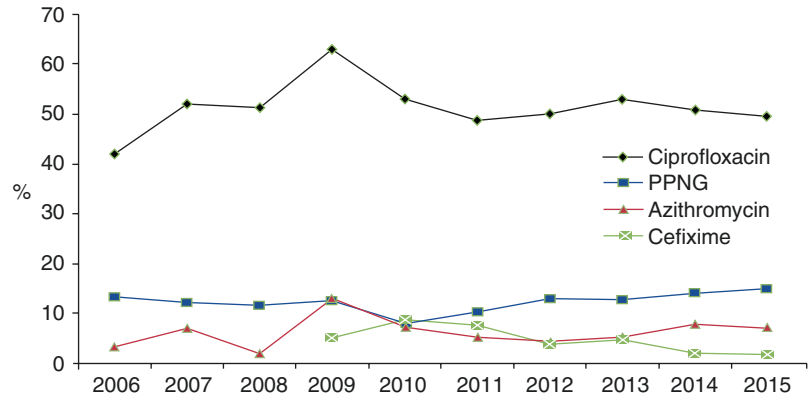

Fig. 19.3 Percentage of resistant gonococci per antimicrobial and year, Euro-GASP 2006–2015 (Reproduced from ECDC, https://ecdc.europa.eu/sites/portal/files/documents/gonococcal-antimicrobial-susceptibility-surveillance-Europe-2015.pdf, Accessed 11 Jan 2018). *PPNG* penicillinase-producing *N. gonorrhoeae*

Croatia, Latvia) to 22% (Greece). In the same year, a total of five isolates with high levels of resistance to azithromycin were documented: three in Ireland, one in Norway and one in the United Kingdom [12]. The highest rate of resistance to azithromycin was observed in MSM and male heterosexual patients (8.1%) and lower among females, 4.9% [12].

On the contrary, the percentage of resistance to ciprofloxacin had a minimum value of 11.1% (Latvia) and a maximum value of 77% (Greece), higher among heterosexual male patients (59.7%) [12]. Globally, in 2015 the levels of resistance to ciprofloxacin were similar to the levels observed in 2014, 49.4% vs. 50.7%, Fig. 19.3 [12].

Finally, the resistance to plasmid-mediated penicillin (Penicillinase-producing *N. gonorrhoeae*, PPNG) was not reporting in some countries (Cyprus, Estonia, Latvia, Iceland) or reported as 32% in others (Austria) with an overall percentage of 14.8, Fig. 19.3 [12].

The integration of molecular typing with epidemiological data has the potential to improve the surveillance and the outbreak investigation. Moreover, the typing system in the routine use across Europe permits to identify emerging clones, drug-resistant determinants. The *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) is in support of the National Reference Labs (NRL) core functions for *N. gonorrhoeae* AMR surveillance.

The ECDC report showed the international spread of G1407 in several European countries [13] with an overall prevalence of 23.3% in 2009–2010 (in Southern and Eastern Europe) [13]. A strong association was evidenced among G1407 gonococci and cefixime, ciprofloxacin resistant profiles. Furthermore, a proportion of G1407 isolates with reduced susceptibility or resistance to azithromycin have been also documented [13].

In Italy, data on *N. gonorrhoeae* AMR are collected through a network coordinated by the Istituto Superiore di Sanità (ISS) with the support of the Ministry of Health. In 2014, the percentage of PPNG decreased significantly from 77%, in 2003, to 7%, in 2012; the percentage of tetracycline-resistant gonococci increased up to 50.5% in 2009 [15]. The percentage of ciprofloxacin resistance gonococci increased from 2003 (38%) to 2012 (64%). The rate of ciprofloxacin-resistant gonococci, accounting for >60%, remained quite stable in the last year in the country [15]. Since the first report of high level of resistance to azithromycin in 2008 [16], the percentage decreased in 2012 up to 14%. The rate of resistant gonococci to cefixime decreased from 11%, in 2008, to 3.3%, in 2012 [15].

From 2009 to 2016, a shift in cefixime and azithromycin susceptibility was observed among 1433 gonococci [17], as a possible consequence of the treatment application of the combined

therapy in the routine protocol [18]. As example, the proportion of azithromycin resistant gonococci decreased up to 2012 and increased afterwards reaching 7.44% in 2016. Furthermore, gonococci with intermediate values of minimum inhibitory concentration (MIC) to azithromycin decreased from 2009 (43.42%) to 2016 (6.97%) [17]. The percentage of cefixime resistant gonococci remained fairly stable in 2014 (3.48%) and, then, decreased up to 1.39% in 2016 [17]. No ceftriaxone resistant gonococci were reported.

G1407 is the predominant clone among the gonococci collected in Italy as also reported in EU/EEA countries [15, 19].

19.2 Antimicrobial Resistance

The high proportion of AMR outlines new scenarios making gonorrhoea as a no more treatable disease [20–22]. To limit the phenomenon the combination of two antimicrobials has been recommended in the practical use [18, 23].

The WHO in the 'Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in *Neisseria gonorrhoeae*' [24] and the ECDC promote specific Gonococcal Antimicrobial Surveillance Programme for the USA [25] and for the EU/European Economic Area (EU/EEA) countries [26], respectively.

AMR in *Neisseria gonorrhoeae* has emerged and expanded towards the new classes of antimicrobials used in the current therapeutic protocols, such as cefixime, ceftriaxone and azithromycin [21, 27–33]. Surprisingly, mutations involved in resistance to antimicrobials no longer in use, as fluoroquinolones, penicillins, tetracyclines and spectinomycin, are still detected in the gonorrhoea genome [31].

Generally the exposure of gonococci to antimicrobials is in favour to select for resistant strains and multidrug-resistant strains (MDR). MDR strains continue to increase globally reducing the available treatment options [24].

Since there is no vaccine against *N. gonorrhoeae,* prevention and control of antimicrobial resistance call for multidisciplinary efforts at national and international levels including an active surveillance on cases and on treatment failures.

Before the global introduction of the combined therapeutic approach, in 2010 by the Center for Disease Control and Prevention (CDC) [23] and in 2012 by International Union against Sexually Transmitted Infections (IUSTI), for the revised European guidelines [18], the antimicrobials used to treat gonococcal infections were classified into three categories. The first category included the recommended antimicrobials, the second those used less frequently and the third group those no longer in use and/or considered inappropriate.

Based on the above categorization, a *N. gonorrhoeae* strain was defined as MDR if resistant to one of the antimicrobials of the category comprising the recommended antimicrobials and to two or more antimicrobials of the category comprising the less frequently used drugs. Furthermore, the term extensively drug resistance (XDR-NG) has been introduced for gonococcal strains resistant to two or more antimicrobials of the category comprising the recommended antimicrobials and to three or more of the category comprising the antimicrobials used less frequently in the therapy for the treatment of gonorrhoea [20].

The first three XDR *N. gonorrhoeae* strains with high level of resistance to ceftriaxone were reported in Japan [34], France [21] and Spain [35].

19.3 Antimicrobial Resistance Molecular Mechanisms

In the era of molecular diagnostic and typing tests, gonorrhoea is increasingly being diagnosed by nucleic acid amplification approaches (NAATs). However, the cultivation of the pathogen is essential to perform the antimicrobial susceptibility tests in the AMR surveillance. In the forefront, whole genome sequencing (WGS) is currently a robust technology to uncover drug resistance mechanisms in *N. gonorrhoeae*, as it can rapidly sequence the complete genome. Rather than using only a limited amount of genetic materials, WGS has markedly enabled

the accuracy of sequencing data for *N. gonorrhoeae*. Moreover, for the application in unraveling drug resistance genetics, this technology also helps in the study of molecular epidemiology, transmission chains, evolution and phylogeny of gonococcus. Other applications of WGS include, but not limited to, its use to identify signatures of convergent evolution and positive selection of new genes associated with drug resistance [36–38].

Many genetic loci have been reported to have putative association with drug resistance in *N. gonorrhoeae*. The presence of single point mutations, mobile elements and exogenous DNA fragments are considered the ways for the antimicrobial resistance among gonococci [30, 39].

The β-lactam antimicrobials, such as penicillin and ESCs, are characterized by the presence of a β-lactamic ring capable to inhibit penicillin binding proteins (PBPs), transpeptidase enzymes. Alterations in PBP-2 decrease their affinity for penicillins and ESCs, and consequently, the susceptibility of the bacterium to these drugs [30, 40]. PBP-2 is encoded by the *penA* gene, which is characterized by a mosaic structure for translocation of exogenous DNA fragments in resistant strains or strains with reduced susceptibility [34, 41]. This exogenous DNA can derive from other *N. gonorrhoeae* strains or from commensal *Neisseria* spp. that cohabit the human pharynx, site of exchange of genetic material [40, 41]. During the last decade, several mosaic *penA* genes have been described with 60–70 amino acid changes in comparison to the wild type gene, determining the resistance profile to both penicillins and ESCs [21, 34, 35].

The mosaic structures *penA-X* and *penA-XXXIV* are known to characterize resistant gonococci or with decreased susceptibility to ESCs; the *penA-XXXIV* is globally widespread including Italy [19, 42, 43]. Moreover, *penA* gene can accumulate amino acid substitutions A501, G545S, I312M and V316 T [34, 41] associated with decreased susceptibility or resistance to ESC.

Mutations in *porB1b* gene define the decreased permeability of PorB1b, one of the most important outer-membrane channel protein. This protein is present in two allelic forms, PorB1a and PorB1b. The amino acid substitution at positions G120 and A121 within the PorB1b loop 3 is associated with resistance to hydrophobic antimicrobials [43, 44].

Furthermore, alterations in *mtr* (multiple transferable resistance) operon and/or in its promoter are associated with resistance to hydrophobic antimicrobials, such as penicillins and ESCs [30, 45]. The *mtrCDE* operon encodes the components of a tripartite efflux pump that expel antimicrobials from the periplasmic space [46]. The MtrD and MtrC proteins assemble into the inner and outer membrane, whereas MtrC is placed in the periplasmic environment and connects MtrD with MtrE [46]. Changes in the regulation mechanism of *mtrCDE* are the main cause of increased susceptibility to hydrophobic agents. The promoter of *mtrR* gene, which encodes a transcriptional repressor, regulates the transcription. The deletion of a single nucleotide (−35A) in the inverted sequence of the promoter causes the increased expression of the operon and consequently, both the loss of *mtrR* transcription and an increase in transcription of *mtrCDE*. Mutations may also occur in the coding region of *mtrR*, such as the substitution of the amino acid alanine, in position 39, with the amino acid threonine (A39T) or the amino acid substituted glycine, in position 45, with aspartic acid (G45D), which alter the DNA binding domain with a consequent increase in *mtrCDE* expression [30, 45].

Finally, among the chromosomal mutations, associated with the resistance to penicillin, there is the alteration in the *ponA* gene, codifying the PBP-1, with the amino acid substitution leucine, in position 421, to proline (L421P) [30, 47].

As well as chromosome mutations, the presence of plasmids mediating penicillin resistance (PPNG) defines high level of resistance to penicillin. $bla_{TEM\text{-}1}$ or $bla_{TEM\text{-}135}$ genes, encoding a TEM-1 and TEM-135-types β-lactamase, respectively [48, 49].

Moreover, chromosomal mutations and β-lactamase production can coexist in the same strain [50]. In several studies [48, 49, 51] was observed how few mutations in $bla_{TEM\text{-}1}$ and a single mutation in $bla_{TEM\text{-}135}$ are more likely to be

associated with extended-spectrum β-lactamase (ESBL) in *N. gonorrhoeae*.

Similarly to plasmid-mediated resistance to penicillin, the resistance to tetracycline is mediated by plasmid with *tetM* gene [52, 53]. The gene can also transfer β-lactamase-producing plasmids between gonococcal strains, and to *Neisseria meningitidis* [54, 55], *Haemophilus influenzae* and *Escherichia coli* [56].

The quinolones, ciprofloxacin and ofloxacin, are the most used for therapy in the past. For those antimicrobials the genetic targets are DNA gyrase and topoisomerase IV, present only in prokaryotes [30, 57]. High levels of resistance are related to mutations at the target site of the *gyrA* gene, encoding the DNA gyrase. Several amino acid substitutions have been described which, when combined, confer a high level of resistance. In particular, the presence of S91 and/or D95 amino acid changes [57]. Multiple mutations may also affect the *parC* gene, that codes for the production of topoisomerase IV, resulting in the amino acid substitutions D86, S87 and/or S88 and associated to resistance [57].

Azithromycin inhibits protein synthesis by binding to the V domain of 23S rRNA, constituting the 50S ribosomal subunit. Alterations, that increase resistance to this antimicrobial, can involve many components. Point mutations may directly affect the peptidyl transferase domain V and, additionally, alterations of the sequence at different sites of this region inhibit the binding of the antimicrobial to the target site. Some examples can be represented from specific mutations which can result in both low-level resistance (C2611T mutation, *E. coli* numbering system) [58] and high level of resistance (A2059G mutation, *E. coli* numbering system) in one of the four or more loci of the 23S rRNA [30, 58, 59].

In addition, a contribution of the efflux pump activity, encoded by *mtr* operon, was also documented for azithromycin resistance. In some macrolide-resistant strains, the *ermB* and *ermF* genes, coding for methylases which modify the 23S rRNA, by inhibiting the binding of the antimicrobial to the target site, have been identified.

Finally, high-level spectinomycin resistance in gonococci is associated with a C1192U (*E. coli* numbering system) single nucleotide polymorphism (SNP), in the spectinomycin-binding region in 16S rRNA, with a deletion of Val25 and with a K26E alteration, in the 30S ribosomal protein S5, encoded by *rpsE* gene [60, 61], whereas the T24P substitution in S5 defines a low-level of spectinomycin resistance [61, 62].

19.4 Treatment Options for Gonorrhoea: Current Treatment Guidelines

In clinical practice, antimicrobial treatment of gonorrhoea is frequently given empirically using the recommended antimicrobials in accordance with treatment guidelines [39].

Due to the emergence and spread of resistant or multiresistant to antimicrobials also due to the misuse or abuse in the use of drugs for the therapies, selecting resistant strains, the antimicrobial susceptibility assays on isolates could be considered strategic to drive the most effective therapy.

A treatment may considered efficacious, by WHO recommendation, when is able to cure at least 95% of infected people [24].

Following the emergence of resistance to ESCs, particularly cefixime, the guidelines for the antimicrobial treatment of gonorrhoea were reformulated internationally. The current guidelines for gonorrhoea therapy recommend, for uncomplicated gonococcal infections of the cervix, urethra, rectum and pharynx, a therapy based on the combined use of two different antimicrobials: a single intramuscular dose of ceftriaxone (250 mg in USA; 500 mg in Europe) plus a single dose of orally administered azithromycin (1 g in USA; 2 g in Europe) [18, 23, 63]. If intramuscular ceftriaxone is not available, the use of cefixime (400 mg in USA and in Europe) given orally is always indicated in combination with azithromycin. For those allergic to cephalosporins, for patients with infections with ESCs resistance and for treatment failure with recommended dual regimen an option may be oral administra-

tion of 2 g of azithromycin plus an intramuscular dose of gentamicin (240 mg in USA and in Europe) [18, 63]. The azithromycin is also recommended in cases of co-infection with *Chlamydia trachomatis*. In alternative, in Europe, for uncomplicated gonococcal infections, it is possible to use ceftriaxone (500 mg) or spectinomycin (2 g) plus azithromycin (2 g) [18].

Azithromycin susceptibility data suggest that the use of this antimicrobial in the recommended treatment guidelines is still correct but it is necessary to monitor it over time for the globally increase of resistance rates [10, 12]. Concern to ESCs, resistance to cefixime is considered predictive for the possible resistance to ceftriaxone [21, 27, 34]. Even though globally the majority of gonococci are susceptible to ceftriaxone treatment failure with dual antimicrobial therapy was documented in UK in 2016 [64]. Consequently, new antimicrobials for monotherapy or for inclusion in new dual treatment scheme need to be evaluated.

19.5 Future Treatment Options

The current scenario for gonorrhoea treatment suggests a more restrictive use of the antimicrobials comprised in the combined therapy with a fix scheme in terms of dosage and time of therapy to preserve antimicrobial effectiveness and moderate the resistance development [65]. Recently, two new combined regimens were evaluated in one randomized controlled trial: gentamicin plus azithromycin and gemifloxacin plus azithromycin, considered for uncomplicated urogenital infections in men and women [66]. There were no serious adverse events except for gastrointestinal symptoms, as nausea and diarrhoea. For all the above reasons, these two schemes might be considered in case of ceftriaxone-resistant strains, treatment failures or allergy to ESCs [66]. Alternatively, azithromycin could be replaced by solithromycin, at least as a temporary solution, and if phase III confirm efficacy, tolerability and safety [67].

Other therapy option could have been represented from spectinomycin in combination with, e.g., solithromycin (CEM-101) [65]. The latter is similar to other macrolides, inhibits protein synthesis, but presents three binding sites for the bacterial 23S rRNA that increase its potency and possibly prevents the onset of resistance [68, 69].

Other antimicrobials have been suggested as alterative monotherapies including ertapenem (injectable), fosfomycin (oral) and gentamicin (injectable) [22, 70, 71]. Gentamicin used as a first-line treatment plus doxycycline in Malawi in 1993 [18, 31, 63, 66, 72, 73], without reporting emergence of resistance in vitro.

Disadvantages in the use of all the above molecules have been reported: rapid resistance selection, in vitro, for the fosfomycin, decreased susceptibility for the ertapenem, lack of data between MICs values, pharmacokinetic/pharmacodynamic parameters and outcome of gonorrhoea treatment for gentamicin, fosfomycin and ertapenem [22, 70]. Consequently, they might be considered together with other antimicrobials only in case of documented resistance to ceftriaxone or allergy to ESCs [65, 68].

Derivates of antimicrobials used in the past have been evaluated in vitro showing high activity against gonococcal strains [65]. Among them, i.e., several fluoroquinolones, avarofloxacin (JNJ-Q2), sitafloxacin, WQ-3810 and delafloxacin have shown relatively high potency against gonococci including those with ciprofloxacin resistance [74–77]. However, in the family of tetracyclines, the fluorocycline, eravacycline (TP-434) and glycylcycline tigecycline were also evaluated [76, 78]. Bicyclic macrolides modithromycin (EDP-420) and EDP-322 displayed activity against azithromycin and ESCs resistant and MDR gonococci, except for high-level azithromicin resistant strains in case of modithromycin and EDP-322 [65, 79].

Recently, new compounds have been developed and have shown, in vitro, powerful activity against gonococcus. Among them several inhibitory proteins of synthesis such as pleuromutilin BC-3781 and boron-containing inhibitor AN3365, LPxC inhibitors, FabI inhibitors such as MUT056399 [76, 80].

New orally antimicrobials are under evaluation in clinical trials such as zoliflodacin (AZD0914/ETX0914) and gepotidacin (GSK2140944) [68, 69, 81–83]. Zoliflodacin, a non-fluoroquinolone inhibitor of topoisomerase II, targets DNA gyrase (especially GyrB) and shows novel mechanisms of action from other antimicrobials. This drug did not induce resistance in 250 isolates including ESCs and fluoroquinolones resistant and MDR [81, 82]. Finally, gepotidacin, is a new non-fluoroquinolone, inhibitor of topoisomerase II that targets DNA gyrase (GyrA subunit) and topoisomerase IV (ParC subunit) but with different binding mechanisms than the other fluoroquinolones, in vitro studies are still ongoing [83].

