Department of Medicine
Department of Molecular Biology & Genetics
DEMOCRITUS UNIVERSITY OF THRACE

European Territorial Cooperation Programme
Greece-Bulgaria 2007-2013
Project Acronym: MEVIR

International Conference on sexually transmitted viral infections: Current diagnostic and therapeutic approaches

November 15 - 17, 2013

Alexander Beach Hotel & Convention Center, Alexandroupolis, Greece

ΙΑΤΡΙΚΗ ΣΧΟΛΗ ΔΗΜΟΚΡΙΤΕΙΟΥ ΠΑΝΕΠΙΣΤΗΜΙΟΥ ΘΡΑΚΗΣ

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The City and the Region

Alexandroupolis is the capital of the Prefecture of Evros in Thrace, with a population of approx. 50,000. It is a coastal town built to a contemporary plan, and has enjoyed its main period of expansion since the liberation of Thrace in 1920. Today, Alexandroupolis is an important commercial, economic and tourist centre in North-Eastern Greece, with transport connections via its railway, port, airport and the modern Egnatia motorway.

The landmark of Alexandroupolis is its famous Lighthouse, located on the scenic coastal promenade of the city. It is of French construction and operated for the first time in June 1880. Other examples of the late 19th - early 20th century architecture include the building of the old Zarifeios Pedagogical Academy and the Leontarideios School (now home to the Ecclesiastical Museum). The Ethnological Museum, founded and curated by Mrs Angeliki Giannakidou, is housed in a neoclassical stone building dating from 1899 and its collection includes agricultural implements and relics, tools and machinery used in traditional crafts, items of everyday life, etc.

Other sites of attraction in the prefecture include: To the west, the village of Makri, with its scenic fisherman's port and the cave of Cyclops, as well as the archaeological site of Mesimvria-Zoni, a Samothraki colony founded in the late 7th century BC and reaching prosperity during the classical times. To the north, the scenic villages of Kirki and Sykorrahi, located in the mountains of Zoni, where Orpheus played his lyre according to the legend. To the east, the Roman baths of Traianoupolis and the town of Feres with its Byzantine domed basilica of Panayia Kosmosoteira, founded by Isaac Komnenos in 1152 AD. Of exceptional natural beauty is the Delta of the river Evros, the protected forest of Dadia and the scenic village of Tychero, with its nearby fossilised forest. Further north, there is the town of Soufli, famous for its long tradition of silkworm breeding and silkware craftmanship, the historic city of Didymoteicho with its Byzantine monuments, and Orestiada, an attractive town built to a contemporary plan. The road then takes the visitor to Nea Vyssa, the location of the popular Ardas Youth Meeting, and finally to Ormenio, at the so-called "Trigono" where the borders of Greece, Bulgaria and Turkey meet. Of particular interest is also the island of Samothraki in the Thracian Sea, 2.5 hours by ferry from the port of Alexandroupolis. The island boasts several sites of great historical significance and remarkable natural beauty, including the Sanctuary of the Great Gods where the famous Nike of Samothraki was found (now housed at the Louvre Museum in Paris).

Within easy access via the Egnatia motorway to the west are other sites of historical importance, including the ancient cities of Maroneia (a city-state reaching prosperity as a democracy and member of the Athenian League), Abdera (the birthplace of the eminent ancient philosophers Leucippus, Anaxarchus and Democritus) and Philippi (built by the Macedonian King Philip II and prospering throughout the Hellenistic, Roman and Byzantine times). Komotini, Xanthi, Kavala and Drama are all historic cities and the centres of modern life in the broader Region of Eastern Macedonia-Thrace, while the island of Thassos is a popular holiday destination and site of important archaeological monuments.

(From the Tourist Guide published by the Region of Eastern Macedonia-Thrace)

The Institution

The Democritus University of Thrace

Democritus University of Thrace was established in July 1973 and begun its operation in academic year 1974-1975. It is named after the famous Greek philosopher Democritus, who hailed from the ancient town of Abdera in Thrace. The administration of the University is based in the campus of Komotini, capital of the Administrative Region of Eastern Macedonia and Thrace. The University currently comprises seven Faculties and eighteen Departments located in the four major cities of Thrace, Komotini, Xanthi, Alexandroupolis and Orestiada. It is a Public Entity with autonomous administration, funded and supervised by the Ministry of Education. Its current student population is approximately 15,000. The University plays an important role in the educational, cultural and economic development of the region, and is one of the largest academic institutions in Greece. It is committed to high quality of teaching and research, promoting its reputation as one of the top universities in the country.

The Department of Medicine

The Department of Medicine was established in 1977 and started to operate in Alexandroupolis in academic year 1984-1985. Despite the early adversities, the Department today demonstrates a high level of quality in undergraduate teaching, and additionally offers a number of postgraduate courses in specialized modern medical fields. It is committed to high standard medical research, as evidenced by an increasing number of publications in peer-review journals and collaborations with research academic institutions both domestically and abroad. The Department strives to advance its scientific and technological status, attracting distinguished scientists from Greece and abroad, as well as competitive external funding. Furthermore, the University Hospital provides vital health services to the local and regional community, while promoting public awareness in major health issues.

The Department of Molecular Biology and Genetics

The Department of Molecular Biology and Genetics is one of the newest departments of Democritus University of Thrace, having commenced operation in 2000. It is the only university department in Greece dedicated to providing a Molecular Biology and Genetics undergraduate and postgraduate curriculum, and was founded with the vision of becoming a centre of innovation and excellence in research and education. The Department contributes to the scientific and technological development of Northern Greece and the broader region through collaboration with renowned institutions nationally and internationally, as well as dissemination of research outcomes in recognised scientific journals and conferences. Research infrastructure has been funded by grants from the Greek Ministry of Education, the Greek Ministry of Economy and Finance, the EU Framework Program 7 etc. Research has additionally been supported by the General Secretariat of Research and Technology, not-for-profit foundations and the industry.

The Project MEVIR

Aim of the project is to influence regional public health policies through the advancement of molecular detection and epidemiological mapping of sexually transmitted viral infections (STVIs) in the cross-border populations of Greece and Bulgaria. The information disseminated to the stakeholders in the two countries, as well as to the general public, is anticipated to raise awareness of STVI prevention and the risks associated with dangerous sexual attitudes. Detection of certain HPV strains and their link to cancer is crucial to the health education of young people and their parents. The project exploits the benefits of long-term partnership between academic institutions in the region and organizations involved in public health policy making.

The scientific objectives of MEVIR are the following:

- Establishment and expansion of a multidisciplinary cross-border network of experts for the surveillance and prevention of STVIs in the region.
- Expansion and operation of molecular diagnostic infrastructure, enabling large scale STVI population screening in the region. Specifically:
  - Upgrading existing high-end equipment
  - Establishing technical experience and transfer of know-how from the Greek to the Bulgarian partners
- Launch of a new molecular diagnostic facility in Kardzhali, Bulgaria, supported by personnel recruitment and training in STVI population screening programs.
- Evaluation of molecular diagnostic methods for STVIs, in terms of workflow management and quality control.
- Establishment of a biobank of clinical specimens and relevant database with STVI-related demographic, socio-economical, clinical and molecular data for the purposes of genetic analysis and validation. Specifically:
  - Determination of suitable strategies and protocols for sample collection, storage and transport
  - Assessment of the effectiveness of STVI diagnosis in reducing the risk for related neoplasias and foetal abortion
- Epidemiological mapping of local prevalence of STVIs and identification of potential high-risk subgroups in the two countries.
- Dissemination of the project outcomes to target groups, involving:
  - Health education of young people and parents
  - Dissemination of project activities to the media and decision-making bodies
  - Endorsement of HPV vaccination as a key element to effective prevention and control of cervical cancer

- Dissemination to the broader scientific community through this international conference

Welcome note

Dear Colleagues and Friends,

We are pleased to welcome you to Alexandroupolis and the Alexander Beach Hotel, venue of the International Conference on "Sexually Transmitted Viral Infections (STVIs): Current diagnostic and therapeutic approaches".