WHO together with the Global Antibiotic Research and Development Partnership (GARDP; http://www.dndi.org/diseases-projects/gardp/) facilitate the development, the clinical evaluations and registration of antimicrobial as well as new treatment schemes for gonorrhoea.

19.6 Concluding Remarks and Perspectives

Seeing as there is no gonococcal vaccine, the public health control of gonorrhoea depends entirely on prevention, sexual contact notification, epidemiological surveillance, diagnosis and, in particular, appropriate antimicrobial treatment. In the forefront, the diagnosis of drug resistance in *N. gonorrhoeae* strains requires speed and accuracy. WGS helps enormously to delineate genes associated with drug resistance. However, a complementary evaluation of putative genes defining the resistance is often required to confirm their role in clinical gonococci-antimicrobial resistant. In the development of new drugs to combat gonorrhoea, new targets should be sought to address the issues of drug resistance that compromise current gonorrhoea therapy. Finally, in response to the increased incidence of *N. gonorrhoeae* resistant or multidrug-resistant an enhanced *N. gonorrhoeae* antimicrobial resistance surveillance system is required to modify treatment guidelines.

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20 HPV Vaccines: An Important Tool for STI Prevention

Cristina Giambi and Giovanni Rezza

20.1 HPV as Oncogenic Agent

Human papillomavirus (HPV) infection is a universally known cause of cancer of the cervix, being responsible for virtually all cases of this tumor. This virus may determine also lesions of other female genital organs, such as the vulva and the vagina and play a role in penis and anus neoplasia, and in head-and-neck cancers (oral cavity, oropharynx, and tonsils), with great impact on neoplasms' burden worldwide [1–5]. In particular, HPV is responsible for 25% of the vulva, 78% of the vagina, 88% of anal cancer, 31% of the oropharynx, and 51% of the penis [6, 7].

In addition to lesions that may determine the development of tumors, HPV is also the cause of genital warts and larynx papillomatosis; these lesions are at high risk of chronicity and relapse, determining high economic and social costs.

There are more than 100 types of HPV, of which 12 have been recognized as oncogenic by the International Agency for Research on Cancer (IARC): HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. HPV types 16 and 18 were classified as carcinogens by the IARC already in 1995 [8] and reported to account for approximately 70% of cervical cancers. HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were included in the IARC carcinogens group in 2011 [9] and are reported to account for 30% of cervical cancers [10–14]. These are defined as high oncogenic HPV types, or simply as high risk, while other types, such as HPV 6 and 11, are defined as low risk. All these types are possible targets of vaccines.

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20.2 First Generation Prophylactic HPV Vaccines: 2vHPV and 4vHPV Vaccines

HPV vaccines, along with the HBV vaccine, represent a new paradigm in cancer prevention.

Three prophylactic HPV vaccines are currently available and marketed in many countries worldwide for preventing HPV-related diseases (Table 20.1). They are prepared from virus-like particles (VLPs) produced by recombinant DNA technology. Purified L1 protein self-assembles to form empty shells that resemble HPV VLPs, able to strongly activate the immune system. However, they do not contain viral genetic material or live biological products, so they cannot multiply and are not infectious.

Between 2006 and 2009, both the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) licensed two prophylactic vaccines against HPV. The quadrivalent (4vHPV) vaccine protects against HPV types 6, 11, 16, and 18. The VLPs are adsorbed to an

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Table 20.1 Characteristics of human papillomavirus vaccines

Characteristics	Bivalent (2vHPV)	Quadrivalent (4vHPV)	9-valent (9vHPV)
Brand name	Cervarix®	Gardasil®	Gardasil 9®
HPV genotypes (VLPs)[a]	16, 18	6, 11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58
FDA licensure	2009	2006	2014
EMA licensure	2009	2006	2014
Manufacturing	*Trichoplusia ni* insect cell line infected with L1 encoding recombinant baculovirus	*Saccharomyces cerevisiae* expressing L1	*Saccharomyces cerevisiae* expressing L1
Adjuvant	500 μg aluminum hydroxide50 μg 3-O-desacyl-4′ monophosphoryl lipid A	225 μg amorphous aluminum hydroxyphosphate sulfate	500 μg amorphous aluminum hydroxyphosphate sulfate
Volume per dose	0.5 ml	0.5 ml	0.5 ml
Administration	Intramuscular	Intramuscular	Intramuscular
Target age group	From 9 years of age	From 9 years of age	From 9 years of age
Recommended schedule before 14/15 years of age	9–14 years: 2-dose schedule, with the second dose given 5–13 months after the first dose	9–13 years: 2-dose schedule at 0, 6 months	9–14 years: 2-dose schedule, with the second dose given 5–13 months after the first dose
Recommended schedule from 14/15 years of age	From 15 years: 3-dose schedule at 0, 1, 6 months	From 14 years: 3-dose schedule at 0, 2, 6 months	From 15 years: 3-dose schedule at 0, 2, 6 months
Indications	Prevention of premalignant lesions and cancers affecting the cervix, vulva, vagina, and anus caused by specific oncogenic HPV types	Prevention of premalignant lesions and cancers affecting the cervix, vulva, vagina, and anus caused by specific oncogenic HPV types; genital warts (condyloma acuminata) caused by specific HPV types	Prevention of premalignant lesions and cancers affecting the cervix, vulva, vagina, and anus caused by vaccine HPV types; genital warts (condyloma acuminata) caused by specific HPV types

Information from summary of product characteristics available on EMA website (Cervarix: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000721/WC500024632.pdf; Gardasil: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000703/WC500021142.pdf; Gardasil 9: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003852/WC500189111.pdf)

[a]*VLPs* virus-like particles

amorphous aluminum hydroxyphosphate sulfate adjuvant. It was approved by the FDA and the EMA in 2006.The bivalent (2vHPV) vaccine protects against HPV types 16 and 18. This vaccine is formulated with a novel adjuvant, AS04, which contains aluminum hydroxide and monophosphoryl lipid A. It was first approved in Europe in 2007 and then in the USA in 2009.

The clinical trials (RCTs) conducted before vaccines' registration showed high immunogenicity and efficacy of both 2vHPV and 4vHPV vaccines.

Both vaccines were proved to induce high levels of serum antibodies against all vaccine-related types in more than 99% of females aged 9–45 years (4vHPV vaccine) or 10–55 years (2vHPV vaccine). A peak antibody titer was observed 4 weeks after the third dose and declined within the first year, then stabilized at a plateau titer after 18 months. The serological response after vaccination is much stronger than the response after natural infection. The highest immune responses were observed in girls aged 9–15 years [15].

Neutralizing antibodies to HPV are thought to be important in protection. However, a serologic correlate of protection has not been identified,

and the minimum antibody level required for clinical protection is unknown.

In the clinical trials, both vaccines showed a high efficacy (90–100%) against infection and cervical lesions associated with HPV-16 and HPV-18 in women not already infected with HPV. The efficacy was lower (50–60%) if measured in the whole study population, including all women independently of their HPV status [16, 17].

Therefore, in order to guarantee the highest efficacy of the vaccines, they should be administered, if possible, before the onset of sexual activity, i.e. before first exposure to HPV.

Since their approval, both quadrivalent and bivalent vaccines have been widely introduced in immunization schedules at the global level. They are routinely recommended during pre-adolescence [15], usually at the age of 11–12 years [18, 19]. In some countries, concomitant catch-up vaccination programs for older ages have been implemented to broaden coverage.

20.3 From 2/4vHPV to 9vHPV Vaccine

In order to extend the protection against a higher number of HPV types, a second generation 9-valent HPV vaccine (9vHPV) was developed. This vaccine includes, beyond HPV 6, 11, 16, and 18, also HPV 31, 33, 52, 58 (phylogenetically similar to HPV 16), and 45 (phylogenetically similar to HPV 18). The main characteristics of the three HPV vaccines are summarized in Table 20.1.

Comparison of 9vHPV against placebo was not acceptable since HPV vaccination is already recommended and implemented in many countries. RTCs comparing 9vHPV and 4vHPV recipients showed non-inferior immunogenicity of the 9vHPV vaccine compared to the 4vHPV vaccine for HPV types included in both vaccines [20]. The efficacy of the 9vHPV vaccine was demonstrated in more than 14,000 women aged 16–26 years vaccinated with the 9vHPV or the 4vHPV vaccine [21]. The incidence of both persistent infection and low-/high-grade disease related to HPV types 6, 11, 16, and 18 was similar in the 9vHPV and 4vHPV groups. The risk reduction for both persistent infection and low-/high-grade disease due to the new vaccine types included in the 9vHPV was very high (almost 97%) for most of the considered endpoints among HPV non-infected women.

The 9vHPV vaccine was approved in December 2014 by the US FDA and granted marketing authorization by the European Commission in June 2015. The US Advisory Committee on Immunization Practices (ACIP) recommended 9vHPV as one of three HPV vaccines that can be used for routine vaccination in February 2015 [22].

In order to estimate the protection induced by the 9vHPV vaccine, it is essential to evaluate the frequency distribution of the different HPV types in cervix cancer and the other tumors of the anogenital apparatus and the oropharynx.

Among women with invasive cervical cancer, the 14 most common HPV types are HPV 16, 18, 45, 33, 31, 58, 52, 35, 59, 39, 56, 51, 68, 66, worldwide [23]. HPV 16 is consistently the most common type and HPV 18 is generally the second most common type. The other HPV types are also quite similar across world regions but the relative importance of each HPV type appears to vary across countries.

HPV oncogenic types included in the 9-valent vaccine are those most frequently associated with cervical cancer. At the global level, in fact, it is estimated that seven HPV types included in the 9-valent vaccine contribute to about 90% of cervix cancer [11, 23, 24].

Figure 20.1 shows the 14 most frequent HPV types associated with cervical cancer worldwide, accounting for 96.4% of all [23]; it is estimated that the inclusion of additional five types could increase of about 18% the potential of protection of the HPV vaccine against cervical cancer: moving from 71.5% (the proportion of cancers associated with HPV 16 and 18) to 89.9% (the proportion of cancers associated with all the seven high-risk types included in the second generation HPV vaccine).

Due to variation in HPV-type specific prevalence and distribution, this proportion varies

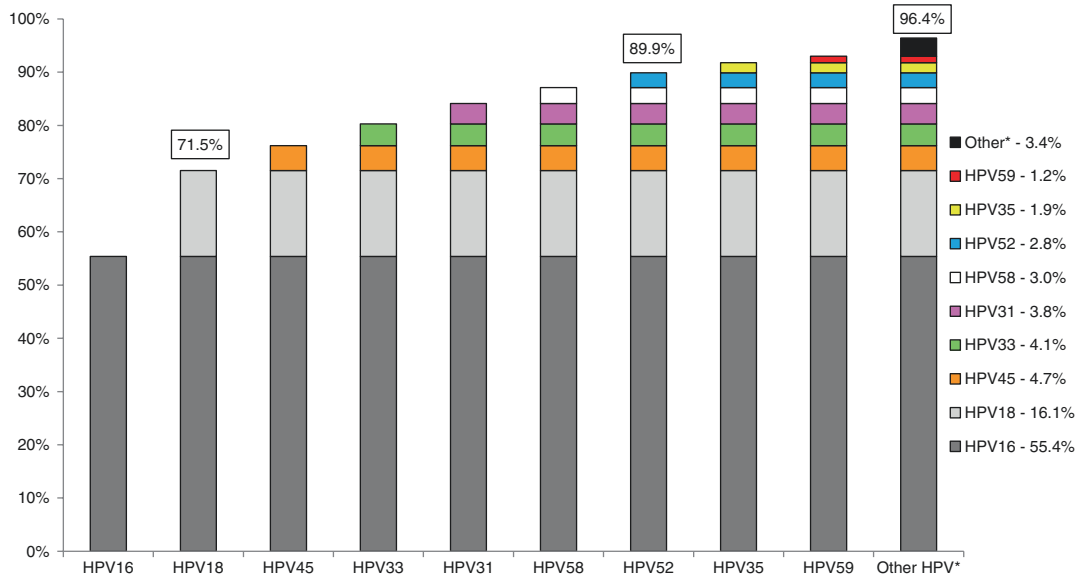

Fig. 20.1 Fourteen most frequent HPV types in cervical cancer worldwide (cumulative frequency) (modified from Zhai and Tumban [23])

Table 20.2 Ten most frequent HPV types in HPV-related cancers by cancer site, worldwide (modified from de Sanjosé et al. [25])

Cancer site	Rank order of HPV types									
Cervix	16	18	33	45	31	58	52	35		
Vulva	*16*	*18*	*33*	*6/11*	*45*	*52*	51	62	42	56
Vagina	*16*	40	*6/11*	*31*	*33*	*18*	*58*	35	39	*45*
Penis	*16*	*18*	*6/11*	22	74	*31*	*45*	*33*	34	*52*
Anus	*16*	*18*	*33*	*31*	*6/11*	*45*				
Oropharynx	*16*	*33*	35	*18*	26	*6/11*	*45*	*52*	*58*	51

Note: the HPV types included in the 9-valent vaccine are italicized

across countries and it is estimated that 9-valent vaccine will offer protection against HPV types associated with about 87.7% of cervical cancers in Asia, 91.7% in Africa, 92% in North America, 90.9% in Europe, 89.5% in Latin America and the Caribbean, and 86.5% in Australia.

HPV type distribution also varies between squamous cell carcinoma (SCC) and adenocarcinoma (ADC), with HPV16 identified more often in SCC than in ADC, and HPV18 and 45 identified more often in ADC than SCC. de Sanjosé et al. reported that in more developed countries, HPV 16 and 18 account for the 59.8 and 10.9% of SCC, respectively, and for 37.8 and 36.2% of ADC ,respectively. HPV 45 is the third most frequent type identified in ADC both in less and more developed regions, accounting for 4.7 and 5.6% respectively [25]. The inclusion of HPV 45 type in the vaccine could increase the protection against ADC and it is especially important because this is a tumor originating from the epithelium of the cervix channel, therefore, less likely to be diagnosed in the early stage through the Pap-test [26].

As reported in Table 20.2, the HPV types newly included in the 9-valent vaccine are those

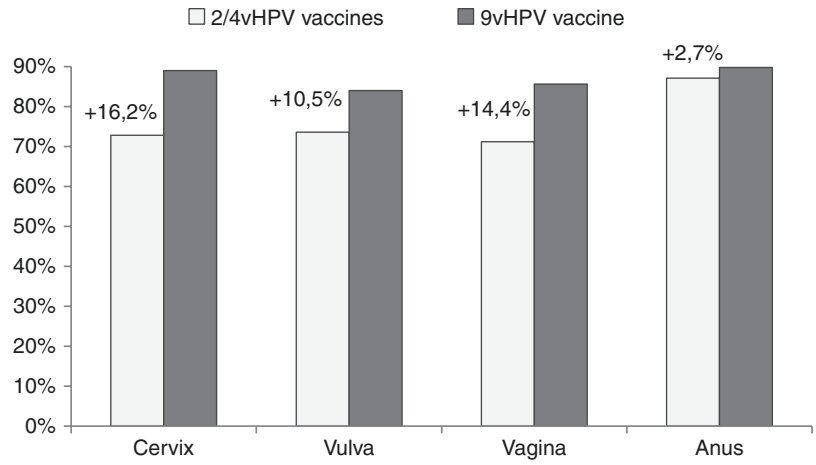

Fig. 20.2 Proportion of HPV-related cancers preventable by vaccines of first and second generation, by cancer site (European data) (modified from Hartwig et al. [27])

most frequently associated with a series of HPV-related tumors other than cervix cancer. With the exception of HPV 40, which ranks second among vagina cancers but is not included in the vaccine, HPV types 33 (second position among oropharyngeal tumors), 31, and 45 are commonly found among several tumor types at the global level [25].

Hartwig et al. estimated that the 9-valent, compared to the bi/quadrivalent, could increase of 16.2% the protection against cervix cancer moving from 72.8% to 89.0% in Europe. In Fig. 20.2 it is reported the proportion of HPV-related cancers preventable by vaccines of first and second generation, for other female cancers [27].

It has been also estimated that the 9-valent could increase of 19% the protection against cervix, vulva, vagina, and anus cancers both among women and men. When including also precancerous lesions in women and men, such as CIN2+, VIN2/3, VaIN2/3, and AIN, the protection would increase of 75% [27]. This is particularly important since the use of HPV vaccine may reduce the burden of invasive procedures and related costs.

As 4vHPV vaccine, the 9vHPV vaccine also protects against HPV 6 and 11, responsible for about 90% of genital warts. Genital warts represent a substantial burden of HPV-related diseases and are at high risk of relapse, determining high economic and social costs. For example, in Italy, an economic study reported that the costs associated with genital warts in men and women corresponded to almost one-quarter of the total costs associated with HPV 6, 11, 16, 18 [28].

Thus, although accurate estimates of the number of cases of cancer, pre-neoplastic lesions, and genital warts that may be prevented are not available, and even though HPV prevalence data vary by geographic area, it is clear that increasing the number of oncogenic HPV types included in the vaccine is convenient in terms of impact on HPV related-disease burden reduction, assuming that the efficacy per single type remains the same.

The potential public health impact and cost-effectiveness of 9vHPV have been explored by mathematical models comparing immunization programs with 9vHPV to different alternative strategies with 4vHPV and 2vHPV under different efficacy, costs, and vaccine coverage scenarios [29–33]. Although the estimates depend on assumptions, different mathematical models performed in USA, Australia, and some European countries [28, 34–36] predict that the switch to the nonavalent vaccine in different settings can further reduce the burden associated with cervical cancer and HPV-related diseases and is highly cost-effective, and even cost-saving in some of them.

However, importantly, the magnitude of the incremental benefits of the nonavalent vaccine in preventing cervical cancer outcomes strongly depends on assumptions regarding the level and duration of cross-protection provided by bivalent or quadrivalent vaccines, coverage rates, and the price of novel vaccines. The 2017 WHO position

paper on HPV vaccine reports that, in high income countries, switching to a nonavalent vaccine program is predicted to be cost-effective or cost-saving if the cost/dose of the nonavalent is assumed to be 10–15% greater than that of the quadrivalent vaccine [15].

20.4 Vaccine Schedules

All HPV vaccines were initially licensed and marketed in a 3-dose schedule (0/1/6 or 0/2/6 months) for recipients starting from the age of 9 years and older. Subsequently, a 2-dose schedule (6–12 months apart) was approved for all the three vaccines for boys and girls before 15 years of age (Table 20.1).

Licensure of the 2-dose schedule was based on the demonstration of non-inferiority of the immune response, comparing the antibody levels after a 2-dose regimen in girls aged 9–14 years to those of the 3-dose schedule in young adult women (15–25 years) in whom the efficacy was demonstrated [37].

In 2014, the WHO recommended to use a 2-dose schedule before 15 years of age [38, 39]. Since the year 2014, several European countries have implemented the 2-dose schedule [40]. In the USA the CDC updated HPV vaccination recommendations introducing the 2-dose regimen in 2016 [19]. The 2-dose schedule has important benefits, such as easier logistics, reduced expenditure, potentially higher acceptance, and fewer side effects. Follow-up studies are needed to assess long-term protection of the 2-dose schedule.

20.5 Duration of Protection of HPV Vaccines

Immunogenicity and efficacy of a 3-dose schedule against infection and cervical lesions associated with HPV 16 and HPV 18 have been proved up to more than 9 years after immunization for 4vHPV and 2vHPV vaccines, and for at least 5 years for the nonavalent vaccine [15, 41].

A 2014 systematic review and meta-analysis was performed to evaluate the duration of protection afforded by HPV vaccines. It included 15 studies focusing on women aged 9–26 years, HPV 16/18 negative, or not yet sexually active. The RCTs included a total of 46,436 participants. During the period of short-term follow-up (median, 3 years), the pooled efficacy of vaccination was 83% (95% CI: 70–90%) for the study endpoint of incident HPV infection (percentage of infections prevented), 90% (95% CI: 79–95%) for persistent HPV infections, and 84% (95% CI: 50–95%) for CIN 2+ lesions. During the period of long-term follow-up (median, 6 years), incident infections were prevented with 94% efficacy (95% CI: 80–98%), persistent infections with 95% (95% CI: 84–99%), and CIN 2+ lesions with 86% (95% CI: 166–99%). The authors concluded that long-term observation does not indicate any loss of antiviral protection after vaccination against HPV 16 and 18 [42].

On the basis of current data, a booster dose is not recommended up to now; long-term follow-up of immunized cohorts will clarify if a booster is needed several years after first series or not.

Antibody titers measured after a 2-dose schedule of the bivalent and quadrivalent vaccines in girls aged 9–14 years remained comparable to those with a 3-dose schedule in women up to 5 years after first vaccination, indicating they have a similar kinetics. Follow-up studies are needed to monitor long-term protection after 2-dose schedule.

20.6 "Real-World" Impact of Bivalent and Quadrivalent HPV Vaccines

Although high efficacy against multiple endpoints was consistently observed in clinical trials, it is essential to document how trial results translate to real-world settings. The real-world benefits of vaccination against associated cancers may not be evident for decades, given the slow progression from infection to invasive disease, but population impact against earlier outcomes (genital warts, HPV infection, and pre-cancer lesions) has already been clearly demonstrated in numer-

ous settings worldwide, subsequent to initiation of HPV vaccination programs.

Vaccine impact first became apparent for HPV infections and genital warts, which have a short incubation period, following exposure to HPV. Availability of population-based comparison data from the pre-vaccine era facilitated the demonstration of vaccine impact. The first available data came from Australia, one of the first countries introducing HPV vaccination programs, where high coverage rates were reached and where cytological screening is implemented at a younger age than in most other countries.

20.6.1 Effect on Ano-Genital Warts and HPV Infections

In Australia, national surveillance data showed marked reductions in the frequency of ano-genital warts, particularly in the youngest age groups at vaccination. A decrease of 92.6 and 72.6% reduction was observed in women <21 and 21–30 years of age, respectively, 4 years after vaccination program implementation (since 2007–2011). Furthermore, significant declines occurred under 21-year-old (81.8%) and 21–30-year-old (51.1%) heterosexual men diagnosed as having genital warts in the vaccination period, consistent with herd protection [43].

The reduction in genital warts was documented in other countries, varying widely depending on setting, age group, and time period considered. In Italy, an observational study was conducted to estimate the trend of hospitalization for genital warts in a region of Northern Italy (Veneto) from 2004 to 2015 [44]. Among females, the rate of overall genital warts remained stable up to 2007 (19.1 per 100,000), then dropped significantly. Among the potentially vaccinated females (12-to-20-year-old), there was a 62.1% decrease in the number of vulvar/vaginal warts from the years 2010–2012 to the years 2013–2015, probably due to an increase in the HPV coverage rate (about 75%). A similar reduction among males was observed in the same period and the same age group for penile warts (68.2%). High vaccine effectiveness against genital warts has been reported among females also in Canada (Ontario) and in Spain (Valencia), and among females and males in Denmark, Israel, and Belgium [41, 45].

More recent studies showed a high effectiveness of 4vHPV on genital warts, using two doses administered 6 months apart, or even more, among females in the USA [46, 47] and, similarly, in Sweden, indicating that two doses of 4vHPV given 4- to 7-months apart are as effective as three doses [48]. Further studies are needed to consolidate these results.

Reduced prevalence of HPV vaccine types has been widely reported both in males and women [41]. Scottish data have also demonstrated substantial declines in HPV infection as the first cohorts of girls vaccinated with 2vHPV enter screening, and confirmed the degree of cross-protection against HPV types seen in the 2vHPV trials [49].

A 2015 meta-analysis including 20 studies from nine countries found that herd protection effects and cross-protection against non-targeted HPV types were dependent on higher coverage being achieved [50]. In fact a significant reduction of HPV type 16 and 18 infections and genital warts between the pre-vaccination and post-vaccination periods was observed in young girls under 20 years both in countries with female vaccination coverage of at least 50% and under 50%. Instead, a significant reduction of HPV type 16 and 18 infections and genital warts was observed in girls older than 20 years and in males (indicating herd effect) and reduction of HPV infections of types 31, 33, and 45 among young girls only in countries with coverage ≥50%.

20.6.2 Effect on Low- and High-Grade Cervical Abnormalities

Subsequently, as successive birth cohorts began cervical screening, a reduced risk of cervical lesions, which take longer to develop, became apparent. High effectiveness of HPV vaccination on CIN2 and CIN3 lesions was demonstrated in several countries [51], with greater effectiveness observed among girls who were younger when

they started vaccination [15, 52–54]. For example, in a nationwide Swedish register-based cohort study, where vaccinated (with 4vHPV vaccine) and unvaccinated women were followed for a mean of 2.6 and 5.1 years, respectively, CIN2+ and CIN3+ among fully vaccinated females <17 years of age at vaccination decreased by 75% and 84%, respectively, compared with unvaccinated or partially vaccinated females; in contrast, among those vaccinated between 20 and 29 years of age, CIN2+ and CIN3+ declined by 22% and 25%, respectively [54].

Again, in a nationwide analysis from Denmark, CIN2+ and CIN3+ declined by 73% and 80%, respectively, in the youngest birth cohort eligible for vaccination (born 1993–1994), and 12% and 22% in the oldest eligible birth cohort (born 1989–1990), compared with unvaccinated women [52]. A similar age-related risk reduction was also observed in Australia [55–57] and USA [58].

In Australia, where declines in CIN following 4vHPV vaccination were first documented in 2014, high-grade CIN rates decreased from 10.9 per 1000 screened women in 2006 (the year prior to vaccination) to 5.0 in 2013, $p < 0.0001$ in the <20 years age group, while continuing to slowly rise in older age groups (women aged 25–29 years and older than 30 years) [55]. In 2016, an update of this data was published, showing that reductions in rates of high-grade CIN in young women were continuing and were demonstrable also in women aged 25–29 years [56].

HPV vaccines effectiveness generally corresponded to vaccine efficacy results from clinical trials. This data refer to the experience from bivalent and quadrivalent vaccines. Follow-up studies of cohorts vaccinated with 9-valent vaccines are needed to estimate the effectiveness of this more recent product.

20.7 Vaccine Safety Profile

Since the licensure of HPV vaccines, over 270 million vaccine doses have been globally distributed, with solid safety data gathered from pre-licensure studies and post-marketing surveillance. A good safety profile of HPV vaccines emerges from all the available data. Injection-site reactions, including pain, redness, and swelling, are the most common adverse events reported for all the vaccines in the pre- and post-licensure phase, more common in recipients of 2vHPV and 9vHPV than 4vHPV. Pain is the most frequently referred local symptom after each dose, being reported by 80% of the cases. Systemic symptoms, generally mild and self-limiting, such as headache, syncope, and fever are reported by 10–30% of the cases [59]. HPV vaccination has also been associated with syncope in adolescents, although this is related to the vaccination process rather than to the vaccine and can be avoided with appropriate care. Although not recommended for use during pregnancy, pregnancy registry data have not found an increase in adverse pregnancy outcomes among those inadvertently vaccinated during pregnancy [15, 60].

Several comprehensive vaccine reviews of pre- and post-licensure safety data found that the incidence of serious adverse events (SAEs) was variable in different studies. In most cases, causal association was studied and no association with HPV vaccination was found, the incidence of these events being similar in both vaccine and control groups [41, 59, 61]. Moreover, in 2017, the WHO commissioned a systematic review of serious adverse events following HPV vaccines, considering as outcomes all SAEs medically significant conditions, new onset of chronic diseases, and death. Data for 73,697 individuals were reviewed and no difference in rates of selected SAEs between exposed and unexposed to HPV vaccine was observed [62].

Although case reports have identified a range of new onset chronic conditions occurring post-vaccination, including autoimmune diseases, evidence from these well-conducted population-based studies has consistently not identified any association between HPV vaccine and such conditions. Data are reassuring that HPV vaccine does not increase the risk of Guillain–Barré syndrome. A review of post-licensure safety surveillance during more than 4 years of routine use of the bivalent vaccine found no patterns suggesting an increased risk of

immune-mediated diseases after vaccination, and observed incidence rates of Bell's palsy and Guillain–Barré syndrome within the expected range in the general population [62]. Similarly, other studies have not detected any association with a range of conditions, including chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). A large, nationwide, population-based study including the first six birth cohorts of girls offered quadrivalent HPV vaccine through the national immunisation programme in Norway, observed an increase in the incidence of CFS/ME among adolescents aged 10–17 years, during 2009–2014, which was similar among girls and boys and not associated with HPV vaccination [61].

Concerns have been raised about complex regional pain syndrome (CRPS) and postural orthostatic tachycardia syndrome (POTS) following HPV vaccination. Despite the difficulties in diagnosing both disorders, reviews of pre- and post-licensure data provide no evidence that these syndromes are a direct effect of the HPV vaccines. In 2015, the European Medicines Agency has carried out a review of available data and the Pharmacovigilance Risk Assessment Committee stated that there is no evidence to support a causal relationship between HPV vaccines and the emergence of CRPS or POTS [63].

The Global Advisory Committee on Vaccine Safety (GACVS) regularly reviews global data on HPV vaccine safety, taking into consideration post-licensure surveillance data and high-quality studies, case reports, and anecdotal concerns arisen since the licensure of HPV vaccines. In June 2017, the GACVS concluded that the available evidence did not suggest any safety concern regarding the use of HPV vaccine [15, 62].

Despite the extensive safety data available for this vaccine, attention has continued to focus on sporadic case reports that raised large attention by the media and, in many cases, negatively impacted on the immunization programs due to concern in the general population. Strengthening of adverse events surveillance systems and their investigation for causality assessment is necessary to collect evidence-based data needed to improve the communication by public health authorities.

20.8 Public Health Value of Universal Vaccination

Although females have been the primary target of HPV vaccines, extending the program towards males may have some benefit. The public health impact of HPV infection is more evident in women; however, high-risk HPV types are also responsible for other significant cancers like anogenital and head-and-neck cancers, not necessarily correlated to female gender.

In USA, during 2004–2008, 33,369 HPV-associated cancers (10.8 cases per 100,000) were notified annually: 12,080 cases were among males (8.1 per 100,000) and 21,290 among females (13.2 per 100,000). Cervical cancer was the most frequently notified, with about 11,967 cases per year, immediately followed by oropharyngeal cancer, with 11,726 cases per year. Furthermore, 2370 head-and-neck cancers were registered among females and 9356 among males. The rate of head-and-neck cancers among males was four times higher than among females (6.2 versus 1.4 per 100,000). Anal cancer was more common among females (1.8 per 100,000) than among males (1.2 per 100,000) [64].

In Europe, Hartwig et al. [65] estimated about 50,000 new cases of HPV-related cancer every year. Even if the highest burden is related to cervical cancer, with about 35,000 new cases/year, other types of tumor represent about a third of all HPV-related cancer cases. Among males, it is estimated that about 9500 new HPV-related tumors are notified every year, including cancer of the anus, penis, and head-and-neck. Also in Europe, most of head-and-neck cancers affect men: among those attributable to HPV, 5834 cases yearly occur in men and 1396 in women. In addition, more than 650,000 new genital warts cases attributable to HPV 6 and 11 occur yearly in Europe, approximately half in men and half in women.

A gender-neutral approach may not only reduce HPV-related diseases in males but also reduce the infection and transmission to females, ultimately reducing the pool of infectious virus in a community (herd immunity). Male vaccination would confer an even greater benefit in settings

where female vaccination rates are low. Moreover, men who have sex with men (MSM), a high-risk HPV group, are less likely to benefit from a female-only vaccination program: even if all girls were immunized, the HPV chain of transmission would still be maintained through MSM.

In addition, universal vaccination would satisfy the principle of equity and equal access to health care, giving men and women the same rights to protection. Previous experience in gender-restricted vaccination programs has demonstrated a substantially lower effectiveness than universal vaccination; instead, universal vaccination would normalize HPV vaccination to become a standard vaccination in pre-adolescents. Moreover, limiting vaccination to girls might increase the psychological burden on girls by confirming a perceived inequality of the sexes [66, 67]. Hence, vaccination of males has recently been incorporated into several national programs in USA, Canada, Australia, and some European countries as Austria, Croatia, and Italy [18, 19, 68].

While clinical benefits of including boys in vaccination programs have been clearly demonstrated, the most debated issue is the cost-effectiveness profile of universal vaccination.

A large number of economic evaluations have been published that assess alternative HPV vaccination strategies [69]. Whether numerous published studies have shown that vaccinating pre-adolescent girls is usually cost-effective if compared to screening only [15, 67], the cost-effectiveness of universal HPV vaccination still remains an open debate.

Assessment of cost-effectiveness of HPV vaccines is heavily influenced by several factors: type of HPV-associated disease considered as outcome, impact of herd immunity, vaccine efficacy and cross-protection, duration of vaccine protection, number of HPV strains included in the vaccine, vaccine price, number of doses per recipient, and country-specific factors, such as HPV prevalence, coverage rates, uptake of cancer screening, and health costs. Thus, it is difficult to compare different cost-effectiveness models.

With regard to cervical cancer protection, WHO stated that if the HPV vaccination coverage in girls is higher than approximately 50%, gender-neutral vaccination is unlikely to be cost-effective (vs girls-only vaccination). Below the level of 50% vaccination coverage among girls, vaccination of boys may be cost-effective in some settings, depending on costs involved, epidemiology of HPV-related diseases, and programmatic issues [15].

In the last ECDC guidance on the introduction of HPV vaccination in European Union countries, universal HPV immunization programs including boys were considered too expensive compared to the potential benefits. This was in accordance with several original studies and review articles on the economic impact of HPV vaccination already published at the time the guidance was issued [67]. However, these models rely on assumptions, such as duration of protection, coverage rates among girls, incidence of HPV-related morbidities in both sexes, and price of the vaccine. Thus, in 2014 ECDC stated that the cost-effectiveness of including boys in HPV vaccination programs should be reassessed when more solid data are available for baseline assumptions, and especially if vaccination costs are significantly reduced in the future [40].

Indeed, many of these factors have changed over time: (a) an increase in incidence of the oropharynx cancers (and in particular of the quota attributed to HPV) has been described, especially in the most developed countries; (b) a two-dose schedule was approved in 2014 for both vaccines when used in younger subjects; (c) the price of the vaccine has greatly decreased worldwide. The latter two parameters are a crucial change for the economic sustainability of universal vaccination programs.

The oldest reviews published on this topic concluded that, with the old parameters, the cost-effectiveness of universal vaccination was controversial and generally unfavorable if compared to female-only vaccination or to strategies increasing coverage among girls. The most recent reviews, instead, suggest that when the burden of disease in men is included in the models then, depending upon coverage, vaccine price, and other factors, universal vaccination may become cost-effective [68, 70, 71].

A 2015 systematic review including 15 economic studies [72] found that the extension of HPV vaccination to boys was cost-effective or potentially cost-effective in nine studies, while six studies indicated that universal vaccination was not cost-effective. However, if the input parameters of the six studies not favorable to universal vaccination were updated with a lower vaccine price and a two-dose schedule, the results would become cost-effective in four out of these six studies.

Concluding, there is a general agreement that economic studies should be updated assuming more recent vaccination costs and immunization schedules, and including all HPV-related clinical outcomes to obtain a more accurate cost-effectiveness profile.

Haeussler et al. found that the universal HPV vaccination strategy was cost-effective when compared with either cervical cancer screening or female-only vaccination within the Italian context, using a dynamic Bayesian methodology and including all HPV-related health states (cervical, vaginal, vulvar, anal, penile, and head-and-neck cancer as well as ano-genital warts) as outcome [73].

20.9 Implications for Cervical Cancer Screening

None of the currently available vaccines offers complete protection against all oncogenic HPV types; any delay in the vaccination age may lead to exposure to HPV; vaccination uptake is still sub-optimal in some areas. For these reasons, HPV vaccination does not eliminate the need for cervical cancer screening programs.

In recent years, primary HPV testing has been evaluated extensively as a cervical screening approach. Numerous randomized controlled trials have been conducted to evaluate the effectiveness of primary HPV DNA testing as compared to cytology-based screening. They have consistently demonstrated that primary HPV testing is more sensitive for the detection of CIN2+ at baseline resulting in decrease detection at subsequent screening rounds (i.e., lower cumulative incidence rate) [74–76]. Several Health Technology Assessments (HTA) of HPV testing as primary cervical cancer screening method were also produced [77, 78]. These documents advised moving to HPV-based screening and provided detailed protocols.

On the basis of this evidence, HPV testing has been, or is going to be, introduced as the primary method of population-based screening for cervical cancer in several countries, as Australia, Italy, The Netherland, New Zealand, and UK [75, 79–81].

Women vaccinated against HPV16/18 are approaching the age for cervical screening. In countries where the screening program starts at age 25, women vaccinated at age 12 will enter the screening program from 2021.

The implementation of HPV vaccination programs could lead to revise cervical cancer screening policies in HPV-vaccinated women, due to the strong reduction in prevalence of CIN2+ among vaccinated women, depending on the lower prevalence of infections by high-risk HPV types, and the lower risk of progression to CIN2+ of infections from non-HPV16/18 genotypes. In addition, the number of clinically relevant lesions will decrease in an even greater proportion compared to the reduction of cytological alterations, and therefore also the positive predictive value (VPP) of cytology for CIN2 + will decrease substantially.

Several model-based analyses have been recently conducted to evaluate how screening policies may be optimized in vaccinated women, clearly indicating that the screening program should be personalized based on vaccination status. Kim et al. demonstrated that, given the expected lower risk of cervical cancer in HPV-vaccinated women, screening can be modified to start at later ages, occur at decreased frequency, and involve primary HPV testing [82]. A microsimulation model, developed by Landy et al. in UK, found that HPV16/18-vaccinated women require three lifetime screens, HPV16/18/31/33/45/52/58-vaccinated women require two lifetime screens, yet unvaccinated women require seven lifetime screens [80].