This international event represents one of the major dissemination activities of the project MEVIR, entitled "Cross border epidemiology of sexually transmitted viral infections in the female population: molecular diagnostic approaches", implemented within the framework of European Territorial Cooperation Programme (INTERREG IV) Greece-Bulgaria 2007-2013. This project is a partnership between the Department of Medicine and the Department of Molecular Biology & Genetics at the Democritus University of Thrace, involving collaborators from Regional Health Inspectorate cross-border organizations of both Bulgaria (Kardzhali) and Greece (Evros).

The major objectives of MEVIR are to promote the molecular diagnosis of STVIs in the female population of Greece and Bulgaria, the epidemiological mapping of local prevalence, and the identification of potentially high-risk population subgroups. The long-term goal is to support prevention of STVIs in the cross-border region between the two countries. MEVIR pursues strengthening of the bilateral scientific potential in the diagnosis and management of STVIs, through research networking and transfer of knowledge. In this context, the durability of the project is secured through the establishment of long-term partnerships between research and academic institutions, and public health organizations in both Greece and Bulgaria.

Our event is designed to foster multidisciplinary interaction advancing improvement of current diagnostic and therapeutic management of STVIs. Our distinguished invited speakers will cover a wide range of topics, communicating their scientific expertise in the field. The current state of the art in diagnosis and prevention of STVIs will be presented, and key issues will be discussed, particularly in relation to the biology, risk, molecular diagnosis, population screening and epidemiology. We anticipate this event to encompass all major scientific and clinical aspects of STVIs, inspire collaborations, and encourage the sharing of know-how.

We welcome your active participation in the conference sessions, which we hope will stimulate constructive discussions involving all delegates. We wish you a wonderful scientific, as well as social, experience and an enjoyable stay in Alexandroupolis.

For the Organizing Committee,

Theodore Agorastos
Ekaterini Alexiou Chatzaki

PROGRAMME

CONFERENCE PROGRAMME

Friday 15 November

17:00pm-19:00pm *Registration & Welcome Refreshments*

Opening session
Moderator: Aikaterini Alexiou-Chatzaki

19:00pm-19:20pm **Welcome Addresses**

19:20pm-19:50pm **Presentation of the region and its customs**
Angeliki Giannakidou (Ethnological Museum of Thrace)

20:00pm-21:00pm *Welcome reception*

Saturday 16 November

Session 1 - Epidemiology and Pathology of Sexually Transmitted Viral Infections
Moderator: Vasilios Lyberis

09:00am-09:30am **Disease burden of sexually transmitted viral infections**
Theodore Constantinidis (Democritus University of Thrace)

09:30am-10:00am **Economic aspects of HIV infected patients in Greece**
Nadia Boubouchairopoulou (National School of Public Health, Athens)

10:00am-10:20am **HPV prevalence in high-grade cervical lesions and cervical cancer in Bulgaria**
Evelina Shikova (National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria)

10:20am-10:40am **HPV prevalence in Greece**
Theodore Agorastos (Aristotle University of Thessaloniki)

10:40am-11:00am **How viral infections affect fertility in women**
George Galazios (Democritus University of Thrace)

11:00am-11:20am **STVIs and fertility treatment**
Mara Simopoulou (National Kapodistrian University of Athens)

11:20am-12:00pm *Coffee break*

Session 2 - New Technologies in Human Papilloma Virus Diagnosis and Control

Moderators: Theodore Agorastos, Katerina Chlichlia

12:00pm-12:40pm **HPV E7 oncoproteins as new markers for detection of cervical cancer and precancer**
Pidder Jansen-Duerr (University of Innsbruck, Austria)

12:40pm-13:20pm **Immune responses to prophylactic and therapeutic HPV vaccines**
Andreas Kaufmann (Charité-Universitätsmedizin, Berlin, Germany)

13:30pm-15:30pm *Lunch break*

Session 3 - Human Papilloma Virus and Cancer

Moderators: Maria Koffa, Jeni Staykova

15:30pm-16:10pm **Biomarkers of oncogenic HPV infection causing significant cervical disease in Scottish women**
Sarah Howie (MRC Centre for Inflammation Research, University of Edinburgh, UK)

16:10pm-16:30pm **Sexually transmitted viruses and cancer**
Nicola Vassilev (Military Medical Academy, Sofia, Bulgaria)

16:30pm-16:50pm **Detection of HPV and other sexually transmitted virus infections in cervical specimens from women in Kardzhali region**
Jeni Staykova (Regional Health Inspectorate, Kardzhali, Bulgaria)

16:50pm-17:10pm **Comparison between cytology and HPV DNA testing in primary screening for cervical cancer prevention: preliminary results from a multicentric study in Greece**
Taxiarchis Katsamagas (Aristotle University of Thessaloniki)

17:10pm-17:30pm **HPV and upper aerodigestive tract cancers**
Maria Riga (Democritus University of Thrace)

17:30pm-18:00pm *Coffee break*

Session 4 - Herpes Simplex Virus: Current and Future Perspectives

Moderator: Penelope Mavromara

18:00pm-18:40pm **The two faces of HSV: An enemy or a friend?**
Roberto Manservigi (University of Ferrara, Italy)

18:40pm-19:20pm **Herpes simplex virus: From infectious pathogen to gene therapy vector**
Cornel Fraefel (University of Zurich, Switzerland)

20:00pm-22:00pm *Dinner at Alexander Beach Hotel Restaurant*

Sunday 17 November

Session 5 - Other STVIs: Key Issues

Moderator: George Sourvinos

10:00am-10:30am **Human Cytomegalovirus: from pathogenesis to novel insights of antiviral therapy through epigenetic and miRNA regulation**
George Sourvinos (University of Crete)

10:30am-10:50am **HIV: The past, the present and the future**
Periklis Panagopoulos (Democritus University of Thrace)

10:50am-11:20am **New insights into the molecular biology of HCV: The role of core protein(s) in hepatocarcinogenesis**
Penelope Mavromara (Democritus University of Thrace & Hellenic Pasteur Institute)

11:20am-11:40am **Hepatitis B virus: clinical spectrum and treatment**
Constantinos Mimidis (Democritus University of Thrace)

11:40am-12:00pm **HBV**
Ioannis Koskinas (National Kapodistrian University of Athens)

12:00pm-12:30pm *Coffee break*

Session 6 - Prevention and Molecular Diagnostics of STVIs: Applications and Quality Management

Moderator: Sotiria Boukouvala

12:30pm-13:00pm **HPV vaccination: An ideal weapon against a steady growing threat**
Theodore Agorastos (Aristotle University of Thessaloniki)

13:00pm-13:30pm **Quality management systems in molecular diagnostics**
Photini Karababa (**TÜV HELLAS**)

13:30pm-13:50pm **Molecular detection of HPV**
Theocharis Constantinidis (Regional Laboratory of Public Health, Alexandroupolis)

13:50pm-14:10pm **Company presentation**

14:10pm-14:20pm **Concluding Remarks / End of Meeting**

ABSTRACTS

Disease burden of sexually transmitted viral infections

Theodoros Constantinidis, Assoc. Professor
Laboratory of Hygiene and Environmental Protection,
Dept. of Medicine, Democritus University of Thrace, Greece