Up to now, current cervical cancer screening guidelines do not differentiate recommendations

based on women HPV vaccination status worldwide. However, the opportunity to tailor cervical cancer screening programs to vaccination status is a matter of debate within the scientific community.

In Italy, a Consensus Conference was organized in 2015 to identify the actions to be implemented in order to optimize the integration of screening and HPV vaccination programs. The Jury considered changing the screening protocols for girls vaccinated in their twelfth year as appropriate. Tailored screening protocols based on vaccination status could be replaced by "one size fits all" protocols only when a herd immunity effect has been reached. Vaccinated women should start screening at age 30, instead of 25, with HPV test. Furthermore, they agreed on a strong rationale for applying longer intervals for re-screening HPV negative women than the currently recommended 5 years, but research is needed to determine the optimal screening time points. For non-vaccinated women and for women vaccinated in their fifteenth year or later, the current protocol should be kept [83].

In Ireland, the Health Information and Quality Authority (HIQA) published an HTA of HPV testing as the primary screening method in 2017, following a request of Ireland's National Cervical Screening Programme. The HIQA advised the Ministry of Health to change to primary HPV screening followed by liquid-based cytology triage at five-yearly intervals for all eligible women aged 25–60 years. They also added that, given their lower risk of developing cervical cancer, screening women vaccinated against HPV at five-yearly intervals may not be cost-effective. However, given the uncertainty about this cohort, screening at five-yearly intervals should be applied also to HPV-vaccinated cohorts while giving consideration to increasing the screening interval as evidence emerges to support the long-term effectiveness of screening women vaccinated against HPV [77].

Concluding, less intensive screening recommendations could be appropriate for women who were vaccinated before sexual debut; different screening algorithms for vaccinated and unvaccinated women are needed, at least until herd immunity data confirms it is safe to reduce screening in unvaccinated women. This requires the linkage of vaccination status to the screening program.

20.10 Conclusions

HPV vaccines represent a milestone in the fight against virus induced tumors. In the 10 years since the introduction of both bivalent and quadrivalent HPV vaccines, population-based comparison data with the pre-vaccine era and observational studies allowed us to assess the global effect on HPV infection, as well as associated disease, in countries that have implemented HPV vaccine public health programs. There is evidence that vaccination significantly reduces the prevalence of high-risk HPV types, the incidence of ano-genital warts, and high-grade cervical abnormalities. More time for follow-up is needed to determine the effect on cancer rates, as carcinomas develop decades after infection.

The 9-valent vaccine provides further opportunities in terms of prevention compared to the first generation products. With the inclusion of HPV types 31, 33, 45, 52, and 58, the vaccine could increase the protection against cervix cancers moving from about 70 (the proportion of cancers associated with HPV 16 and 18) to 90%. It is also estimated to provide a high reduction of the burden of pre-neoplastic cervical lesions from the current 46–82% [27]. Moreover, there is an additional protection fraction regarding tumors involving the anus, vulva, penis, vagina, and oropharynx.

A universal HPV vaccination program could greatly reduce the incidence of new HPV-related diseases by direct boys immunization but also by indirect protection of unvaccinated girls. Taking into account all HPV-related clinical outcomes, the more recent vaccine price and the 2-dose schedule, the extension of HPV vaccination to males could be cost-effective.

Concluding, HPV vaccines appear to confer an advantage in terms of public health contributing to greatly reduce the burden of HPV-associated disease.

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21 Molecular and Immunological Strategies Against *Treponema pallidum* Infections

Mark C. Fernandez and Lorenzo Giacani

21.1 Introduction

Syphilis continues to be a significant global and public health problem, with an incidence of nearly 12 million new cases every year and a global burden of at least 36 million infected individuals [1]. Because the agent of this disease, the spirochete *Treponema pallidum* subsp. *pallidum* (*T. pallidum* hereafter) can cross the placenta, syphilis is also a major cause of stillbirth and perinatal morbidity and mortality in settings with poor prenatal care [2]. Furthermore, syphilis is a demonstrated co-factor in the transmission and acquisition of the human immunodeficiency virus (HIV), and mathematical models suggest that improved syphilis control would decrease HIV incidence as well [3–5]. Concern is also caused by the widespread genetic resistance to macrolides developed by *T. pallidum* and the fact that this class of antibiotics is no longer a treatment option in substitution of benzathine penicillin G [6, 7]. The recent global re-emergence of syphilis supports that the current measures to contain the spread of this disease are no longer sufficient. This emphasizes, of course, the need for enhancing surveillance networks and improving case/contact finding and treatment, but also the need for new research tools to deepen our still limited knowledge on the networks of transmission of syphilis and the development and spread of antibiotic-resistant strains. To this end, a major innovation in the field is represented by current efforts to develop *T. pallidum* molecular assays and typing methods. These approaches aim at detecting antibiotic resistance and to differentiate strains of *T. pallidum* and, at the same time, facilitate molecular epidemiology studies, and possibly identify *T. pallidum* strain types associated with specific clinical manifestations of the disease. In parallel, the recent identification of new vaccine candidates for syphilis and the introduction of innovative vaccine designs in the syphilis field are indication of renewed interest in syphilis vaccine development, even though the peculiar biology of *T. pallidum* along with the inability to grow this pathogen in vitro inevitably hinders progress toward this ambitious goal. This chapter will focus on the

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past and current efforts to develop, implement, and improve a molecular typing method for *T. pallidum* strains and a practical syphilis vaccine.

21.2 Molecular Typing of *Treponema pallidum* Subsp. *pallidum* Strains

Molecular typing of *T. pallidum* has been increasingly adopted in countries with resurgent syphilis [8] with the overall goal of identifying genetic diversity in circulating *T. pallidum* strains and better define the epidemiology of syphilis. If combined with the patient travel history as well as clinical, demographic, and behavioral records, molecular epidemiology data should facilitate the understanding of *T. pallidum* acquisition and transmission dynamics, and inform current and future syphilis control and elimination campaigns. Furthermore, early data suggest that there may be a link between defined genetic traits in *T. pallidum* and the likelihood of developing specific clinical manifestations in infected patients, such as ocular syphilis and neurosyphilis [9–11], even though such associations have yet to reach statistical significance.

21.2.1 Typing Methods

In 1998, at the American Centers for Disease Control and Prevention (CDC), Pillay and colleagues developed the first molecular method to be ever applied to group *T. pallidum* strains into discrete typing units [12]. Their method relied on the identification of 1) the number of 60 bp-repeats in the *T. pallidum* acidic repeat protein (*arp*) gene (*tp0433*) by means of amplification of the repeat-containing portion of the gene, and determination of the number of repeats based on the amplicon size and of 2) the restriction pattern length polymorphism (RFLP) generated by digestion with the *MseI* enzyme of three small amplicons obtained from the paralogous genes *Treponema pallidum* repeat (*tpr*) *E* (*tp0313*), *tprG* (*tp0317*), and *tprJ* (*tp0621*) by means of multiplex amplification [12]. Although the role of the proteins encoded by these genes in syphilis pathogenesis is not fully understood, the presence of inter-strain variability within the *arp* and the *tprE/G/J* loci made them suitable candidates for strain typing [13, 14]. The original *tprE/G/J* RFLP patterns were designated by Pillay et al. as "a" through "g" [12]. Since then, additional restriction patterns have been described in *T. pallidum* strains and called "h" through "q" [15–17], while the "r" pattern was found in *T. pallidum* subsp. *pertenue* (the causative agent of the endemic treponematosis yaws) Samoa D isolate [18] (Fig. 21.1a). To date, the shortest and longest number of *arp* repeats identified are three and 25 [32–34], corresponding to PCR amplicon sizes of 512–1,832 bp, respectively (representative samples are shown in Fig. 21.1b) due to the fact that in addition to the 60-bp repeats, 5′- and 3′-flanking regions of the gene are also amplified. Although nucleotide polymorphisms can be identified within the *arp* repeats, and there are at least eight different types of repeats (Type I–III, Type II–III, and Type IV–VII), such differences are not taken into account by the CDC typing method [13]. As a consequence of this typing system, strain types began to be identified with an alphanumeric code representing the number of *arp* repeats followed by the *tprE/G/J* restriction pattern. Hence, a *T. pallidum* strain determined to contain 14 *arp* repeats and a "d" RFLP pattern for *tprE/G/J* would be called "14d." To improve the discriminatory power of this approach, Katz et al. [35] introduced a third component to the typing system, represented by the amplification and sequencing of a poly-G homonucleotide repeat within the *tp0279* gene, with the goal of identifying sub-types within *T. pallidum* types. A sub-type would be designated based on a combination of the number of *arp* repeats, the RFLP pattern of the *tpr* genes, and the number of G residues within *tp0279*. This sub-typing approach, however, was short lived, likely because of increasing experimental evidence that poly-G tracts in *T. pallidum* are highly variable within a strain, and therefore not ideal targets for typing [36, 37].

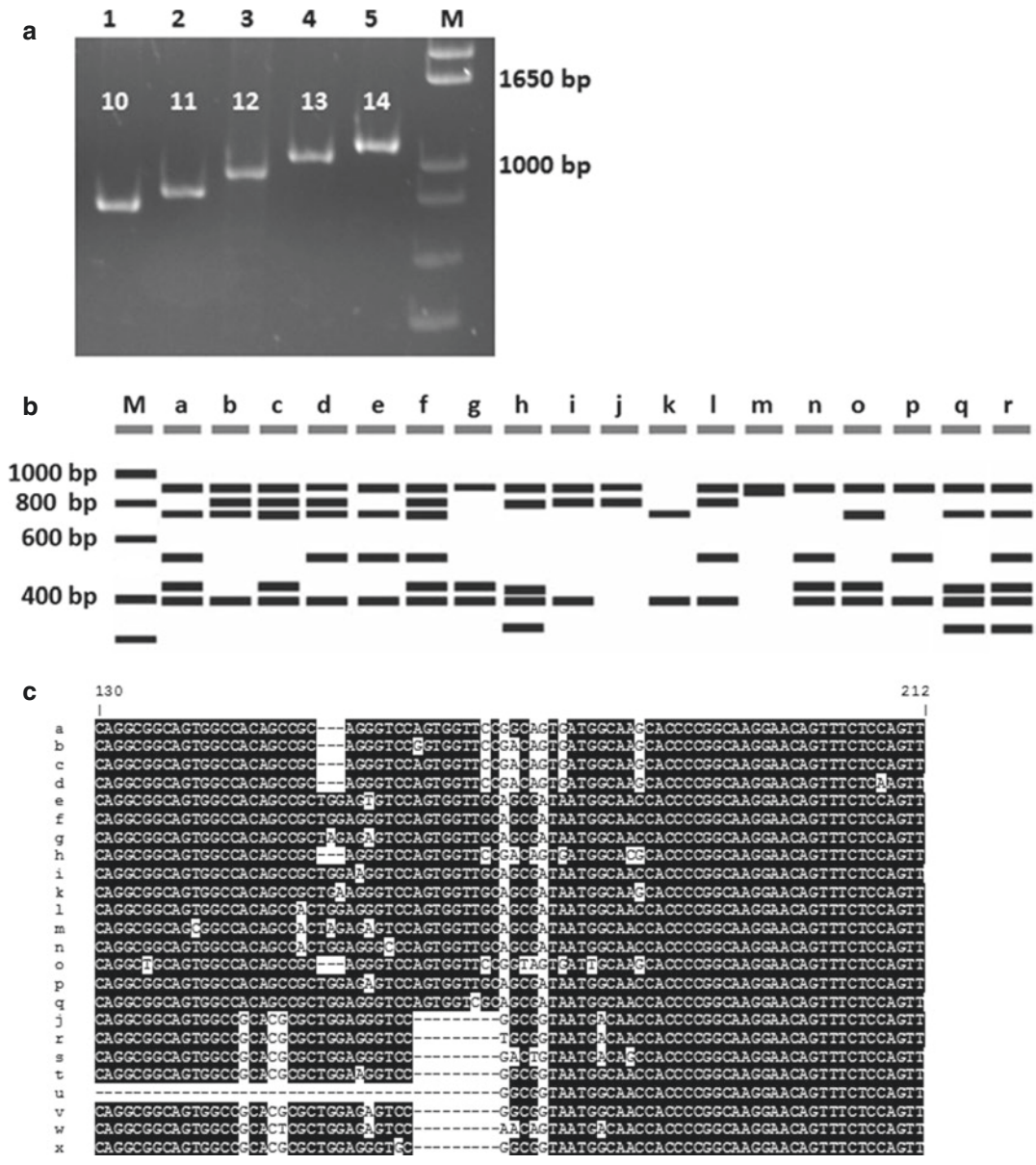
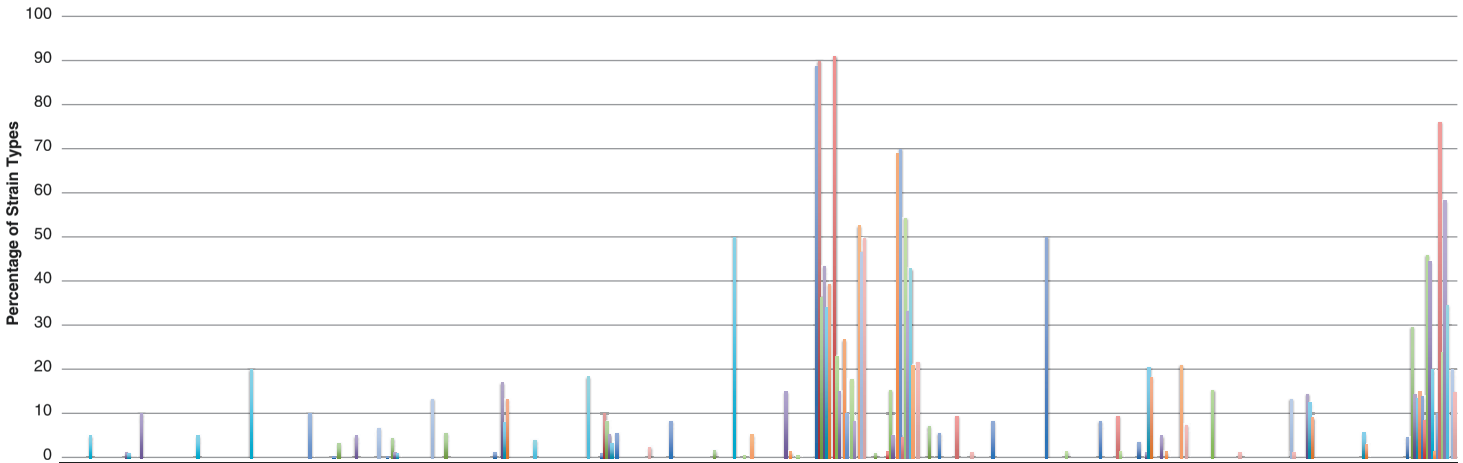


Fig. 21.1 Targets of the enhanced CDC typing protocol for *T. pallidum*. (**a**) Representative samples of the *arp* PCR showing different amplicon sizes, each different by 60-bp. Number of repeats is shown above each band. Sample 1–5: *arp* amplicons; M: molecular size marker. Gel picture courtesy of Charmie Godornes. (**b**) Schematic representation of the RFLP patterns of *tprE/G/J* amplicons digested with *MseI*. Published references for each RFLP type are as follows: a–g [12], h, j, k [19], i [20], l [16], m, n [17], o [21], p [16, 22], q, r [15, 23]. Modified from reference (17). M: Molecular weight marker. (**c**) Sequences of *tp0548* types "a" through "x." *tp0548* sequences from different strains of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*. Nucleotide coordinates shown above the alignment are based on the Nichols strain genome (GenBank AN AE000520.1). Published references for each *tp0548* type are as follows: a–i [11], j [15], k [24], l [25], m, n [26], o [27], p [28], q [29], r–u [30], and v–x [31]

Twelve years after the publication of the CDC typing system, Marra et al. [11] demonstrated that the addition of a new typing target to the ones described above would significantly increase the discriminatory power of the CDC typing method without changing its overall level of technical complexity or the bench time required to perform the procedure. The newly selected target was an 83-bp highly polymorphic region of the *tp0548* gene, predicted to code for a putative surface-exposed protein involved in long fatty acid transport across the bacterial outer membrane. By adding this target, 173 samples studied by Marra and colleagues could be divided into 25 unique strain types, while the original CDC two-component typing system would have separated them in only 14 types. Currently, there are 18 published *tp0548* variants from *T. pallidum* strains, designated "a" through "p" [31] (Fig. 21.1c). There are however additional *tp0548* variants that were isolated from *T. pallidum* subsp. *pertenue* (variants "r" through "w") and *T. pallidum* subsp. *endemicum* (the agent of Bejel, variant "j") and the unclassified Fribourg-Blanc isolate of *T. pallidum* (variant "u") [31]. For type nomenclature, Marra's approach uses the same code introduced by the CDC, to which the letter corresponding to the *tp0548* sequence is added. For example, a 14d strain with a *tp0548* "f" sequence would now be designated as 14d/f. This enhanced CDC typing method is currently the most widely used *T. pallidum* typing system. Marra and colleagues have also demonstrated the efficacy of their method as an epidemiological tool to track the changing landscape of strain variability in syphilis patients in Seattle. In their study, the authors demonstrated that the 14d/f strain was predominant from 2001 to 2004, to be then replaced by the 14d/g type [11], demonstrating a practical use of the typing system. According to the current method of strain typing, the 14d/f and 14d/g strains are the most prevalent worldwide [38]. This may indicate some significant fitness advantage or increased virulence of these types over others. Interestingly, the shift from 14d/f to 14d/g as the most common circulating type in Seattle reported by Marra and colleagues coincided with an increased incidence of syphilis in the same region [11], which could indicate higher virulence for this strain type. This prevalence seems to be relatively consistent: a recent strain typing study by Giacani et al. [39] suggests that most strains circulating in Northern Italy are 14d/g. This is also the predominant type in other European countries, as reported by studies conducted in Ireland, England, France, and the Czech Republic [15, 24, 38, 40]. An updated global distribution of *T. pallidum* strains is provided in Fig. 21.2.

21.2.2 Clinical Relevance of Strain Typing

In their original publication of the enhanced CDC typing method, Marra et al. also observed an association between the 14d/f type with the development of neurosyphilis [11], even though that specific linkage was not always confirmed in studies by others [41, 42]. A similar association between molecular type and clinical manifestations of syphilis was investigated during a recent increase in cases of ocular syphilis in the United States [9], but no significant correlation was found between ocular involvement and *T. pallidum* types identified in these patients [43]. Although so far we have been unable to establish a significant connection between *T. pallidum* genetic variants and specific disease manifestations, the importance of such studies cannot be overstated. As mentioned, in addition to causing neurological involvement, *T. pallidum* can cross the placenta and cause congenital infection. In the United States alone, the rates of congenital syphilis (CS) have increased from 9.1 cases per 100,000 live births in 2012 to 11.6 cases in 2014. Of all CS cases reported in the United States in 2014 ($n = 458$), 5.5% of the infants affected were stillborn, and 1.7% newborns died within 30 days of delivery [44]. Such scenario warrants increased efforts to determine if any association exists between strain type and baleful CS outcomes. As clinical strain typing data continues to be generated from patients with specific manifestations, overrepresented strains should be studied also in vivo using the rabbit model of syphilis infection. This could help attribute virulence characteristics to specific types empirically.