Viral infections caused by Human Pappiloma virus, Herpes Simplex Virus, Human Immunoinsuffieciency Virus, Cytomegalovirus, Hepatitis B & C Viruses, Molluscum-contagiosum Virus, can be transmitted via sexual contact and produce a bright spectrum of non-neoplastic but also neoplastic-malignant diseases (papillomata, condylomata accuminata, genital herpes, AIDS, Kaposi sarcoma, hepatitis B & C, Molluscum-contagiosum, hepatocellular cancer, cancer of the cervix, anus, vulva, vagina, oropharyngx, laryngx, probably oesophagus and lungs and others). In both men and women the yearly incidence of genital warts is 2%, and the patients suffer from stigma of STD, discomfort with sexual activity, and treatment related pain. There are approx. 470.000 new cases of cervical cancer worldwide each year, and the disease causes 230.000 deaths. The majority of cases arise in the developing world, and in many countries cervical cancer is the most frequent malignant disease in women. Still, a disturbingly high number of new cases are seen in Europe, where 40 women die every day of the disease, in spite of widespread and costly screening programs the last 30-40 years. Approx. 95.000 new cases are seen yearly in the developed world, and 5-10 times as many women receive surgical treatment for cancer precursors (CIN II and III) every year. Recent studies indicate sequelae after treatment of premalignant cervical disease to increase the risk of both late abortion and prematurity. Of clinical significance is also the fact that palliative care in the frequently occuring end stage of locally advanced cervical cancer may be particularly demanding in several aspects. As compared to other cancers, cervical cancer occuring mainly in postmenopausal women, cervical cancer is much more prevalent in women of fertile age (peak 35-55 years), many of whom care for small children. Additionally, a tendency of the mean age of cervical cancer getting lower in countries with a high frequency of HIV infections have been observed, adding to the disease burden.

Economic aspects of HIV infected patients in Greece

John Kyriopoulos[1], MD, MSc, MPH, PhD, Kostas Athanasakis[2], BScHS, BScEcon, MSc, PhD, Nadia Boubouchairopoulou[3], BSc, MSc

[1]Professor, Department of Health Economics, National School of Public Health,196 Alexandras Avenue, 11521, Athens, Greece.
[2]Research Fellow, Department of Health Economics, National School of Public Health,196 Alexandras Avenue, 11521, Athens, Greece.
[3]External Collaborator, Department of Health Economics, National School of Public Health,196 Alexandras Avenue, 11521, Athens, Greece.

Background: HIV / AIDS infection is a human's immune system disease with a pandemic impact. According to WHO, the virus bearers are approximately 34 million globally, while in Greece patients are estimated at 12,500 (2012). Consequence of infection is therefore the need for continuous monitoring and administering antiretroviral treatment for life, burdening both the patient physically, psychologically and financially, but also society in the disease burden.

Objectives: The purpose of this study is to present the effects of HIV / AIDS infection in the morbidity burden and healthcare cost in Greece. Specifically, to assess the resource use by health state of HIV patients as indicated by the STARTMRK clinical trial as well as to estimate the partial cost of each resource type in an annual basis.

Methods: The initial estimate of the direct cost performed retrospective study. Data from 447 patients monitored in a General Hospital of Athens were collected through their medical records. Medical confidentiality was preserved throughout the course of the study. The survey involved outpatient visits, hospitalization, antiretroviral therapy, i.e. services and treatments that patients received from 7/2/2012 to 07/02/2013 as well as demographical data. Patients were stratified in 18 health states according to the number of CD4 cells/mL and the viral load as defined by the clinical study STARTMRK. The initial study was then followed by an expert panel to confirm the figures but also for recording new data such as hospitalization both inside and outside ICU, emergency visits and the consumption of health services 30 days before death. The data processing was performed by Microsoft Office Excel 2007 and IBM SPSS Statistics 20.0 programs.

Results: The annual direct cost for each patient is calculated at 6,861€, of which 85% refers to antiretroviral therapy. Annual cost of providing simple healthcare services (inpatient cost, outpatient and emergency visits, CD4 tests, viral load tests, genotypic resistance tests) regardless of health state is computed at 2,422€. Certain exceptions aside, the healthcare services' use was found to gradually increase from 1 to 18 health state, a result that can be attributed to the decrease of CD4 T-lymphocytes and to the increase of viral load combined, leading to prolonged hospitalization and an extended average laboratory tests.

Conclusions: As anticipated, the disease direct cost increases as CD4 T-lymphocytes decrease, while the largest portion of expenses is related to antiretroviral therapy. Estimating costs and resources used to counter the infection in Greece is essential to the timely and proper planning of specialized services.

HPV prevalence in high-grade cervical lesions and cervical cancer in Bulgaria

Evelina Shikova MD PhD[1,2], Zina Ivanova MSc[2], Violeta Popendikyte MSc PhD[3], Aurelija Zvirbliene MSc PhD[4], Gancho Ganchev MD PhD[5]

[1]National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
[2]IEMPAM-BAS, Sofia, Bulgaria
[3]Thermo Fischer Scientific/Fermentas, Vilnius, Lithuania
[4]Institute of Biotechnology of Vilnius University, Vilnius, Lithuania
[5]SHATO, Sofia, Bulgaria

Background. Bulgaria has one of the highest rates of cervical cancer incidence and mortality in the EU. At the same time there is no organized population-based cervical cancer screening in the country and the HPV vaccination program only started one year ago. The aim of this study was to assess the type-specific prevalence of HPV in high-grade cervical lesions and cervical cancer in order to evaluate the potential benefit of HPV vaccination, HPV-based screening and other cervical cancer prevention programs in Bulgaria.

Methods. DNA was extracted from 278 cervical specimens, collected from women with confirmed histological diagnosis - 96 with invasive squamous cell carcinoma (ICC) of uterine cervix and 182 with high-grade squamous intraepithelial lesions (HSIL). All samples were analyzed for the presence of HR HPV using a newly developed test system by Fermantas/Thermo Fisher Scientific in cooperation with the Institute of Biotechnology in Vilnius. A multiplex PCR-based system (Fermantas/Thermo Fisher Scientific) was used for genotyping of screen positive samples.

Results. HPV was detected in 91.7% of cervical cancer patients and in 85.2% of patients with HSIL. Overall, sixteen HPV genotypes (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, - 58, -59, -66, -68, -73, -82) have been identified in cervical specimens. The most prevalent type in both, ICC and HSIL cases, was HPV16 found in 65.6% and 48.4%, respectively. The second most common type in cervical cancer was HPV18 (8.3%) and in HSIL - HPV31 (14.8%).

Conclusions. Numerous HPV types were detected in this study, but the high proportion of HSIL and ICC caused by HPV16 and HPV18 confirmed the utility of HPV16/18 vaccines for Bulgaria. The obtained information is also helpful for monitoring the potential changes in HPV type-specific prevalence and role in cervical cancer in the country, e.g. potential replacement of HPV16/18 with nonvaccine genotypes as a result of HPV vaccination.

HPV prevalence in Greece

Theodoros Agorastos, Professor

4th Dept. of Obstetrics and Gynecology, Hippokrateio Hospital, Aristotle University of Thessaloniki, Greece

Given the lack of an organized cancer registration network in Greece the only possible approach for valid comparable epidemiological data is the use of mortality and morbidity indexes per a certain time period (usually the calendar year) and per geographical region. Usually, this refers to 100,000 residents to allow direct comparative studies. For the population of Greece, recent data regarding cervical cancer define morbidity at 9.3/100,000 women and mortality at 1.8/100,000 women. Thus, in Greece, incidence is stable, or shows a mild reduction over the last twenty years, whereas in Europe there is a clear rate decline. On the other hand, rates regarding mortality due to other types of uterine malignancies show a falling trend in Greece as well as in other European countries.

How viral infections affect fertility in women

Georgios Galazios, Professor of Obstetrics and Gynecology, Director of IVF**, Democritus University of Thrace,** Greece

- Infertility, a major problem of modern medicine is defined as the inability of sexual active couples to achieve pregnancy after 12months. Infertility affects nearly 20% of reproductive aged couples.
- Viral infections of the genital tract play a crucial role in the pathogenesis of infertility. The main viral infections that affect infertility are the infections from HSV, HPV, HIV and CMV virus.
- Symptomatic infection with HSV causes much physical discomfort, psychological disorders and interferes with sexual relations. Subclinical infections which are not infrequent contribute to the sexual transmission of the disease. An association of HSV infection with recurrent abortions and with preterm labor and prematurity has been suggested Serious neonatal infections are possible through vertical transmission of HSV from mother to child these are associated with high morbidity, including permanent neurologic handicaps and even death if left untreated. Further there is no information about a potential association of genital HSV infection with reduced cervical mucus quality and a cervical factor in involuntarily childless patients. Moreover the results of a recent study indicate that HSV, by affecting the most important semen parameter sperm count, plays an important role in male infertility.
- Human papillomavirus is one of the most sexually transmitted viruses witch comprise a group of small DNA viruses that infect both cutaneous and mucus squamous epithelia Alvarez Fernandez et al. found in 1998 that the presence of the HPV in the female lower genital can cause tubal factor infertility. Obviously though, these viruses are much more harmful in the cervical region and surgical procedures for cervical intraepithelial neoplasia(cin) though less invasive nowadays, still cause a large number of mutilations that compromise.
- HPV in semen was associated with impairment of sperm parameters, especially a reduction of sperm motility.
- HIV may impair sperm parameters by itself and certainly deteriorates the outcome of concomitant genital infections. In addition, the specific anti- HIV therapies can devastate the male reproductive system. HIV has a negative impact also on female fertility but is not clear to what extent this is due: to the activity of the virus itself, or to other genital infections whose course is deteriorated by the HIV infection, or even to the side effects of therapy.
- CMV presence in the genital tract of sub fertile patients is considerable, but findings do not suggest that sexual transmission is a frequent route of infection or that CMV infection is a significant cause of infertility.
- Campaigns informing the public on the effects of high risk sexual behavior and encouraging people to seek controls on their genital health status and to get timely treatment seem to be the best way to limit the impact of sexually transmitted viral infections.

STVIs and Fertility Treatment

Simopoulou Maria B.Sc.,M.Sc.,Ph.D.

Clinical Embryologist/Geneticist, Lecturer of Physiology, National and Kapodistrian University of Athens Medical School

Infertility refers to the biological inability of a person to contribute to conception, implantation or carrying a pregnancy to full term. According to WHO Infertility is the inability to conceive a child. A couple may be considered infertile if, after two years of regular sexual intercourse, without contraception, the woman has not become pregnant. Its prevalence lately increases especially due to social reasons (couples delaying conception due to busy lifestyle, increased professional obligations in the Western world). Statistics may vary depending on the definition of infertility and its duration. Infertility is of multifactorial etiology from 20% accounting for male factor infertility (obstructive, infectious, hormonal etc), 66% accounting for female factor (tubal, endometriosis, ovarian, hormonal etc), and an interestingly rising percentage of 14% attributed to unexplained infertility. One of the factors related to infertility are infections that go undetected, undiagnosed and untreated. The infertility investigative work up of the infertile couple includes mandatory testing for STVIs for both partners as required by the In Vitro Fertilization Boards of Approval of the National Health System in Greece (EOPYY). Viral infections have been shown to contribute to male infertility either by direct toxic effects on cells in the male reproductive tract, and/or indirectly by causing a local inflammatory, or immunological, reaction. All infections should be treated prior to IVF. Assisted Reproduction Technology (ART) has contributed to the birth of millions of babies worldwide. It has become the answer to infertility helping couples becoming parents. The basic IVF approach in a nutshell involves ovarian stimulation employing stimulation protocols with gonadotrophins according to the patients' hormonal and reproductive profile, oocyte retrieval, sperm preparation and fertilization, embryo culture, evaluation of implantation potential and embryo selection for embryo transfer with subsequent cryopreservation of the remainder embryos. In Vitro Fertilization can help patients with chronic diseases such as HCV and HIV to maintain the ability to reproduce worldwide providing the required recommendations and guidelines are in place. This is why we are here today to contribute to better detection of STV infections leading to decreasing infection related infertility and better practice in Assisted reproduction and IVF laboratories that treat seropositive individuals for infertility irrespectively of etiology. Top of the list on STVIs in ART are HIV I+II, HepC, HepB, CMV. The major risk involved in treating such patients involve contamination of the following: Seronegative partners in serodiscordant couples, seropositive partners with different strains, contamination of the couple's embryos, contamination of other patients attending the centre at the same time and/or their embryos, and finally contamination of the IVF specialists involved in treating the seropositive patients. This presentation focuses on guidelines on protection of the staff exposed, other patients and their gametes/embryos, and owns patient's gametes and seronegative partners. The aim is to help contain the disease while not violating the basic human right of access to treatment. ART centers require special infrastructure to treat these couples and this is discussed in this presentation from special scheduling to special culture conditions and handling. Attention and code of practice is discussed when treating serodiscordant couples depending on which partner is seropositive. There have been numerous reports on effective semen preparation techniques and molecular testing to ensure use of non viral spermatozoa from seropositive patients employing gradient centrifugation and washes at certain conditions, which cannot eliminate risk but can certainly contain it. In particular CREAThE (Centers for Reproductive Assistance Techniques to HIV couples in Europe) report on >4500 inseminations of HIV-negative female partner with HIV positive sperm subjected to preparation and separated from the semen with none of the women or the hundreds of children produced being infected. ESHRE (European Society of Human Reproduction) report on >15,000 cycles of intrauterine inseminations/IVF employing washed sperm without reported HIV transmission. Finally informed consent and counseling and their necessity are discussed. Viral screening policies differ between ART Centers, and different countries. At present different protocols are observed for detection and quantification in both serum and semen and there are differences in processing techniques and sensitivity of assays. We need worldwide accepted guidelines to be in place to promote better ART services for these special patients.

HPV E7 oncoproteins as new markers for detection of cervical cancer and precancer

Pidder Jansen-Dürr, PhD

Leopold-Franzens-Universität Innsbruck, Institute for Biomedical Aging Research, Innsbruck, Austria AND Tyrolean Cancer Research Institute, Innsbruck, Austria,
on behalf of the PIPAVIR consortium (www.pipavir.com)

Objectives: The main cause for the development of cervical cancer and precancer is a persistent infection by human papillomaviruses (HPVs) of the "high-risk" group. The integration of the viral high-risk DNA into the host genome often leads to a dysregulated expression of the viral proteins E6 and E7, which are the major transforming oncoproteins of HPVs. Current cervical cancer screening relies mainly on cytological analyses (Pap smear), which suffer from frequent false-positive and false-negative results. Our finding that E7 oncoproteins are expressed continuously in biopsies from cervical carcinomas indicates that high-risk HPV E7 proteins may be useful markers for the detection of cervical cancer and precancerous lesions.

Methods: We developed and characterized a set of rabbit monoclonal antibodies that detect E7 proteins from various high-risk HPVs with high sensitivity and specificity. Diagnostic tools based on these antibodies have been developed and were validated with cervical smears. Results of a clinical study will be presented and discussed.