	4d/c	7d/f	10c/c	10d/c	10d/g	11d/f	12d/f	12e/f	13d/f	13d/g	14a/f	14b/c	14d/c	14d/d	14d/f	14d/g	14e/f	14f/c	14f/f	14j/g	15d/f	15d/g	16a/e	16d/f	17d/f	Other
Shanghai, China						0.33	0.33		1.3		1				88.8						3.6					4.63
Nanning, China											10				90											0
Hunan, China						3.3	4.4	5.5			8.3		1.66		36.5	1.1	7.2									29.66
East China		1.3					1.3		17.1		5.3				43.4						1.3			14.5		14.4
South China		1.1					1.1		8		3.4				34.1						20.5			12.5	5.7	13.4
North China									13.2						39.4						18.2			9.1	3	15
Taiwan											5.56	8.33					5.56	8.33	50	8.33						13.9
Tuva, Russia															91	1.4										8.5
Lima, Peru															23.1	15.4						15.4				46
Buenos Aires, Argentia		10				5								15	15	5					5					44.5
Madagascar	5		5	20									50													20
Paris, France														1.41	26.8	69					1.41					1.41
Dublin, Ireland					10										10	70										10
Antwerp, Belgium																4.76	9.52			9.52						76.18
Denmark													0.51	0.51	17.77	54.31			1.52	1.52						23.88
London, UK															8.3	33.3										58.3
Italy									4.08	18.37						42.86										34.69
San Francisco, United States													5.26		52.63	21.05					21.05					0
Baltimore, United States						6.67	13.33								46.67								13.33			20
Seattle, United sates											2.48				49.69	21.74	1.24				7.45	1.24	1.24			14.89

Fig. 21.2 Global distribution of *Treponema pallidum* major strain types. References for the reported data are [21] for Shanghai, [11] for Nanning, [168] for Hunan, [34] for East, South and North China, [59] for Taiwan, [42] for Tuva, [169] for Lima, [51] for Buenos Aires, [11] for Madagascar, [15] for Paris, [11] for Dublin, [28] for Antwerp, [170] for Denmark, [39] for Italy, [40] for London, [11] for San Francisco and Baltimore, and [43, 65] for San Francisco. "Other" category includes partially typed samples. Numbers in table below graph represent strain type percentages

Neurological and ocular invasion by *T. pallidum* in rabbits have been already documented [45, 46], and a rabbit model of congenital syphilis has also been reported [47–49].

21.2.3 Sequence-Based Multi-Locus System for Typing (MLST)

The enhanced CDC typing method is the most widely adopted approach to type *T. pallidum*, with over 2000 samples typed in the past seven years from virtually all over the world. Nonetheless, this typing protocol is complex to execute, time-consuming, and sometimes results are hard to interpret. Therefore, development of a sequencing-based multi-locus system for typing (MLST) would be desirable. Such approach would no longer rely on the procedures to identify the number of *arp* repeats or the restriction patterns of the *tprE/G/J* amplicons. The need for a MLST typing scheme became more impellent after unexpected differences in strain type were seen in specimens collected in parallel from different anatomical sources of the same patient [50]. These results led the authors to believe that the *arp* and *tpr* loci were genetically too unstable to be typing targets. Confidence in the enhanced CDC typing protocol, however, was strengthened by both Pillay et al. [12] and Marra et al. [11], who showed that a strain type was stable with repeated rabbit passages of the Nichols, Sea81-4, and Chicago isolates of *T. pallidum*, respectively. Additional issues with the enhanced CDC typing system include poor and inconsistent amplification of the *arp* target from patient samples, especially from blood [19, 51, 52], and inability to perform phylogenetic analysis of *arp* and *tprE/G/J* types, which would allow for a finer analysis of strain-strain relatedness, potentially improving epidemiological tracking of *T. pallidum*. Alternative MLST methods based exclusively on sequencing of variable loci are currently under scrutiny in the author`s laboratory and others [24, 50, 53]. Currently, the only published sequence-based typing approach is based on the analysis of a 1206 bp fragments of the *tp0136* gene, a 629 bp fragment amplified from each of the two *T. pallidum* 23S rRNA genes and a large fragment (1065 bp) of the *tp0548* reported to be more discriminative than the 83 bp fragment used by the enhanced CDC method [51]. Additional possible targets include the *tp0304*, *tp0346*, *tp0488*, *tp0515*, and *tp0558* genes, used by Nechvátal et al. to differentiate SS14-like from Nichols-like strains of *T. pallidum* [54].

Compared to the enhanced CDC typing system, this MLST approach is in its infancy, with less than 200 published fully typed samples [24, 28, 51, 53]. In the future, improved sequencing-based methods for *T. pallidum* typing will be facilitated by the increasing number of whole genome sequences from historical and recent strains and isolates. Currently there are 42 *T. pallidum* subsp. *pallidum* published genomes, 86% of which were released in 2016. As existing genomes continue to be analyzed and new ones are published, new genes and gene combinations with higher discriminatory power than any typing method already described may be discovered.

21.3 Syphilis and the Threat of Antibiotic Resistance

Syphilis has been successfully treated with penicillin for over 70 years now, since the introduction of this antibiotic at the end of World War II, and benzathine penicillin G (BPG) remains the drug of choice for treatment unless the patient reports allergy to this medication. In such cases, antibiotics such as doxycycline, tetracycline, ceftriaxone, and amoxicillin are the recommended alternatives. In a world where antibiotic resistance is a growing problem for global and local health, *T. pallidum* has remained exquisitely sensitive to BPG, and no evidence whatsoever of genetic resistance to this antibiotic has ever emerged in this pathogen. Widespread within the syphilis research community is also the opinion that acquired genetic resistance to β-lactams will not arise in *T. pallidum*, mostly because the syphilis agent seems to lack mechanisms for horizontal gene transfer. On the other hand, development of endogenous resistance would likely require a complex multi-step mutation/selection process to convert one of the pathogen's penicillin-binding proteins (PBPs) into a *bona fide* β-lactamase [55,

56]. *T. pallidum* certainly has the capacity for endogenous genetic changes that can lead to antibiotic resistance (as described below for resistance to macrolides), and the appearance and selection of mutations that could lead to a true penicillin-resistant strain cannot be completely excluded. Nonetheless, because this scenario has yet to manifest in spite of a continuous use of BPG, the threat of a penicillin-resistant *T. pallidum* seems unlikely for the time being.

The need for intramuscular injections of BPG, and the possibility of an allergic reaction to penicillin led some clinicians to use alternative antibiotics for treatment, such as macrolides, including erythromycin, azithromycin, clarithromycin, and spiramycin as first-line drugs for early syphilis. Macrolides could be administered in a single oral dose [57], avoiding painful BPG injections and also issues related to treatment adherence. In 2003, however, azithromycin treatment failure was first documented in a small patient population in San Francisco [58]. In the 14 years since, macrolide resistance rates in *T. pallidum* have soared globally. [24, 26, 32, 39, 40, 42, 59–63]. Currently, there are two known mutations in the 23S rRNA genes of *T. pallidum* that have been experimentally linked to macrolide resistance. These mutations are adenine to guanine transitions at residues 2058 or 2059 and are readily identifiable by RFLP screening [64–66]. Macrolide-resistant *T. pallidum* appears to be uncommon only in those countries where the use of these antibiotics is not widespread [51, 67]. At this time, macrolide resistance is the only clinically significant obstacle in syphilis treatment, and this class of antibiotics is no longer recommended. The global prevalence of macrolide–resistant *T. pallidum* is shown in Fig. 21.3. Although no mutations linked to doxycycline resistance have ever been described in *T. pallidum*, resistance to this antibiotic could

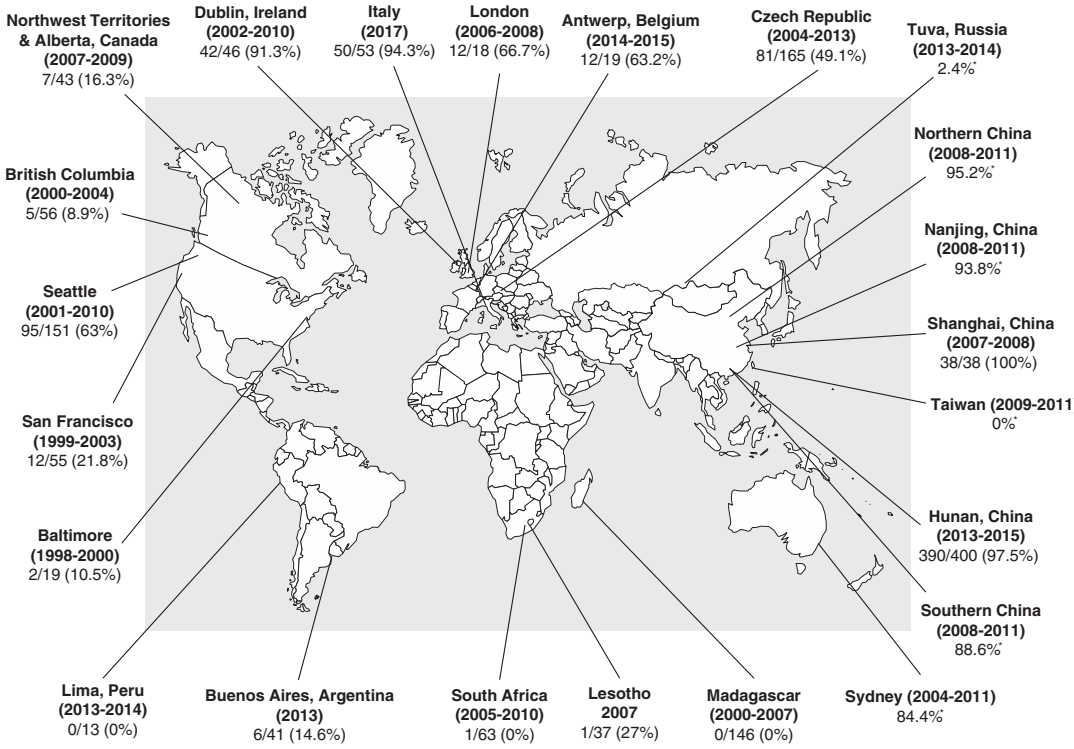


Fig. 21.3 Prevalence of macrolide resistance in *T. pallidum* by country, city, or geographical area. Values represent all samples tested to date (∗Sample number unclear). References for the reported data are [39] for Italy and, moving clockwise, [40] for London, [28] for Antwerp, [53] for the Czech Republic, [42] for Tuva, [171] for N. China, [172] for Nanjing, [60] for Shanghai, [59] for Taiwan, [168] for Hunan, [171] for S. China, [26] for Sydney, [67] for Madagascar, [32] for Lesotho and South Africa, [51] for Buenos Aires, [169] for Lima, [63] for Baltimore, [63] for San Francisco, [63, 65] for Seattle, [61] for British Columbia, [173] for Northwest Territories, and [63, 174] for Dublin

potentially arise as quickly as they did for macrolides. Poor adherence to doxycycline treatment could expose *T. pallidum* to sub-therapeutic doses of this antibiotic and induce a selective pressure fostering the appearance of resistance-inducing mutations. Single point mutations that confer such resistance have been already described in the 16S rRNA gene of *E. coli*, *H. pylori*, and *B. hyodysenteriae* [68, 69].

21.4 The Rationale for a Syphilis Vaccine

Syphilis is very amenable to control by vaccination: there is no known animal reservoir for *T. pallidum* and simple diagnostic tests, now available also in a convenient point-of-care format [70], allow for an easy determination of syphilis prevalence in target populations or geographical areas, so that interventions can be appropriately focused. On the other hand, unfortunately, the relatively low incidence of syphilis with respect to other curable sexually transmitted infections [1], the low cost of diagnosis and treatment [57, 71], and the fact that this infection affects somewhat marginalized populations and minorities reduce the global interest in syphilis vaccine development.

21.4.1 The Quest for an Effective Syphilis Vaccine.

In 1973 Miller reported what is to date believed to be the only successful vaccination experiment to have achieved durable sterile immunity against syphilis [72]. In his work, Miller immunized Dutch belt rabbits intravenously with 3.7 billion γ-irradiated *T. pallidum* (Nichols strain) cells over a seven-month period, with weekly or semi-weekly injections. Ten days after the last immunization, animals were challenged intradermally (ID) with infectious Nichols cells but did not develop any visible lesions in the following 3-month observation window. Although the animal became positive for to both non-treponemal and treponemal serological tests, which is generally consistent with active infection, asymptomatic infection in the challenged animals was ruled out by performing rabbit infectivity tests (RITs). For the RITs, following euthanasia, testicular tissue and lymph nodes from the challenged animals were homogenized and injected into naïve rabbits that did not become infected. This result supported that there were no live treponemes in the transferred tissues and confirmed the efficacy of Miller's immunization approach. In the same work, an additional group of rabbits was still protected following challenge with *T. pallidum* one year after receiving the last immunization, showing the durability of the immunity induced in these animals [72]. Due to its very laborious nature, this study was never reproduced or independently confirmed but, nonetheless, provided a much-needed proof of concept that a vaccine against syphilis is possible. Miller himself acknowledged that this procedure could not be translated into humans for many reasons, including the exhausting immunization protocol and the fact that the *T. pallidum*-specific serologic reactivity induced by the vaccination would not be distinguishable from that caused by natural infection, in case the subjects underwent syphilis testing. Nonetheless, Miller's work had embedded pivotal concepts that still guide investigators currently involved in the quest for a syphilis vaccine. Such concepts include, for example, the need to preserve the structure of the antigens inducing sterile immunity, which was suggested by the use of a physical (γ-irradiation) rather than a chemical method to inactivate *T. pallidum* cells. Additionally, a series of studies preceding Miller's successful experiment suggested that the preservation of the *T. pallidum* outer membrane (OM), the cellular compartment that most likely harbors the protective antigen(s), was essential for success. Furthermore, the use of a high dose of treponemal cells in combination with a prolonged immunization cycle supported that the protective antigen(s) were either weakly immunogenic or poorly expressed in *T. pallidum*. In previous attempts using lower doses of γ-irradiated treponemes and shorter immunization periods resulted in only partial or no protection from infection [73, 74].

21.4.2 Early Syphilis Vaccinology: Whole Cell and Subunit Vaccines

Miller's experiment is still the only successful case over a very long series of failures to induce protective immunity in susceptible animals with killed or attenuated *T. pallidum* cells. The last vaccination experiments using whole-cell preparations date back to the early 1990s and were performed by Fitzgerald [75], who believed in the now-discounted theory that *T. pallidum*'s ability to escape immune clearance during early syphilis was caused by the downregulation of the initial Th1-mediated immune response in favor of a Th2 response. Fitzgerald, therefore, immunized rabbits with heat-inactivated *T. pallidum* cells and subsequently tried to support a Th1-type response with the administration of cyclophosphamide, monophosphoryl lipid A, and indomethacin. This approach, however, failed to induce sterile immunity and even increased pathogen dissemination to distant sites following challenge [76, 77]. Investigators also attempted immunization with different *Treponema* species, based upon their antigenic relatedness to *T. pallidum* and their ability to be grown in vitro. All immunizations with *T. phagedenis* [78, 79], *T. refringens* [79, 80], *T. minutum*, *T. ambiguum*, *T. microdentium* [79], *T. paraluiscuniculi* [81], and *Spirochaeta aurantia* [82], however, resulted in absent or, at best, partial protection after challenge with *T. pallidum*.

At the same time, the increasing understanding of antigenic relatedness among different treponemal species fostered the use of subcellular fractions and recombinant proteins as experimental vaccines in place of whole cells. For example, Hindersson et al. [83] immunized rabbits with purified endoflagella from *T. phagedenis* to try and induce sterile immunity against syphilis. The rationale for selecting such antigen was justified by the presence of shared epitopes between the *T. phagedenis* and *T. pallidum* homologous proteins [84], even though Hindersson was well aware of the lack of surface exposure of this cellular structure. The outcome of the experiment showed no protection following infectious challenge. A similar experiment that also failed to provide protective immunity was later attempted by Champion et al. [85] with purified *T. pallidum* endoflagella. Based on the disappointing results of these studies compared to Miller's work, the general concept emerged that antigens exposed on the surface of *T. pallidum* would be the most likely targets to induce sterile immunity. This belief launched an active effort in many laboratories to identify the antigens residing on *T. pallidum* surface at the interface between the pathogen and the host. Such focus on surface-exposed targets was also supported by an improved knowledge of the pathogenesis of the disease, and specifically that immune clearance of *T. pallidum* in early lesions is mediated by phagocytosis of opsonized *T. pallidum* cells by activated macrophages [86]. Investigators, therefore, inferred that targets of the opsonic antibodies were perhaps the key to a successful and practical syphilis vaccine.

In the 1980s the identification of putative *T. pallidum* surface-exposed antigens proteins seemed to be easily achieved. Libraries of *E. coli* expressing *T. pallidum* antigens were engineered, and *T. pallidum*'s major immunogens began to be identified based on the reactivity of *E. coli* colonies with sera from syphilis-infected rabbits or patients [87–89]. Proteins like the *Treponema* membrane protein A (TmpA, or TpN44.5), B (TmpB), and C (TmpC, TpN35) [90, 91] were among the first isolated antigens. The membrane location of the Tmp proteins was supported by the evidence that they were synthesized in *E. coli* as larger precursors, that the amino-terminal sequence was predicted to contain a signal peptide for the sorting of the mature peptide to the bacterial envelope, and finally that fractionation studies of *E. coli* cells expressing the Tmp proteins showed the tendency of these antigens to localize in the hydrophobic phase containing the cell membranes. Nonetheless, protective immunity was partial or absent following immunization/challenge studies with recombinant Tmp proteins [92, 93]. Later studies of radiolabeled fatty acid incorporation into *T. pallidum* membranes lead to the conclusion that these antigens were in reality lipoproteins, and their observed hydrophobicity

was actually due to protein acylation with lipid moieties that anchor the antigens to the periplasmic side of the inner membrane (IM), rather than to actual membrane-spanning domains of the protein [94, 95]. Lack of protection using these antigens in immunization/challenge experiments would, therefore, be explained by the lack of surface exposure. The list of *T. pallidum* periplasmic lipoproteins initially mistaken for OM antigens, unfortunately, is not short [94–103].

21.4.3 The Impediments to the Identification of Protective Antigens

Understanding the failures described above becomes easier when the limitations posed by working with *T. pallidum* are considered. Such limitations hamper the ability of syphilis investigators to apply many approaches to identify surface-exposed antigens and therefore putative vaccine candidates in *T. pallidum.* First, as mentioned, the syphilis spirochete cannot be propagated in vitro, and the bacterial cells rapidly die outside of a susceptible host. The New Zealand white rabbit is currently the only means available for in vivo propagation of *T. pallidum,* achieved by serial intratesticular (IT) passages of the syphilis agent from an infected animal into a naïve rabbit approximately every ten days. However, the slow growth rate of *T. pallidum* (doubling time is ~30–33 h) [104, 105] and the rapid onset of both innate and adaptive host immune responses in the rabbit make it very difficult to recover large numbers of organisms from an infected animal, prohibiting experimental approaches that requires high numbers of live bacterial cells. The failure to maintain *T. pallidum* viability in culture [106–108] also precludes genetic manipulation of the pathogen, preventing any strategy to functionally inactivate, delete, and complement genes and analyze directly how lack of potential virulence factors influence the pathogenesis of the disease. The ongoing quest for vaccine candidate antigens finds obstacles also in the biochemical composition and structural organization of the *T. pallidum* envelope. Unlike more conventional Gram-negative bacteria, the *T. pallidum* OM does not contain lipopolysaccharide [109–114], and the similarity between the lipid composition of the outer and inner membranes [115] discourages the use of separation techniques to isolate OM fractions from the cytoplasmic membrane, in that such methods inevitably result in contamination of the OM fractions with periplasmic antigens [95]. Furthermore, although in most Gram-negative bacteria the peptidoglycan layer is associated with the inner leaflet of the OM, this layer overlies the cytoplasmic membrane in *T. pallidum* [116, 117]. Such feature is responsible for the extremely fragile nature of the *T. pallidum* OM and difficulty in applying common immunological methods (i.e., surface immunofluorescence staining or immunoelectron microscopy) without compromising the integrity of the OM and exposing internal antigens to the immunological probes. Last but not least, the OM of *T. pallidum* was shown by freeze-fracture and deep-etch electron microscopy to have an unusually low density of integral membrane proteins [118, 119], which causes this cellular compartment to be only modestly immunoreactive and certainly does not facilitate the identification of such rare antigens.

21.5 Animal Models in Syphilis Research and Vaccinology

The rabbit (*Oryctolagus cuniculus*) is the most widely used animal model to study experimental syphilis [120], and has advantages over guinea pigs, hamsters, mice, and nonhuman primates. Such advantages mostly reside in the parallels between the experimental infection in rabbits and natural syphilis infection in humans that are not seen when smaller rodents are used instead [93, 121, 122]. Like humans, rabbits are highly susceptible to *T. pallidum*, with an identical ID_{50} [104, 123], and develop skin lesions following ID infection that are clinically and histologically similar to primary human chancres [120, 124]. The repertoire of *T. pallidum* antigens that elicit humoral immune responses is also similar in rabbits and humans, as are the

IFNγ-predominant cytokine milieu at the inoculation sites, and the development of partial immunity to reinfection [120, 124]. As in humans, rabbits remain chronically infected for a lifetime unless adequately treated [120]. Secondary lesions are sometimes seen in infected rabbits, but not manifestations of tertiary syphilis [120]. The disadvantages of the rabbit model include the high cost of the animals, the lack of inbred strains, and the lack of species-specific immunological reagents. The rabbit model is however excellent for assessing post-immunization protection against syphilis. Following immunization and ID challenge with *T. pallidum* on their shaved backs, test and control animals are monitored for lesion appearance and progression. Lesion appearance and progression on the skin of control rabbits devoid of fur closely resemble that of primary chancres in humans, and the degree of alteration of this course in immunized rabbits is proportional to the level of immunity, with complete immunity resulting in no clinical or serological evidence of infection. Partial immunity may manifest as the development of atypical lesions, such as flat non-ulcerating lesions, and a significantly lower burden of *T. pallidum* cells in most challenged sites. Partial protection, however, does not prevent the establishment of the infection, demonstrable by seroconversion of the animals.

Hamsters and guinea pigs have been used as alternative models [121, 125, 126] but mostly because of the availability of inbred strains. However, hamsters have reduced susceptibility to the syphilis spirochete compared to the other *T. pallidum* subspecies (subsp. *endemicum* and *pertenue*, the causative agents of endemic syphilis and yaws, respectively), and guinea pigs must be inoculated with extraordinarily high numbers of treponemes to induce lesion appearance [126]. The different histopathology of skin lesions between guinea pigs and humans also disfavor this animal model with respect to the rabbit. Although infection can be experimentally achieved in mice, these animals do not develop lesions, and therefore the model has not been deemed adequate [127, 128].

21.6 Current Vaccine Candidates

The modest immunological reactivity and the protein-poor content of the *T. pallidum* OM [86, 98, 99, 129–136] discussed above have not discouraged investigators from applying alternative approaches to identify surface-exposed antigens. Among all of these approaches, indirect analytical methods, such as comparative genomics, were the most effective in paving the way for the experimental identification of *T. pallidum* putative surface antigens. The initial search for homology between *T. pallidum* predicted proteins and previously characterized OM proteins of related bacteria, such as the oral pathogen *T. denticola*, has allowed the identification of an important first group of candidates, known as the *T. pallidum* repeat (Tpr) proteins, which are homologous to the *T. denticola* major sheath protein (Msp) known as a surface-exposed virulence factor with both porin and adhesin proprieties [114, 137–139]. Since their discovery, the Tpr antigens have been intensively investigated to confirm the original hypothesis of their surface exposure [14, 139–152]. Although only a few of those antigens have been unequivocally shown to reside within the OM, immunization/challenge experiments using laboratory-produced recombinant proteins based on the Tpr sequences have thus far produced the most promising results and are currently considered the most likely vaccine candidates for syphilis. The combination of in silico tools for prediction of signal peptides, membrane-spanning domains, and protein cellular location, has also led to the identification of the lipoprotein Tp0751. This protein was subsequently shown to encode for a laminin/fibronectin/fibrinogen binding protein [153–155], to be surface-exposed, and to likely foster pathogen dissemination to distant bodily sites from the site of primary infection by positioning the syphilis agent in the proximity of the vascular endothelium and also by acting as a proteolytic enzyme [156, 157]. Like the Tpr antigens, Tp0751 is regarded as a promising vaccine candidate for syphilis.

21.6.1 Tpr Antigens

The identification of the 12-membered family of genes coding for the Tpr antigens (named TprA-TprL) is one of the major findings of the *T. pallidum* genome project [113], in that these hypothetical proteins were immediately identified as potential virulence factors, surface-exposed antigens, and vaccine candidates [114, 139]. Some of the *tpr* genes had been identified earlier using subtraction libraries and other methods [139, 158], but the exact number of the genes composing this family (named *tprA-tprL*) was not anticipated until the genome sequence was released. These *tpr* genes account for a high percentage (~2%) of the small *T. pallidum* genome [139], suggesting an important role for these antigens in the survival of an organism that has lost many biosynthetic capabilities during its evolution to become an obligate human pathogen [113]. The Tpr antigens are divided into three subfamilies by their predicted amino acid homology: Subfamily I (TprC, D, F, and I), Subfamily II (TprE, G, and J), and Subfamily III (TprA, B, H, K, and L) [139]. Within the Subfamily I and Subfamily II members, the proteins have conserved amino- and carboxyl-terminal regions, with central domains that are variable in sequence and length, allowing the differentiation of the individual proteins [139]. Among Subfamily III members, the *tprK* gene undergoes extensive sequence diversification shown to accumulate during infection [140, 142, 149, 159]. In more detail, *tprK* heterogeneity is restricted to seven discrete variable regions (named V1–V7) [140], mostly located in the last half of the gene; these variable regions are flanked by conserved ones.

In all of the immunization studies performed with recombinant peptides representing an entire Tpr antigen or only a portion of the predicted protein, none of the challenged rabbits were fully protected. In several of these studies, however, significant alterations were noted in the appearance, progression to ulceration, and content of detectable *T. pallidum* following infectious challenge. Although incomplete, this level of protection using recombinant antigens was unprecedented in syphilis vaccinology and significantly contributed to bolstering the enthusiasm and the hope of many investigators that a syphilis vaccine was closer than ever. Antigens able to induce such level of protection were the TprK [139, 141, 160], TprI [145], and the TprF full-length peptides [161], the TprC/D and TprJ central regions, the amino-terminal conserved region of Subfamily I Tprs (TprC/D/F/I) and the carboxyl-terminal domain of Subfamily I members [161] as well as hybrid forms of the antigens (Fig. 21.4). Further studies identified the amino-terminal portion of the TprK antigen as the fragment inducing the highest level of protection, compared to the central and carboxyl-terminal regions [160].

There is obvious reason to be enthusiastic about the Tpr antigens as vaccine candidates, and studies are ongoing to refine the size and number of antigens to use and the immunization protocols. The preliminary studies described above strongly suggest that significant levels of immunity are induced by the amino-terminal regions of TprK and the Subfamily I Tprs. Ongoing work in the authors' laboratories is focusing on the combined use of these two antigens with a third one, the Tp0751 adhesin.

21.6.2 Tp0751

Attachment to host cells or tissue components is a crucial initial step in the pathogenic process that leads to the establishment of infection. Although *T. pallidum*'s ability to attach to various cultured mammalian cell types was first reported in the 1970s [162–164], the discovery of the receptors on the surface of *T. pallidum* that mediate such interactions is relatively recent. Among *T. pallidum* adhesins, Tp0751 was found to bind laminin, fibronectin, and fibrinogen [153–155] and to be the target of opsonic antibodies, findings that altogether strongly support its surface exposure [156]. Investigation of the protective ability of Tp0751 in immunization/challenge experiments led to the intriguing discovery that although immunity to Tp0751 does not confer protective immunity, it is nonetheless able to inhibit the dissemination of *T. pallidum* to distant

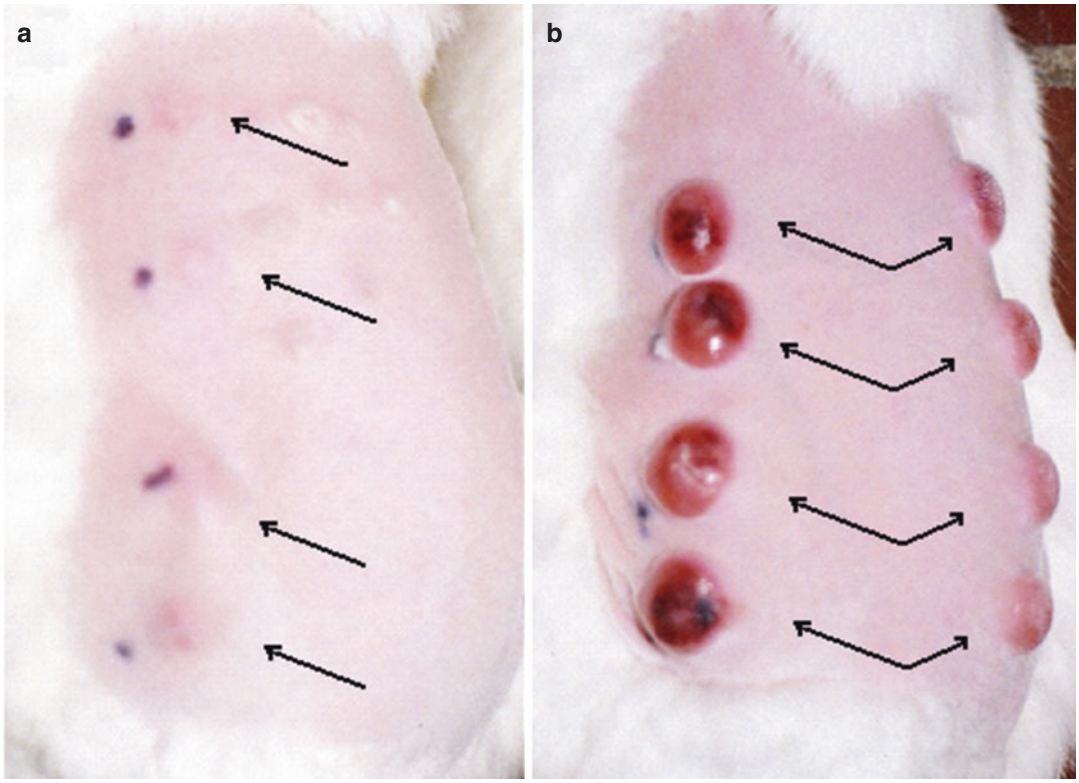


Fig. 21.4 Lesion development following intradermal challenge with *T. pallidum* (Nichols strain) in rabbits. (**a**) Rabbits immunized with a chimeric Tpr protein containing the NH_2-terminus of Subfamily I Tprs and the TprG central region. (**b**) Unimmunized animals. Pictures show shaved rabbit backs, where the treponemes were injected. In immunized rabbits, smaller lesions (arrows), containing fewer treponemes and not progressing to ulceration are observed compared to control animals at the same time point after challenge. In both animals, black dots mark the left of the site where treponemes were injected

organs in rabbits. Furthermore, lymph nodes recovered from Tp0751-immunized animals failed to induce a productive infection when injected into naïve rabbits in RIT experiments. These results collectively reiterate that Tp0751 immunization inhibits treponemal dissemination [156]. The combined use of the NH_2-terminal regions of TprK and the Subfamily I Tprs and Tp0751 currently is, in the authors' opinion, the most promising experimental vaccine for syphilis. These components could, in fact, work synergistically to (1) effectively reduce the treponemal burden in early lesions by favoring *T. pallidum* opsonophagocytosis (the Tprs) and (2) to inhibit *T. pallidum* dissemination from the site of infection (Tp0751). This innovative vaccine formulation is currently being experimented in the authors' laboratory.

21.7 Adjuvant Selection and Innovative Approaches to Syphilis Vaccine Design

There has also been wide variability in the immunization protocols used, particularly with regard to the duration of immunization, the dose of antigen, and the route of antigen administration. These factors are critical to successful immunization studies, and yet optimal protocols are determined almost completely empirically. There is no way to determine the correct combination of these parameters without actually conducting the lengthy and labor-intensive experiments. Adjuvant selection is another critical variable and, with no knowledge of markers of protec-

tive immunity, little guidance can be offered on adjuvant selection. Many recent immunization studies performed in the authors' laboratory have used an adjuvant containing monophosphoryl lipid A (a TLR4 agonist from *Salmonella enterica* serovar Minnesota), synthetic or natural trehalose dicorynomycolate from *Mycobacterium bovis*, cell wall skeleton from *Mycobacterium phlei*, with squalene and tween-80 as emulsifiers. Additional alternatives for the adjuvant portion of the vaccine are currently being explored, with particular attention to formulations already licensed for human use.

Live attenuated bacterial (LAB) vaccines still offer great promise to modern vaccinology [165, 166]. A LAB-based vaccine design has not been reported yet for syphilis, although it is currently being experimented in the author's laboratory. The administration of *T. pallidum* antigens expressed by a living carrier that is related to the syphilis agent but not pathogenic could provide several advantages. These include the natural adjuvant proprieties of the bacterial components administered jointly with the specific antigen but, above all, the potential for delivering an immunogen in its truly native conformation and in its natural cellular compartment. This, in turn, could foster a humoral response against conformational epitopes in addition to linear ones. To deliver *T. pallidum* antigens to the animal using a LAB vaccine we are currently using an engineered non-pathogenic strain of *Borrelia burgdorferi* (*B. burgdorferi* hereafter) that has permanently lost the ability to infect mammalian hosts and therefore only works as an antigen carrier/adjuvant once injected in the rabbit. This strain is also overexpressing OspC, a *Borrelia* lipoprotein known to be a TLR2 agonist, similar to *T. pallidum* lipoproteins [167]. Preliminary vaccination studies using *B. burgdorferi* expressing *T. pallidum* putative surface antigens like TprK (already shown to be partially protective when administered as recombinant proteins) are underway in the laboratory (Giacani et al. unpublished).

21.8 Conclusions

It is clear today that to establish infection, *T. pallidum* uses multiple strategies to evade the immune response of the host. These strategies exploit the limited surface immunogenicity due to a remarkable paucity of integral OM proteins, antigenic variation of putative surface antigens, phase variation, and likely additional but yet-unidentified mechanisms. The discovery of these multi-faceted strategies has strongly underlined the necessity to identify those immunogens that were ultimately responsible for the success of Miller's experiment and use them to develop an equally effective but more practical vaccine. Even with effective therapy and cheap diagnostic tools, syphilis control is only partially achieved throughout much of the world, and a vaccine would significantly help any disease control and eradication intervention. In the meantime, the application and improvement of existing methodologies for strain typing to unveil transmission networks of the disease and rates of antibiotic resistance, as well as the continuous work to improve our limited understanding of syphilis pathogenesis will hopefully remind our scientific community and general audience of the fact that syphilis is still a relevant public health problem that we shall not ignore.

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Natural History of Human Papillomavirus Anal Infection

22

Maria Gabriella Donà and Massimo Giuliani

22.1 Introduction

Human papillomaviruses (HPVs) are ubiquitous viruses that infect at least once in a lifetime virtually all individuals. More than 40 types of HPV are sexually transmitted and infect the ano-genital tissues. At least 13 of these ano-genital HPVs are considered carcinogenic to humans (high-risk types, HR-HPVs), and are recognized as the cause of several pre-cancerous and cancerous lesions in both men and women [1]. HPV-associated cancers account for almost 5% of all cancer cases worldwide and include those of the genital tract (uterine cervix, vagina, vulva, penis) as well as the anal canal and the head and neck region.

The incidence of genital HPV infection is very high among sexually active women. Cervical infections are detectable soon after the first exposure at sexual debut, and a cumulative incidence of 42% can be measured in 15–19 year-old women, whereas it decreases to 12% in those over 44 years [2]. Most cervical HPV infections, however, including those caused by oncogenic HPV types, are resolved spontaneously. Half of them clear within 6 months and the great majority within 20–24 months [3, 4]. Only a minority of HR-HPV infections persist and lead to the development of pre-cancer and cancer.

In recent decades, many efforts have been made to prevent cervical cancer with population-based screening programmes based on cervical cytology and colposcopy, with the aim to detect and treat pre-cancerous and cancerous lesions. After the successes reached in the control of cervical cancer in developed countries, interest in the pathogenic role of HPV infection partly shifted from female to male infections, and a multitude of studies have recently focused on the natural history and clinical course of HPV infection in males. The dramatic re-emergence of sexually transmitted infections (STIs) observed after the year 2000 in Western countries among high-risk male groups (i.e., men who have sex with men-MSM, HIV-1 infected patients) has further contributed to the promotion of studies which aim to describe the burden of genital and anal HPV infections in these populations. Alongside cross-sectional investigations, cohort studies have contributed to defining other relevant epidemiological measures of the infection, such as incidence (acquisition), and duration (clearance/persistence).

A particular interest of clinicians and researchers emerged for anal HPV infection. This was boosted by an increase in the incidence of anal squamous cell carcinoma (ASCC) recently observed in many developed countries in both

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genders. Annually, there is an estimated 27,000 new cases of anal cancer worldwide, with a female to male ratio of 5:1. In Europe, a total of 154,208 deaths due to malignant neoplasms of the colon, rectosigmoid junction, rectum, anus and anal canal were reported in 2015 [5].