Conclusion: Our results suggest that the detection and quantification of E7 proteins in cervical smears is feasible and provides a promising alternative solution for the reliable detection of HPV-driven precancerous lesions.

Immune responses to prophylactic and therapeutic HPV vaccines

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More than 99% of cervical carcinoma and most premalignant dysplasia of the cervix uteri are associated with human papillomavirus (HPV) infection. Also other dysplasia and malignancies of the anogenital and oropharyngeal epithelia are associated with HPV infection. Most HPV infections – that are very common – regress spontaneously due to an immune reaction. Viral gene expression leads to antigen presentation by the infected cells tagging them with strong viral antigens for the immune system. Many therapeutic and prophylactic vaccine candidates have been developed over the past decades. Two prophylactic HPV vaccines have been developed and approved in 2006 that showed very high efficacy and sustained immunogenicity in all studies. As protective mechanism high antibody titers are induced directed against the major capsid protein L1. These antibodies have virus-neutralizing activity preventing the initial infection of host cells. However, also strong helper T cell responses are induced that are necessary to stimulate B cell differentiation and antibody isotype switching. These helper T cells itself could have a protective effect and a cytostatic/cytotoxic activity on HPV-infected keratinocytes augmenting and supporting the efficacy of the prophylactic vaccines. New vaccination strategies also rely on the minor capsid protein L2 that has shown a much wider cross-reactivity with other HPV types. In parallel many different strategies of therapeutic vaccines have been developed and tested in clinical trials showing mostly disappointing results so far. This is due to several reasons like i) necessity of stimulation of an immune system that has already failed, ii) immune resistance of the tumor cells that have evaded a strong immune response of prolonged period of time, iii) initial trials were done in patients with end stage disease. Recently trials in earlier disease stages and in patients with premalignant dysplasia have shown promising results. The use of strong adjuvants and local immune stimulation may further enhance this initial success.

Biomarkers of oncogenic HPV infection causing significant cervical disease in Scottish women

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Scotland has a national archive of liquid based cytology (LBC) specimens collected over the past 10 years from different centres. This has a generic ethics approval to supply samples for HPV related research. Together with our unique form of patient record linkage in the NHS in Scotland this provides a unique resource to examine biomarkers of disease in well characterised clinical samples.

We have examined High Risk (HR)-HPV typed (Luminex extended genotyping) specimens from the National HPV archive that showed A) normal cytology and no infection; B) normal cytology with HR-HPV infection; C) CIN1 with HR-HPV infection; D) CIN2 with HR-HPV infection and E) CIN3 with HR-HPV infection.

As many countries are moving towards HPV typing as the primary test for cervical screening there will be an excess of women identified with HR-HPV infection (approximately 10% of all samples screened) compared to those who need further examination for significant disease by colposcopy (approximately 20% of HR-HPV positive women). There is thus a need to develop adjunct, reliable laboratory tests to discriminate significant infection in LBC samples. We have assessed for presence of cytokines and cancer stem cell markers at both protein and mRNA levels. We have found protein to be more reliable as this is better preserved by the transport medium used.

We have further examined expression of markers in cervical biopsies from women with HR-HPV+ve squamous cell carcinoma. Data will be presented to show the distribution of proteins associated with malignancy and with the process of autophagy, a molecular process known to be important in the regulation of cell stress, holding an important role in the regulation of intracellular infection, immunity and the pathogenesis of a wide range of cancers.

Sexually transmitted viruses and cancer

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The authors briefly review: (1) the causative link between viruses and human malignancies – beginning with Epstein-Barr virus and Burkitt's lymphoma, and (2) the concept of 'venereal diseases' in its evolution towards the concept of 'sexually transmitted infections' or STI.

Too often people understand STI as something that causes vaginal discharge. Vaginal discharge might be the clinical manifestation of STI, but this relationship is far from mandatory and could be classified as follows:

- vaginal discharge that is STI, e.g. trichomoniasis, candidosis;
- vaginal discharge that is not STI, e.g. senile colpitis, atopic vulvovaginitis;
- STI that might appear as vaginal discharge, but not always, e.g. chlamydiosis, HPV infection;
- STI that are never the direct cause of vaginal discharge, e.g. hepatitis B, AIDS.

The agents of STI occupy quite various levels in the taxonomy of organisms: arthropoda, protozoa, fungi, bacteria, viruses. Both DNA and RNA viruses can cause STI. Sexually transmitted viruses that display the narrowest link with neoplastic growth are HPV, HbV and HIV. Amongst them HPV assumes the uncontestably leading position, being the cause of cancers in at least eight organs, namely cervix uteri, anus, vagina, vulva, penis, tongue, tonsils and oesophagus.

Fortunately, HPV infection (as well as HbV infection) is preventable by means of vaccines. Unfortunately, anti-HPV vaccines will have their impact on cancer morbidity and mortality in some (if any) future. Once having made their appearance, the sequels of HPV infection at the cellular level, namely intraepithelial neoplasia progressing towards invasive cancer are successfully treatable if discovered at a sufficiently early stage. The main difference between (1) preventing HPV infection and (2) successfully treating its sequels is that prevention does not cause any inconvenience, while treatment could be the cause of reproductive problems, and is inevitably the cause of regular (and therefore distressing) contacts with medical care.

Detection of HPV and other sexually transmitted virus infections in cervical specimens from women in Kardzhali region

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Background. The incidence of viral sexually transmitted infections has increased considerably in recent years. Human papillomavirus (HPV) infection is the most prevalent sexually transmitted infection and the main cause of cervical cancer. The role of other virus infections is not clear, although they have been suspected as possible cofactors in HPV-related oncogenesis. The purpose of this study was to determine the prevalence of HPV, herpes simplex virus (HSV) type 1 and type 2, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human immunodeficiency virus (HIV) in the cervical specimens obtained from women from Kardzhali region. We report here our preliminary results.

Methods. The study enrolled 52 women aged 17–57 years from Kardzhali. Exfoliated cervical specimens were obtained from all women. Each participant was interviewed by use of a standardized questionnaire. Cervical samples were tested by real-time (RT) PCR assay for the presence of HPV, HSV type 1 and type 2, CMV, EBV and HIV. In addition, genotyping of HPV positive samples was performed.

Results. Viral DNA was detected in 34 (65.4%) of the cervical samples. Rate of multiple infections was 21.2%. 19 women (36.5%) were HPV-positive. Of them, 11 (21.2%) were HR-HPV-positive. Genotyping showed that HPV6 was the most prevalent HPV type. HPV16, HPV18, HPV31, HPV52, and HPV56 were also detected. HSV DNA was found in 16 (30.8%) women, of them 15 were positive for HSV-1 DNA. CMV and EBV were detected in 5.8% and 9.6% of women, respectively. All specimens were negative for HIV.

Conclusions. This is the first study in Bulgaria on evaluation of status of five virus infections in cervical samples by RT-PCR. Our preliminary results indicate a high proportion of infected women. Because the size of study group is small, caution is needed in generalizing the findings from this study. Further surveys on prevalence of HPV and other sexually transmitted virus infection involving larger groups of women are needed.

Comparison between cytology and HPV DNA testing - using the Cobas 4800 system, Roche® - in the context of primary screening for cervical cancer prevention. Preliminary results from a multicenter greek study.

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Objectives: Comparison of the performance of cytology versus HPV DNA testing in the context of primary cervical cancer screening.