The increase in ASCC incidence has been described particularly in individuals at higher risk, such as women with a history of cervical cancer [6], MSM [7], immunosuppressed individuals, especially those infected with HIV-1 [6, 8], and transplant recipients [9]. In the USA, the rate of anal cancer incidence in HIV-infected individuals increased annually by 32.8% between 1996 and 2000. Thereafter and until 2008, a plateau was reached. Incidence rates declined from 2008 to 2012 at an annual rate of 7.2% [10]. Today, in the era of effective combination antiretroviral therapy (cART), ASCC is one of the most frequent non-AIDS-defining malignancies in the HIV-infected population [11] and several studies confirm a higher incidence of ASCC and its precursors in HIV-infected individuals compared to HIV-uninfected counterparts [7].

22.2 Viral Life Cycle

HPVs are epitheliotropic viruses which show a strict tropism for the squamous stratified epithelium. The HPV life cycle in its target tissue has been widely investigated. Although the key steps have been mostly clarified for cervical infection, we still lack comprehensive information regarding the biology of HPV infection and its ability to cause malignant transformation at anal level. Our understanding is poor also for other anatomical sites, such as the oropharynx.

In the stratified squamous epithelium, proliferation of the basal and suprabasal cells is tightly regulated. HPV initially infects the cells of the basal layer, probably through a micro-wound that favours the access of viral particles into basal cells [12]. Once the virus is inside the cell, the viral DNA accesses the cell nucleus. Early proteins required for the replication of the viral genome, i.e., E1 and E2, are now expressed. In the basal cells, the viral DNA is maintained at a low copy number. Once these stem-like cells proliferate, one of the daughter cells loses its proliferating ability, differentiates and moves up towards the upper layers of the epithelial strata. Importantly, HPVs have developed mechanisms to keep differentiated cells in a proliferative status, since the virus requires the cell machinery for the replication of its genome. The expression of the viral proteins E6 and E7 is essential for this step. In fact, they serve to maintain the differentiated cells in a replication competent status in order to exploit the expression of the cellular genes involved in DNA synthesis for the amplification of the viral genome. In the upper layers of the epithelium, viral replication occurs at high levels, and numerous copies of the viral genome are synthesized. In addition, the late proteins required for virus assembly, i.e., L1 and L2, are produced. The newly assembled viral particles are then released by shedding, through desquamation of the upper layers of the infected epithelium. Importantly, E6 and E7 early proteins, which are responsible for promoting the proliferation of the infected cells and are thus essential for the HPV life cycle, also play a major role in HPV-induced carcinogenesis. However, while in the productive infection their expression is tightly regulated, the HPV-driven carcinogenic process is characterized by the over-expression of these proteins, for instance, as a result of viral integration.

It is well known that the preferential target of HPV in the uterine cervix is represented by the cells of the squamocolumnar junction (SJ), i.e., the junction between the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix [13]. The anus contains a similar junction between the stratified squamous epithelium of the anal canal and the rectal columnar epithelium (anal transition zone, TZ). This histologic transition is usually at level of the dentate or pectinate line (Fig. 22.1a). Squamocolumnar junctions are particularly prone to HPV infection and associated transformation. Interestingly, in recent years, a new model has been developed regarding the cells involved in HPV-mediated carcinogenesis. In the uterine cervix, a region has been identified which

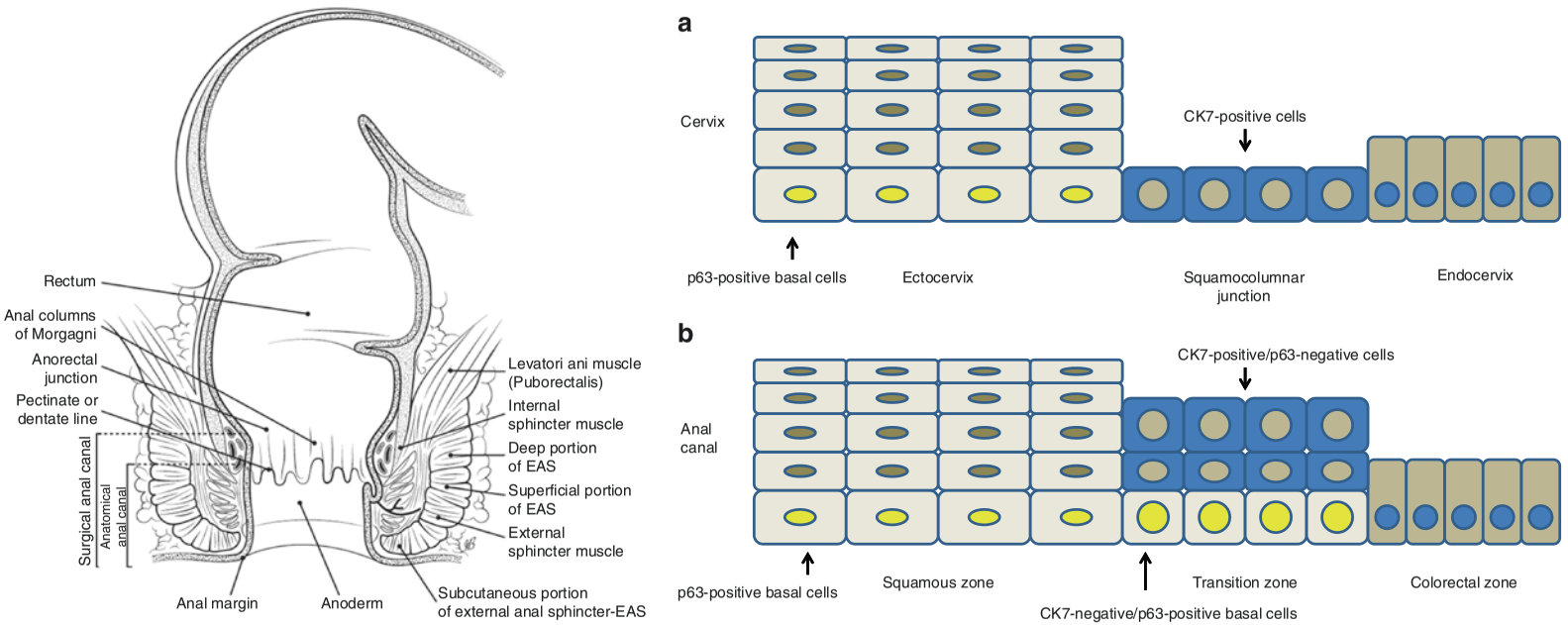

Fig. 22.1 A schematic representation of the anatomy of the anal canal; the dentate or pectinate line is shown (reprinted from Management of Fecal Incontinence-Current Treatment Approaches and Future Perspectives by Mongardini M. and Giofrè M. [62], Chap. 1, p. 3; B) Schematic representation of the cervical squamocolumnar junction (**a**) and of the anal transition zone (**b**); the cervical transition zone is formed by a single layer of cytokeratin (CK) 7-positive cells, whereas the anal transition zone is characterized by the presence of a multilayer of CK7-positive cells upon a single layer of p63-positive basal cells (modified from Yang et al. [14])

is characterized by the presence of a single layer of cuboidal cells (junctional cells) that seem to represent the preferential target of infection and site of development of HPV-associated neoplastic lesions [13]. These cells, of embryonic origin, are characterized by a unique gene expression profile, which makes them different from the epithelial cells of the transformation zone. Importantly, it appears that while infection of the mature squamous epithelial cells of the ectocervix and transformation zone is likely productive, with a low oncogenic potential, infection of the junctional cells may initiate the process that leads to transformation. It is interesting to note that the anal transition zone differs from the cervical transition zone, since it is formed by a multilayered epithelium. Additionally, the profile of the biomarkers which characterizes the cervical SJ cells is different from that of the anal TZ cells [14] (Fig. 22.1b).

Interestingly, the outcome of HPV infection appears to be related to the type of infected cells. A productive infection can be established in the canonical HPV target tissue, i.e., the stratified squamous epithelium. In this case, the virus completes its life cycle, viral progeny is produced and released by the infected tissues. Other types of cells, such as those present at the transformation zones, may be susceptible to the infection but less permissive to the virus. In this case, an abortive infection is probably established and a process of HPV-induced transformation of the target cells may initiate.

22.3 Anal HPV Infection

HR-HPVs are implicated in the development of nearly 90% of anal cancer cases [15]. HPV16 (75–80%) and HPV18 (about 3.5%) represent the most prevalent types in this neoplasia. Populations at high-risk for sexually transmitted infections, such as MSM and HIV-1 infected subjects, harbour an increased risk for anal cancer development. It is noteworthy that anal cancer incidence among HIV-infected subjects has increased despite the implementation of cART [7]. The use of cART has not affected the incidence of this neoplasia in spite of its ability to suppress the HIV load and restore immunity through an increase of CD4+ T cells. The improved survival of HIV-infected subjects may be the reason underlying this phenomenon, since the prolonged survival of cART-treated patients may have provided sufficient time for the development of anal cancer compared to the pre-HAART era. Nonetheless, findings in this regard are conflicting, since some studies have evidenced a protective effect of cART regarding the risk of anal (pre)cancer development [16–18].

Because of the etiological association between HPV infection and anal cancer and the increasing incidence of this neoplasia in recent years, particular attention has been paid to the burden of anal HPV infection and its natural history. However, this has been less investigated compared to that of cervical infection.

Since anal HPV is very common among populations at high-risk for sexually transmitted infections, its natural history has been mostly investigated in MSM and HIV-1 infected individuals. Sparse data are available on women and men who have sex with women (MSW). These populations may in fact harbour anal HPV infections as a result of non-penetrative sex, transmission from the cervix, auto-inoculation or partner-mediated inoculation. Data on the natural history of anal infections may help clarify why certain populations are characterized by a higher anal cancer risk and may be extremely useful to evaluate the impact of prophylactic HPV vaccinations.

22.4 Methodological Challenges in the Study of HPV Natural History

22.4.1 HPV Testing Methods: Which One?

Studies on HPV natural history require the collection of clinical samples repeatedly, usually once every 6 months, and the analysis of all of them with the same, reliable HPV test throughout the entire prospective study to ensure that all the

HPV test results are comparable. A large variety of HPV assays are commercially available, and many in-house variants of these also exist [19]. They differ profoundly in terms of number of HPV types detectable, analytical sensitivity and specificity. Some of them provide information only on the presence of HPV nucleic acids (DNA or mRNA), whereas others provide data both on HPV presence and genotyping. The use of genotyping assays to test sequential specimens of a longitudinal cohort makes it possible not only to assess HPV status and estimate incidence and clearance rates for HPV infection as a whole (any HPV), but also to estimate these parameters for individual genotypes. Thus, the choice of the HPV testing method is pivotal in order to obtain data in this regard. To acquire detailed information on anal HPV natural history, it appears important to choose a method that allows the detection of a large spectrum of genotypes and that has a good performance in detecting HPVs in multiple infections, which are very common in the anal canal. Tests with these characteristics may not be suitable for clinical applications, but are extremely useful, if not indispensable, in research settings. A number of HPV-DNA based genotyping assays have been developed. In the majority of the cases, they are microarray-based (e.g., PapilloCheck®, CLART® HPV 2) or rely on reverse line-blot hybridization (e.g., Linear Array® HPV Genotyping test, INNO-LiPA® HPV Genotyping). The latter two assays are among the most widely used genotyping methods. They are both based on a multiplex PCR that targets a region of the L1 viral gene, followed by a hybridization step to HPV-specific probes, which can be performed in a fully automated fashion [19]. Both these assays have a high analytical sensitivity and can detect a broad spectrum of HPV genotypes. In detail, the Linear Array® HPV Genotyping test can detect 37 mucosal HPVs, included both high-risk and low-risk types (HPV 6, 11, 16 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108). The INNO-LiPA® HPV Genotyping assay is able to detect a variable number of genotypes, according to the different versions of the test that have been developed over time. The latest version, INNO-LiPA® HPV Genotyping Extra II, can individually identify 32 genotypes (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 70, 73, 81, 82, 83, 89). The Linear Array® HPV Genotyping test and the INNO-LiPA® HPV Genotyping (version INNO-LiPA25), which have been compared in several large studies, have demonstrated a very good concordance in the detection of carcinogenic HPVs [20]. However, the Linear Array® has proved to be more efficient in detecting individual genotypes, in revealing multiple infections and in detecting more HPV types in cases of multiple infection [20]. The use of this assay may thus provide unique advantages in the study of anal HPV natural history, because of the very high frequency of multiple infections at anal level and broad panel of HPVs detectable. Indeed, the Linear Array®, which is very sensitive for HPV detection and genotyping in ano-genital samples [21], has been used widely in longitudinal studies of genital, oral and anal HPV infections [22–27].

22.4.2 Parameters of the Natural History: Not a Simple Matter

Studies on the natural history of anal HPV infection aim to provide data on its basic parameters, which are incidence, clearance, persistence (or duration of infection or time-to-clearance). Unfortunately, the definition of these parameters is challenging. For instance, this is evident in the definition of clearance. Since HPV infections are evaluated through detection of HPV-DNA, lack of detection does not necessarily imply a cleared infection, but might be due to the fact that the number of HPV-DNA copies is lower than the detection limit of the assay used. In some instances, a status of viral latency may be established. In this condition, viral DNA is not detectable, and lack of detection is interpreted as viral clearance, whereas the virus has not actually been eliminated by the immune system (Fig. 22.2). Similarly, incident infections can truly represent acquisition of a new infection (or re-infection), but can also result from the reactivation of an

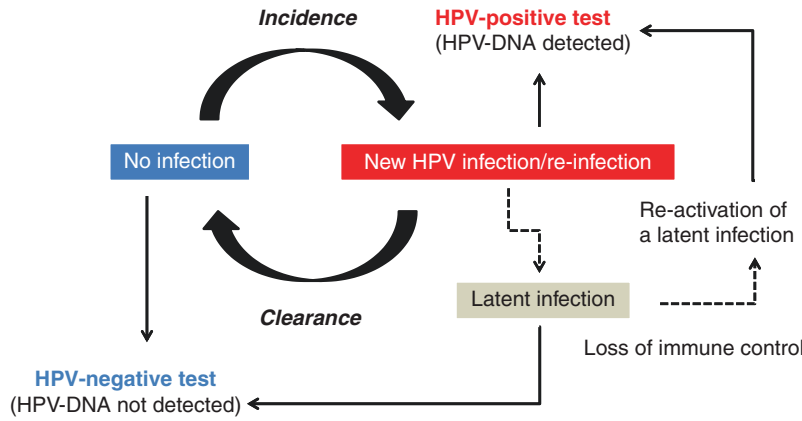

Fig. 22.2 Schematic model of the natural history of HPV infection. Incidence represents the acquisition of a new HPV infection or re-infection in an uninfected individual, as a result of recent sexual activity. Clearance represents the eradication of the infection by the immune system of an infected subject. Incidence and clearance are estimated based on the detectability of HPV-DNA through HPV testing, which does not truly identify the uninfected vs. infected status. An HPV-positive test may result from acquisition of a new infection, re-infection, or reactivation of a latent infection once the immune control is lost. An HPV-negative test may indicate HPV clearance (the subject is truly uninfected) or may result from a latent infection (the virus is present but undetectable since the viral load is below the test detection limit)

already existing infection that was previously below the assay detection limit, as a result of fluctuations of the viral load above and below this limit.

In addition, estimates of parameters of natural history may be deeply biased by the interval between samplings. In fact, if time between anal sample collection is longer than the median duration of infection, incident infections may be missed, because infections may have been acquired and cleared within the interval. An excessive interval may also lead to incorrect (upward) estimates of persistence, because an infection present at baseline may be cleared and then reacquired (re-infection) during the interval. It is worth noting that the exact time of these events (either acquisition or clearance of infection) cannot be directly observed, it can only be inferred to happen in the interval between the observations. Estimates of time-to-clearance may also be biased as a consequence of the interval between follow-up visits. Natural history studies are thus complex, and also expensive, because serial samplings at short intervals are required. Intervals between 2 and 12 months have been used, but in most studies, a 6-month interval has been chosen, since it has been shown to be appropriate for cervical and non-cervical HPV infection, including the anal one [23]. For instance, the median duration of genital male infection is 5.9 months [22], therefore setting follow-up visits every 6 months means it is unlikely that acquisition and clearance events will go undetected.

Estimates for parameters of natural history may be provided for the anal infection by any HPV type, by HR-HPVs or individual HPV genotypes (e.g., HPV16). Incidence is usually defined as a positive test (acquisition of infection) following a negative one (the infection was not present at the previous visit). However, more conservative incidence estimates require at least two positive tests following the baseline visit. This is the definition used in vaccine studies (Fig. 22.3). In most of the prospective investigations, clearance is defined as a negative test following a positive one. However, more stringent criteria may be applied, and only in cases where there are two consecutive negative tests, is an infection considered as cleared by some authors. Similarly, persistence may be defined as having either two or three consecutive HPV-positive tests.

It must be noted that measures of incidence, clearance and persistence are deeply affected by

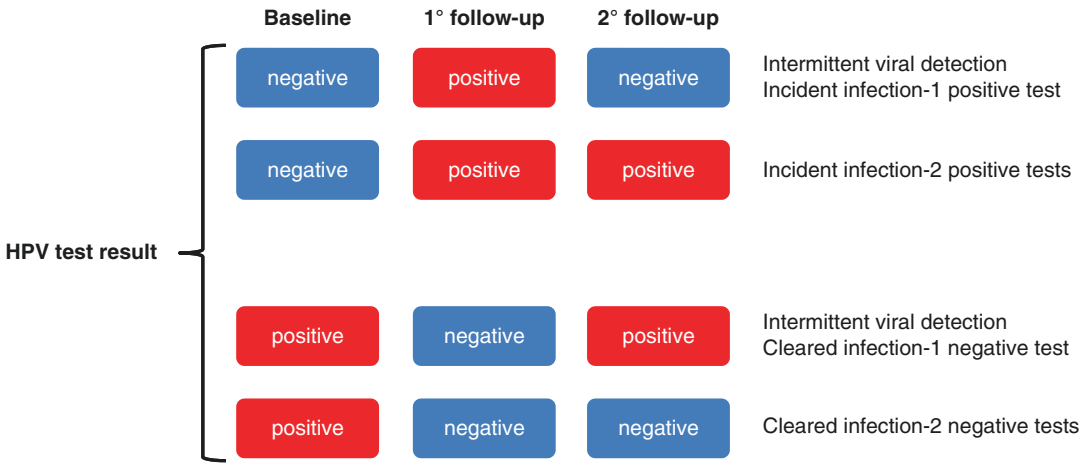

Fig. 22.3 Schematic representation of the possible HPV test results in a prospective study and of the definition of incident and cleared infections based on the transitions between an HPV-negative and positive test (incidence) and vice versa (clearance). One positive test following a negative one may be interpreted as an incident infection, but a more conservative definition requires two positive tests following a negative result at baseline. Similarly, a stringent definition for clearance requires two sequential negative tests in an individual that was HPV-positive at baseline. One negative test followed by re-detection of the same type/s identified at baseline suggests an apparent rather than a real clearance of the infection

the characteristics of the population investigated in the study, e.g., HIV status, demographic characteristics, behavioural factors, source of recruitment.

22.4.3 How to Analyse Data on HPV Natural History?

The natural history of HPV infection has specific characteristics that are not common to other infectious diseases or other fields of research. These features mean that the researchers must make precise choices both during the study design and data analyses. Indeed, specific data analysis models must be identified and used to produce accurate results. Some authors involved in in-depth studies on the natural history of HPV infection have tried to provide effective answers to these needs using a statistical approach. Importantly, they have provided estimates on the diffusion of HPV infection comparing the results obtained with new models to those produced by classic approaches (i.e., standard logistic regression analysis or Cox models) [28].

Ano-genital HPV infections commonly harbour multiple genotypes. Nonetheless, infections by each HPV type must be considered as separate events to describe the role of the individual HPVs in studies on natural history or to assess HPV-associated pathological pathways. In fact, there is a growing focus on type-specific HPV infection or infections by oncogenic HPVs stratified by risk level rather than grouped in broad categories (e.g., any oncogenic HPV type). Despite the fact that multiple HPV infections are commonly detected, it may be important to estimate prevalence, incidence, clearance and persistence of each individual HPV in order to evidence possible type-specific behaviours. Some authors have shown that the probability of detecting any given HPV type increases among individuals who are currently positive for at least one other HPV type [29–31].

Notably, HPV infections are mostly cleared by the immune system but they can be later reacquired because of a non-protective immune response, or can re-activate after a period of viral latency. Therefore, a peculiar characteristic of HPV natural history studies is that they require serial HPV testing over time. Importantly, longitudinal studies have shown that repeated mea-

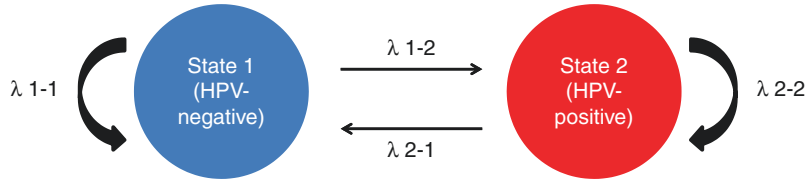

Fig. 22.4 Diagram of the homogeneous two-state Markov model. State 1 represents the HPV-DNA negative state, state 2 the HPV-DNA positive state. All possible transitions from one state to the other are shown by the arrows and are characterized by λ transition intensity: λ1–1 persistence in susceptibility; λ1–2 acquisition (incident infection); λ2–1 clearance; λ2–2 persistence in infection. Modified from Donà et al. [27]

sures involving the same individual and the same anatomical site tend to be correlated. These characteristics require powerful tools to analyse all the events, to measure many variables punctually, and to estimate the transitions from one state to another (e.g., HPV-negative to HPV-positive, HPV-positive to HPV-negative) or the perseverance in the same state (i.e., persistence of infection) using series of repeated measures. Statistical models that do not use all the available data (e.g., across all the follow-up visits and all HPV types detected) may be inefficient and may produce inaccurate results [28].

Marginal and mixed-effects models have been described as effective statistical strategies to address the issues in HPV research. These methods have shown a greater efficiency compared with standard logistic regression analysis or Cox models, because they are able to estimate subject-specific associations as well as mixed-effect models, while the marginal models are able to estimate population-averaged associations [28].

The Markov model is a statistical model of data analysis that is particularly appropriate for the study of HPV natural history. A two-state Markov model is a stochastic method for randomly changing systems based on the assumption that future states do not depend on past states [27, 32, 33]. To estimate incidence, clearance or persistence of HPV infection, each transition between infection states (i.e., negative/positive) is estimated independently of the previous state. Using the Markov model, the probabilities of transition between any two states are estimated jointly. The incidence is modeled as the rate of transition from a negative to a positive state, and clearance as the rate of transition from a positive to a negative state (Fig. 22.4). Notably, using this model, multiple infections detected or cleared during a visit are treated as a single event.

It is important to note that, even if the appropriate methods/models to study HPV natural history have been partly identified, these are rarely employed for this type of investigation. The use of these methods requires specific statistical knowledge and tools which are not always available in HPV research settings. Nonetheless, a larger use of the appropriate models should be encouraged in order to obtain correct estimates for the parameters of interest.

22.5 Incidence of Anal HPV Infections

Because of the above-mentioned variability in the definition of parameters of natural history, time between samplings, and HPV detection methods used, wide ranges of incidence, clearance and duration have been reported for anal HPV infection. A recent review of the literature reports incidence of anal HPV infection ranging from 21.3 to 46.2/100 person-years among men and from 14 to 56.3/100 person-years among women [34]. Importantly, incidence is consistently higher in MSM than MSW, irrespective of the HIV serostatus [23, 35]. A recent study conducted on over 400 HIV-positive individuals found a significantly higher incidence in MSM compared to MSW both for HPV 16 and 18 [36]. In fact, HPV16 incidence at 48 months was 24% for MSM and 7% for MSW. For HPV18, the corresponding figures were 13% and 4%. Similarly, incidence rate was higher for

MSM than MSW and women in another study conducted on HIV-positive individuals [37]. However, in other studies, the incidence rate of anal infection among women was reported to be as high as in MSM. Interestingly, the investigation by Beachler et al. [37] also compared incidence and persistence of anal and oral infections, and found a higher incidence (and persistence) of anal vs. oral infections. This represents an indication that the natural history of HPV infection depends also on the anatomic site infected. This may affect the acquisition and clearance of the infection because of differences in immunologic milieu and/or sexual behaviour associated with transmission.

Higher incidence rates in MSM than MSW have also been observed among HIV-negative subjects. In a study that analysed anal HPV natural history in over 2000 HIV-negative men recruited in Mexico, Brazil and USA, a significantly higher incidence was found in MSM and men who have sex with men and women (MSMW) compared to MSW [38]. High rates of incident infections have also been reported in young MSM (median age: 21 years) [39].

HIV serostatus affects the acquisition of anal HPV infection. Indeed, among MSM, HIV-positive individuals usually show higher incidence rates in comparison with their HIV-negative counterparts, as shown by Moji et al. for incidence of HR-HPV types [40] as well as in a Thai study [41].

Regarding the individual HPV genotypes, HPV 16 and 18 are among those with the highest incidence rate [34]. However, other genotypes, e.g., HPV 51, 52 or 59, may also display similar or even higher incidence [39, 42, 43]. In a study conducted in Italy on HIV-negative MSM, HPV51 (14.5/1000 person-months) and HPV52 (13.2/1000 person-months) showed the highest incidence, while for HPV16 was estimated an incidence rate of 9.1/1000 person-months [27]. Similarly, HPV51 showed the highest incidence in an investigation on HIV-positive MSM [32], while in a study on over 1000 HIV-negative subjects, HPV52 showed the highest incidence, followed by HPV 59 and 16 [23].

22.5.1 Risk Factors for Incidence

Risk factors for the acquisition of anal HPV infection have not been investigated in all studies of natural history so the body of data is quite limited. Additionally, while certain associations are consistently reported, some variables only sporadically emerged as risk factors for incident anal infections. In some instances, no risk factors have been indentified for anal HPV or HR-HPV infection in men, as in an investigation conducted on 612 HIV-positive MSM enrolled in Spain [32].

Variables of sexual behaviour are among the factors more commonly associated with anal HPV incidence. Being MSM was found to be associated with incident anal infection in a cohort of HIV-positive individuals [44]. Incident HPV16 infection has been found to be associated with recent anal sex in HIV-positive MSM [36, 43]. Receptive anal sex emerged as a risk factor for incident anal HPV infection also among HIV-negative MSM [27]. Lifetime and recent number of receptive partners, together with condomless sex, emerged as risk factors for incident anal infection among young HIV-negative MSM [39, 45]. Interestingly, the number of new oro-anal partners has been shown to be associated with incident infection, indicating that oro-anal contacts may play a role in the acquisition of anal infections, also in the absence of receptive sex [43]. Other lifestyle factors may increase the risk for anal HPV incidence. Although not commonly reported, incident HPV16 infection has been found to be associated with alcohol consumption and incident HPV18 infection with marijuana use in the 6 months preceding the enrollment among HIV-positive subjects [36].

Several risk factors for anal HR-HPV infection in women have been reported, although a very limited number of studies have been performed in this regard. Condom use (possibly due to the use of the same condom for both vaginal and anal sex) and a lifetime number of partners $\geq$6 were found to be risk factors in the Hawaii HPV cohort study [46]. This study also found a significantly lower risk of acquiring anal

HR-HPV infection in women ≥45 years of age compared to those <25 years. Anal sex was significantly associated with increased risk of incident anal infection by any HPV type, while little association between alcohol drinking and smoking and incident anal HR-HPV infection was found. By contrast, smoking has been found to be a risk factor for incidence of anal HR-HPV infection in HIV-positive women [47].

Regarding HIV parameters, especially those related to immunosuppression, associations have been inconsistently observed. A study conducted on HIV-positive MSM found no effect of nadir CD4+ count on anal HPV incidence [40]. In a cohort of HIV-positive MSM and MSW, time since HIV diagnosis was instead associated with incident infection [44]. In a cohort of HIV-positive women, the risk of incident anal infection increased with advanced HIV infection (late vs. early CDC stage) [47].

22.6 Clearance of Anal HPV Infections

The clearance rate for prevalent anal HPV infections has been reported to vary between 14.6 and 66.7/100 person-years among men [34]. HPV clearance of prevalent infections in women appears similar, while a much higher clearance rate has been found for incident infections (89.3/100 person-years) [48]. In contrast with the findings regarding incidence, which tends to be higher among MSM, clearance tends to be higher among MSW. In fact, Patel et al. reported that, at 48 months, 31% of MSM had cleared prevalent HPV16 infection vs. 60% of MSW, although in this study, the difference was not significant, as was the case also for HPV18 clearance [36]. Decreased anal clearance of HR-HPV infection is usually found in HIV-infected compared with HIV-negative MSM [40]. Importantly, a very recent study on HIV-positive women found that clearance of anal infection is lower than that of cervical infection, while their incidence is similar [49]. This may explain the higher prevalence of anal vs. cervical infections observed in several studies.

Among HR-HPV types, the lowest (or one of the lowest) clearance rate is consistently observed for HPV16 [23, 27, 32, 39, 40, 50]. This finding is not limited to MSM, since also among women, anal infection by HPV16 clears more slowly than that caused by other HR types [26].

22.6.1 Risk Factors for Clearance

As also observed for incidence, only a few studies have investigated the risk factors associated with clearance. In a recent study on MSM, only history of anal sex at baseline (with or without condom) was associated with failure to clear prevalent infection by HPV16 [36]. Similarly, clearance was found to be reduced in HIV-infected men practicing receptive sex [44]. Age has been shown to affect anal HPV clearance in some studies. Geskus et al. observed that HIV-positive MSM aged below 25 years had the highest clearance rate, while clearance was reduced in MSM >30 years [32]. In the same study, it was observed that HIV-positive MSM with high HIV-RNA load had a lower clearance. However, not all the parameters of HIV infection and/or HIV-related immunosuppression affect anal HPV clearance. A study conducted on more than 300 HIV-infected individuals showed a non-significantly decreased clearance in individuals with a lower CD4+ count [51]. No effect of nadir CD4+ count was observed in MSM by Moji and collaborators [40]. This study also showed a significantly decreased clearance of anal infection by HR-HPVs in HIV-positive MSM compared to the HIV-negative counterparts. Importantly, this was observed after adjusting for sexual behaviour, indicating that HIV status per se affects the features of anal HPV infection, independently of sexual habits. This might have implications regarding the risk for anal cancer in HIV-infected MSM. In fact, it is well known that anal cancer incidence may be 30–70 fold higher in these individuals than in the general population and increases several fold compared to what is observed for HIV-uninfected MSM [52].

Among women, clearance of anal infection was delayed by tobacco smoking and anal sex

[48]. On the one hand, these findings might indicate that smoking is a risk factor for anal cancer since it favours HPV persistence, on the other hand that continuous exposure to HPV through anal sex may favour re-infection.

22.7 Persistence

Median duration of anal HPV infections varies widely, depending on the characteristics of the target population. As previously mentioned, longer persistence is generally reported for HIV-infected men compared to immunocompetent individuals as a result of a slower clearance. For HPV16 and 18, time-to-clearance between 30 and 40 months has been reported both for HIV-positive MSM and MSW [42, 50]. In fact, the mean retention time for HPV16 in HIV-positive MSM was 36 months in the study by de Pokomandy et al. [50], a duration very close to the estimate found in Spanish HIV-positive MSM and MSW (35.4 months) [42]. In HIV-uninfected individuals, a shorter duration has been reported. In a cohort of Italian HIV-negative MSM, a median duration of 9.6 months and of about 17 months was calculated for HR-HPV types and HPV16, respectively [27]. All the other HR-HPVs showed a shorter time-to-clearance compared with HPV16. Differently, in another study on HIV-negative MSM, the longest duration was not calculated for HPV16 but for other HR-HPVs, i.e., HPV33, 18 and 31 (24–27 months for prevalent infections) [53]. Interestingly, this study also estimated the median duration for incident anal infections and this was much lower than that observed for prevalent infections. For incident infections, the median time-to-clearance was 10.8 months for any HPV type, 6.9 months for HPV16 and 8.1 months for HPV18. It is worth noting that in HIV-negative MSM, the median time-to-clearance for genital HPV infection has been estimated as 6.3–9.4 months for any HPV and 6.5–11.8 months for HR-HPV types [54].

In comparison with the observations on HIV-infected individuals, among which the mean retention time was similar for MSM and MSW, a higher persistence has been observed in HIV-negative MSM compared to HIV-negative MSW [23]. These findings suggest that among HIV-infected subjects, HIV infection affects persistence of anal infections and overrides differences by sexual orientation. Conversely, in HIV-uninfected individuals, anal infections appear to be persistent in MSM and transient in MSW.

Among women, a median clearance time of 5 months has been found for HR-HPV types [48]. Interestingly, in this study, HPV16 cleared more rapidly than other HR-HPVs, showing a median duration of 4.5 months, while HPV18 cleared in a median of 7 months. HPV59 displayed the longest duration of infection (almost 12 months). In a very recent study, up to 93% of anal infections by HR-HPV types detected at baseline in HIV-positive women were persistent at 6 months, especially those caused by HPV16 [55].

22.7.1 Risk Factors for Persistence

Persistence is intimately linked to HPV-driven carcinogenesis. In the cervical cancer model, it is well known that the establishment of a persistent infection is a key step in the HPV-associated transformation. Patel et al. found that MSM with persistent HPV 16 or 18 infection were more likely to have cytological abnormalities compared to those with transient infections [36]. Therefore, it is important to understand the factors that affect persistence of HPV infection in the anal canal. Both host and viral characteristics may play a role in this regard. As already mentioned, tobacco smoking increases persistence of anal HR-HPV infection in women [48]. Similarly, current smoking has been reported to increase persistence among HIV-negative MSM [23]. In this same study, persistence was associated with age, but only for MSW. In fact, older MSW (45–70 years) showed increased persistence. Another study on HIV-uninfected MSM found several factors associated with the persistence of HR-HPV types [53]. In particular, a sexual relationship longer than 2 years reduced the risk of persistence, whereas having ≥1 partner in the

last 3 months or >2 partners lifetime increased the persistence of anal infection. In young MSM, it has been shown that the lifetime number of receptive anal sex partners is significantly associated with persistent infection by oncogenic HPVs [39].

Several viral factors, such as the risk group (high-risk persist longer than low-risk types), specific HPV genotype or variant (non-European variants of HPV 16 and 18 show longer persistence), as well as concomitant infection with other types, have been shown to affect persistence of cervical infection among women. Among the viral characteristics, viral load appears to play an important role, also in the male anal infection. Recently, it has been reported that HPV 16/18 viral load is an independent determinant of type-specific persistence in MSM [56]. Notably, no difference in terms of HPV viral load was observed between HIV-positive and HIV-negative MSM, suggesting that the higher persistence of anal HPV in HIV-positive subjects cannot be explained by different viral loads. Interestingly, genital EBV infection has been found to be associated with increased persistence of anal HR-HPV in HIV-positive MSM [57].

Among heterosexual women, it has been observed that HPV16 persistence in the anal canal significantly increases with concurrent cervical infection with this same genotype, recent anal sex, and no condom use during anal sex [26]. Importantly, association with cervical infection suggests that transmission of HPV infection between the anus and cervix is very likely. It appears that the anus and cervix may act as a reservoir of infection for each other. It is worth noting that prevalence of anal infection among women may be significantly higher than cervical infection [49, 55].

Regarding HIV-related parameters, inconsistent findings have been reported. Beachler and collaborators found that in HIV-positive MSM, MSW and women, a lower count of current CD4+ T cells (i.e., a more severe immunosuppression) is only associated with prevalence but not persistence of anal infection [37].

22.8 Transmission

Transmission dynamics of HPV infection remain largely unexplored and particularly difficult to investigate, although many mathematical models have been developed. Several studies have been performed on heterosexual couples in order to clarify the transmission of genital infection between man and woman [58–61]. Differently, the transmission dynamics of anal HPV are largely unknown. Exposure to anal infection is mainly dependent on penetrative sexual intercourses (receptive anal sex) with an HPV-infected partner, i.e., a partner with a penile infection. Other routes of transmission that involve digital or oral contacts may play a role, although less frequently and less efficiently.

Only one study conducted on young MSM has estimated transmission probability from penis to anus and vice versa [45]. This study evidenced that the probability of transmission of infection by the HPVs included in the quadrivalent vaccine from penis to anus is significantly higher than from anus to penis. In fact, the authors estimated that transmission probability per partner from penis to anus is 50% on average, ranging from 33.7% for HPV16 to 85.7% for HPV6. Differently, they calculated a transmission probability per partner from anus to penis of 7.6% for HPV16 and 4.6% for HPV6. Estimates on transmission probability from penile to anal infection largely exceed those calculated in heterosexual partners. Indeed, Burchell and collaborators calculated a per-partnership transmission probability of 20% [60].

22.9 Conclusions

MSM are at increased risk for anal HPV compared to MSW. Consequently, they are at higher risk for developing HPV-associated lesions in the anal canal, and ultimately, anal pre-cancer and cancer.

The available data suggest a high incidence for anal HPV infections, particularly in MSM, with HPV16 being among the HR-HPV geno-

types with the highest incidence and the lowest clearance rate. HIV status, sexual behaviour (number of partners, same-sex intercourses, sexual practices, among others), lifestyle habits (e.g., smoking status) may deeply affect HPV incidence, clearance and persistence. MSM and HIV-infected individuals consistently show higher incidence of anal HPV infection in comparison with MSW and HIV-uninfected subjects, respectively. Importantly, HIV-infected men show a substantially longer time-to-clearance compared to the HIV-uninfected counterparts. A longer persistence has also been demonstrated in MSM compared to MSW, although in this regard, data are still insufficient.

Virus-related factors, such as the specific HPV genotype or carcinogenic risk group, also play a role, especially affecting the persistence of the infection.

The pivotal role of anal HPV in the development of anal cancer warrants further investigations on the natural history of this infection. The identification of the factors, both viral and host-associated (non-modifiable, such as host-genetics, and modifiable, such as behavioural characteristics), that affect acquisition and in particular persistence of anal infections might help define strategies to prevent/control these infections and consequently the development of the associated lesions.

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