Methods: In 4,106 women aged 25-55 years, who visit the outpatient clinics of OB/GYN Univ. Departments in Greece's largest cities for cervical screening purposes, a liquid-based (ThinPrep®) Pap test is performed. After the cytologic evaluation the material collected is used to detect DNA of 14 oncogenic HPV types using the COBAS 4800 System, Roche® [16 &18 separately, and 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 as a pool].
Considering as objective for cervical cancer screening the detection of ≥CIN2 (CIN2+) lesions, women found positive (cytology ≥ASCUS and/or hrHPV DNA) in the first round are subjected to colposcopic evaluation (+/- biopsies, according to the colposcopic findings). If colposcopy proves normal, women are subjected again to HPV DNA and Pap testing in 1 year. Then, if both tests negative, reevaluation in 3 years, if one of these tests positive, colposcopy (+/- biopsy).

Results:

Preliminary results

HISTOLOGY	PAP TESTING		HPV DNA TESTING	
52 CIN1	(-)	31	(-)	6
	(+)	21	(+)	46
33 CIN2+	(-)	18	(-)	0
	(+)	15	(+)	33

Conclusion: HPV DNA testing using the Cobas 4800 System, Roche® seems to be by far more sensitive than cytology as a method of primary screening for cervical cancer.

HPV and upper aerodigestive tract cancers

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In 1995, the International Agency for Research on Cancer first classified HPV types 16 and 18 as carcinogenic to humans. Although HPV types 16 and 18 are the most dominant types implicated in cervical cancer (verrucous carcinoma and squamous cell carcinoma) in all continents, more recent evidence has expanded the list of carcinogenic HPV types to include a total of 13 mucosotropic anogenital HPV types as being definite or probable carcinogens (grade 1 or 2a)[1,2] .

HPV testing of biopsy specimens including FNAs and brushing specimens can establish the diagnosis of HPV-related oral carcinoma. HPV testing of cervical lymph node specimens can also establish the HPV status of these carcinomas. HPV testing of FNA material of cervical lymph node metastases from unknown primaries can aid in directing the search for the primary site to the oropharynx[3].

Patients with HPV-associated head and neck squamous cell carcinomas (SCC) have a distinctly different clinical profile from squamous cell carcinomas associated with tobacco and alcohol use. The HPV-related head and neck SCC occur in a younger age group and most commonly in the oropharynx[4]. Infection rates in oropharyngeal SCC range from 20% to more than 90% in different studies, depending on geographical factors and the detection method used. In the last decade, the incidence of HPV-related oropharyngeal SCC has increased relative to the total group of head and neck SCC[5].

Although recently overshadowed by its involvement in oropharyngeal carcinogenesis, the classical location of HPV infection in the upper aerodigestive tract has been the larynx. However, with laryngeal papillomatosis being caused by low-risk HPV types 6 and 11, recurrent respiratory papillomatosis may develop into SCC in percentages as low as 1.2%[6]. The most common HPV type detected in laryngeal cancers is HPV16. Low-risk HPV are uncommonly detected, and might represent incidental "bystander" rather than viral integration and viral-mediated. The most common HPV type detected in oral cancers is also HPV16. Regarding sino-nasal cancers, the published findings support the role of low-risk HPV in the etiology of benign Schneiderian inverted papilloma, and the hypothesis that high-risk HPV(most commonly HPV-16) is responsible for malignant progression of inverted papilloma[7].

Recent data on treatment response indicate that patients with HPV-related SCC demonstrate a better response to radiation therapy and an overall improved survival compared to patients with non-HPV associated tumors[8]. HPV positivity has been shown to correlate with lower risk of tumor progression and increased sensitivity to ionizing radiation with or without associated chemotherapy[9]. The above data have given rise to the assumption that HPV positive patients may undergo deintensified treatment, without compromising treatment efficacy. However this hypothesis remains to be verified by prospective clinical trials. Besides the overall better survival of patients with HPV-

positive tumours, their treatment may be further improved by the implementation of strategies that may promote the immune response to eradicate the virus, inhibit viral DNA replication, specifically target viral oncoproteins or have an effect on deregulated signal transduction pathways specific for HPV-positive tumour cells[5]. Current guidelines for the treatment of oropharyngeal SCC have not incorporated specific treatment modalities for HPV-related tumors. The development of such treatment options is still in a preclinical phase or in early clinical trials.

Patients with HPV positive sinonasal SCC have significantly improved 5-year progression-free and overall survival rates. As far as oral and laryngeal SCC is concerned, however, HPV positivity has not been associated with significant improvement in overall survival, time to disease progression or disease specific survival[7].

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The two faces of HSV: An enemy or a friend?

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are important human pathogens that cause a variety of diseases from mild skin diseases such as herpes labialis and herpes genitalis to life-threatening diseases such as herpes encephalitis and neonatal herpes. HSV is a neurotropic virus, that after initial lytic replication in epithelial cells enter sensory neurons and is transported retrogradely along axon to the neuronal soma, where the viral genome enter the latent state. In a fraction of neurons the virus is periodically reactivated and the infectious virus particles are transported by anterograde axonal transport at or near the site of initial infection. The latent state is a characteristic of the peripheral nervous system (PNS), whereas spread to the central nervous system (CNS), either from the PNS following reactivation or as a new infection via the olfactory route, can end either in latency or productive replication. A growing body of epidemiological and experimental data points to HSV infection as a possible co-factor in the progression of Alzheimer's disease (AD). At this regard, it has been shown a relationship between HSV axonal transport and proteins involved in AD and it has been demonstrated that the products of HSV genome interact with many AD susceptibility genes and proteins. In particular, there are data linking HSV-1 with the main neuropathological features of the disease, amyloid plaques and neurofibrillary tangles, which comprise mainly beta-amyloid and abnormally phosphorylated tau, respectively. If the pathogenic cascade occurring in human brain and leading to AD is proven to be triggered by a series of minor HSV-1 reactivations in CNS, over a long period of time, then a possible new approach to AD treatment could be antiviral therapy and/or therapeutic HSV vaccines.

A number of studies have elucidates the role of HSV products in viral replication, immune response to infection, immune evasion from the host system and pathogenicity. This research has allowed the development of HSV vectors for human diseases and HSV-vector vaccines. These major HSV-derived vectors include: (i) amplicons for gene delivery vectors; (ii) replicative-defective HSV recombinants for vaccine vectors or gene therapy of neuropathies; (iii) replication-attenuated HSV recombinants for vaccine vectors or oncolytic virotherapy. The development of HSV vectors for human use faces substantial challenges, both in the scientific ad regulatory fronts. However, these technologies are finally achieving their efficacy in both animal and human. The translation of these new technologies will facilitates vaccine development against HSV diseases, such as genital herpes, as well as other pathogens diseases, such as tuberculosis. Moreover, HSV-oncolytic vectors are emerging as one of the most effective and powerful therapeutic approach for solid tumors.

Herpes simplex virus: From infectious pathogen to gene therapy vector

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Herpes simplex virus type 1 (HSV-1) is a common human pathogen with a worldwide seroprevalence of up to 80% in adults. The virus causes lytic infection at the site of host entry and lifelong latent infection in the proximal trigeminal ganglia. Most commonly, lytic infection takes place in orofacial mucosal epithelia cells, but also genital mucosa epithelia cells and cutaneous sites can become infected. In some cases infection can cause severe meningo-encephalitis and kerato-conjunctivitis that can lead to permanent neurological damage with high mortality or corneal blindness, respectively.

Over the past three decades, two different approaches have been taken in parallel to turn pathogenic HSV-1 into a safe and efficient gene delivery vector. One strategy was to progressively delete selected genes from the viral genome and replace them with therapeutic transgenes of interest. Depending on which viral genes are deleted, these so called recombinant HSV-1 vectors are replication-defective or replication-conditional and may be applied in somatic gene therapy, cancer gene therapy and vaccination. The second approach was based on the observation of defective HSV-1 genomes upon serial passaging of wild type HSV-1 at high multiplicities of infection. Defective genomes retained only a fraction of the HSV-1 genome, including a DNA packaging/cleavage signal and an origin of DNA replication. These two *cis* acting HSV-1 elements cloned in a bacterial plasmid formed the backbone of the so called HSV-1 amplicon vector, which can be equipped with therapeutic transgenes of interest. HSV-1 amplicon vectors are replication-defective as they do not encode any HSV-1 genes, but they can be propagated and packaged into HSV-1 virions by using helper virus-dependent or helper virus-free strategies. HSV-1 amplicon vectors can accommodate foreign DNA of up to 150 kbp, which allows not only the insertion of entire genomic loci but also the addition of various other elements, such as promoters, enhancers or inducible systems for regulated gene expression, marker genes to enable visualization of fusion proteins or transduced cells, immune modulatory or other therapeutic genes, and genetic elements from other viruses to create hybrid vectors.

This talk will focus primarily on the design and application of HSV-1 amplicon vectors.

Human Cytomegalovirus: from pathogenesis to novel insights of antiviral therapies through epigenetic and miRNA regulation

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Human Cytomegalovirus (HCMV) is a widespread virus that infects 40-90% of the world's popupalation. It can cause life-threatening disease to immunocompromised individuals and it is the most common viral infection in newborns. Healthy adults usually remain asymptomatic or rarely present with an infectious mononucleosis type of syndrome when first infected by the virus and subsequently harbor an asymptomatic latent infection that is prone to reactivation in the setting of immunosuppression or if they become critically ill. HCMV is a large double stranded DNA virus and its 230 kb long genome encodes approximately 180 viral proteins. Viral gene expression during HCMV infection occurs in a temporally regulated manner and it is characterized by three sequential and interdependent waves of transcription: immediate-early, early and late. Among the mechanisms regulating HCMV gene expression, epigenetic changes have been shown to be reversibly implicated. Earlier studies have confirmed that immediate-early gene transcription can be altered by the acetylation status of histone H3 associated with the major immediate-early promoter. We recently identified that productive HCMV infection is controlled by histone H3K27 trimethylation, through the Polycomb Repressive Complex 2 (PRC2), affecting the HCMV negative regulator named GFI1. Parallel studies on the miRNA profile during HCMV lytic infection, identified clusters of host-miRNAs belonging to the NF-κB inflammatory and the PRC2 chromatin networks which are dynamically induced as a response to the virus. Using a novel drug discovery approach it was found that both networks are specifically regulated by specific compounds, which are reported to exert robust anti-HCMV effect in vitro by inhibiting the expression of immediate-early viral genes and thus imposing an early/premature stop to the virus lifecycle.

HIV: The past, the present and the future

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HIV-1 is a retrovirus responsible for a pandemic worldspread disease. Since 1983 when the virus was identified more than 35 million individuals have been infected. In Greece the cumulative number of reported HIV infections (including AIDS cases) by 31/12/2012 was 12689 (81.6% males). Overall the main route of transmission is male-to-male sexual contact. Hence in 2011 an outbreak among injecting drug users was recorded. That led to an increase of the coinfections incidence (HIV/HCV/HBV).

In 1987 the first antiretroviral drug was administered to HIV (+) individuals (AZT, Zidovudine), whereas now more than 20 agents are available. We use them in combination as an antiretroviral regimen (three active agents at least). The goal is to inhibit maximally viral replication, allowing re-establishment and persistance of an effective immune response that will delay or prevent HIV-related morbidity. Fully undetectable levels of virus are the target of therapy for all patients. The enormous evolution of pharmacokinetic and pharmakodynamic studies help us to develop new, easier, more effective and less toxic antiretrovirals. Combinations of 3 or 4 drugs in one pill, known as Single Tablet Regimen (STR) are available.

Additionally over the past few years there has been a remarkable increase in our knowledge of the variation in human genome. In parallel genotyping technologies have advanced significantly and allow sufficient throughput to accommodate genome-wide approaches. This knowledge has allowed the implementation of pharmacogenomics in the clinical management of HIV infection. Despite the fact that only one case of HIV-infected individual has been reported as cure (the Berlin patient), new strategies such as gene therapy, immunomodulators and new drugs are in the pipeline. The future will be different and interesting.

New insights into the molecular biology of HCV: the role of core protein(s) in hepatocarcinogenesis

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HCV is a small positive sense RNA virus of the *Flaviviridae family* (hepacivirus genus) with severe health consequences for infected individuals. HCV is a major cause of chronic human liver diseases that can progress to cirrhosis and hepatocellular carcinoma (HCC). Approximately 130 million people worldwide are chronically infected with the virus. The global burden of HCV-associated HCC is difficult to estimate, but HCV infection has become the leading cause for HCC in many countries, and together with HBV are implicated in more than 70% of HCC cases. No vaccine exists to prevent HCV infection and the current antiviral treatment is inadequate in a relatively significant proportion of patients.

HCV is unable to integrate into the host genome. Its positive sense, single-stranded RNA genome (9,6 kb) is translated in an IRES-dependent mode to generate a single polyprotein which is proteolytically processed by host and viral proteases into three structural proteins (core, E1 and E2), p7 and several nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). In addition, a protein known as ARPF or F or core+1 that is encoded by the core coding region has been recently identified. Most if not all of the viral proteins are multifunctional and in addition to their basic functions in supporting new virion production and viral genomic replication, viral proteins interact with host proteins/pathways in ways that antagonize host antiviral immune responses and otherwise alter the cellular environment to favor virus production. Thus, an important prerequisite for understanding the pathogenesis of viral infection is to understand how viral proteins interact with the host cell machinery to support viral replication and evade host antiviral responses.

HCV exhibits a considerable degree of sequence diversity resulting into six major genotypes and more than a hundred of different subtypes. All currently recognized HCV genotypes are hepatotropic and pathogenic. HCV genotypic/genetic variability affects virus infectivity and pathogenicity, thereby influencing the rate of disease progression, the risk of HCC as well as the response to current anti-HCV therapy.

HCV core protein appears to have the most important function in hepatocarcinogenesis. Apart of its role in virion formation, core has a diverse range of functions and interacts with many cellular proteins and pathways. HCV core modulates apoptosis, reactive oxygen species (ROS) formation, lipid metabolism and steatosis, cell growth, transformation, angiogenesis and immune responses. Core protein regulates the activity of several tumor suppressor proteins and cyclin/cyclin-dependent kinase complexes involved in cell-cycle control and tumor formation. Additionally, core activates important cancer signaling pathways, including the Raf/MAPK and the wnt/ß catenin pathways and regulates the activity of several transcription factors including retinoid X-receptor (RXR), nuclear factor-kappa B (NF-κB), CCAAT enhancer binding protein-α (C/EBPα), activating protein-1 (AP-1), SREB and PPAR-α. Most importantly, transgenic mice (C57BL/6) expressing core protein under the control of HBV promoter developed steatosis and HCC. The effect of HCV genetic variability on the core protein functions as well as the role of the newly discovered core+1/ARF protein on HCV- associated HCC will be discussed.

Hepatitis B virus: clinical spectrum and treatment

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Hepatitis B has a wide spectrum of manifestations including subclinical hepatitis, icteric hepatitis and fulminant hepatitis during the acute phase; and asymptomatic carrier state, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) during the chronic phase.

Approximately 70% of patients with acute HBV infection have subclinical or anicteric hepatitis, whereas 30% become icteric. Acute liver failure develops in approximately 0.1 – 0.5% of patients. The incubation period lasts 1-4 months. A serum sickness-like syndrome may develop during the prodromal period. This is followed by constitutional symptoms such as low-grade fever, malaise, anorexia, nausea and vomiting, and right upper quadrant or mid-epigastric pain. Jaundice usually appears as the substitutional symptoms begin to subside. Clinical symptoms and jaundice generally disappear after 1-3 months but some patients may have prolonged fatigue, even after normalization of aminotransferase levels. Elevation of ALT and AST levels, up to 1000-2000 IU/L, is typically seen during the acute phase, with ALT levels higher than AST levels. Prothrombin time, which reflects hepatic synthetic function, is the best indicator of prognosis. In patients who recover, normalization of aminotransferases usually occurs within 1-4 months, followed by normalization of bilirubin levels. Persistent HBsAg lasting more than 6 months indicates progression to chronic infection

Approximately 50% of patients in low or intermediate areas and the majority of patients in high prevalence areas develop chronic HBV infection without experiencing an acute hepatitis. Many patients are asymptomatic, while others may have non-specific symptoms such as fatigue and mild right upper quadrant discomfort. Some patients experience exacerbations that may be asymptomatic, mimic acute hepatitis or manifest as liver failure. Physical examination may be normal or there may be stigmata of chronic liver disease. In patients with cirrhosis, additional findings such as jaundice, splenomegaly, ascites, peripheral oedema and encephalopathy may be present. Laboratory investigations including liver profile and blood counts may be normal in some patients, but most patients have mild to moderate elevations of AST and ALT levels. During exacerbations, ALT levels may be as high as 50 times the upper limit of normal and α-fetoprotein (AFP) levels of up to 1000 IU/L may be seen. Progression to cirrhosis is suspected when serum AST levels are superior to ALT levels, when there is evidence of hypersplenism (decreased leukocyte and platelet counts) and impaired hepatic function (hypoalbuminemia, prolonged prothrombin time and hyperbilirubinemia).

Extrahepatic manifestations occur in 10-20% of patients with chronic HBV infection. Mediated by circulating immune complexes, acute hepatitis may be heralded by a serum sickness-like syndrome. Vasculitis associated with HBV may affect large-, medium- and small-sized vessels in multiple organs. The course is highly variable and the mortality rate is high. Hepatitis B virus-related membranous glomerulonephritis is more often found in children and 60% of the cases undergo spontaneous remission. In adults the course may be progressive and response to interferon poor. Other extrahepatic manifestations including essential mixed cryoglobulinemia and aplastic anemia have also been reported.

To date, two classes of antiviral drugs have been approved by the Food and Drug Administration for the treatment of hepatitis B, immunomodulators (interferon [IFN]-α and pegylated-interferon [PEG-IFN]-α) and nucleos(t)ide analogs (lamivudine, telbivudine, adefovir, tenofovir [TDF], and entecavir [ETV]). Of these, ETV, TDF, and PEG-IFN-α are the most effective and are currently recommended for anti-HBV therapy. However, these therapies are less than optimal because of their low rate of surface antigen clearance; thus, there exists a significant unmet medical need for safe and efficacious new anti-HBV drugs.

HPV vaccination: An ideal weapon against a steady growing threat

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The most important malignant consequence of HPV infection is cervical cancer, but recent research indicate increased risk also for other anogenital and certain oropharyngeal cancers, and possibly also lung and esophageal cancer. So far, these aspects are insufficiently researched, and the consequences of vaccination are thus hardly evaluable at this point. However, we are today just at the doorstep to the new era of primary prevention of cervical and other HPV-related cancers as anti-HPV-vaccines are commercially available. So far, the final picture of their place and consequences is by far determined or known due to several independent factors, namely the spectre of HPV disease burden, the composition of the vaccines, the mode of implementation of vaccines and whether the actual country already have screening programmes. To date, randomized clinical trials with histologically verified CIN 2/3 as endpoints have been published with by quadrivalent vaccine against types 6, 11, 16, 18 as well as with bivalent vaccine against HPV type16 and 18. The striking preventive effect common for all of them is just impressive, leading to enthusiasm and considering this development to be a new paradigm in cervical cancer prevention: primary prevention rather than secondary prevention by Pap smears. The selected HPV types are the clinically most relevant ones, but still at least ~15 other HPV types may cause infection and disease in the urogenital and oropharyngeal mucosa. Therefore we must probably consider these vaccines to be the first generation, and expect new polyvalent vaccines to be introduced in the future.

Quality management systems in molecular diagnostics

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The certification and accreditation of the Clinical Laboratories aim to improve the quality of patient care. Laboratory medicine is the backbone in medical treatment, diagnosis and prevention. Laboratory diagnostics influence 70–80% of hospital health care decisions and cost between 3–5% of total health care expenses.

The concept of laboratory accreditation is defined by ISO/IEC as formal recognition that the testing laboratory is competent to carry out specific tests or specific types of tests.

ISO standard 15189:2007, Medical laboratories – Particular requirements for quality and competence, encompasses all the assessment criteria specified in the policy statement and as such should form the basis for the accreditation of laboratories. A single accreditation body operates in each country. In Greece the official accreditation body is ESYD.

The core points in laboratory accreditation systems are internal and external quality control and educational activities.

Laboratory accreditation is mandatory in several European countries, while in others it is expected to become so in the near future. In Greece, all clinical laboratories, since 2011, should be certified and apply internal and external quality control for all tests provided (Law 4025/2011 art.34). The same law obliges laboratories that perform laboratory testing for other health providers to be accredited according to ISO 15189:2007

The five most important points for the recognition of the technical competence of a Molecular Diagnostics laboratory are:

- The infrastructure of the Laboratory.
- Selection of appropriate laboratory methods-Good laboratory practice. The selection includes the application characteristics of the methods, the analytical characteristics and the clinical validation and utility.
- The validation and verification of methods depending on whether it is a new laboratory approach or standardized method of manufacturer.
- Internal quality control at appropriate intervals and the use of appropriate control samples.
- External quality control with successful participation in proficiency testing. These are offered by organizations as UK-NEGAS, EMQN, CAP, QCMD, UNIQ, INSTANT etc. who suggest proficiency testing schemes accredited by the international standard ISO 43-1.

The above elements are needed to bring molecular QC practice up to the same level as other laboratory disciplines. Therefore, it is crucial to determine error rates, to adopt traditional QC protocols in order to prevent failure, to introduce current proficiency requirements and built-in software to facilitate QC strategies.

Molecular Detection of HPV

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Human Papilloma Virus (HPV) is the most common sexually transmitted disease either in its clinical (warts on cutaneous epithelium and genital warts) or asymptomatic presentation in men and women.

The Papillomaviruses are small double-stranded DNA viruses. The main characteristic of viruses is display absolute species-specificity: human papillomavirus (HPV) only infect humans. The viral genome is circular with size is close to 8.0 kb and is enclosed in a protein shell made from the major (L1) and minor (L2) capsid proteins. It can be divided into 3 domains: a noncoding upstream regulatory region (URR), an early region with 6 genes (E6, E7, E1, E2, E4 and E5), which are involved in DNA replication, transcriptional regulation and cellular transformation, and a late region encoding two genes (L1 and L2) which encode capsid proteins [1]. There are more than 100 different HPV genotypes that have been divided into two groups: low-risk and high-risk based on their oncogenic potencial.

The main interest in HPV relates to its causative role in different cancer, especially as cause of cervical cancer. Numerous medical societies such as the American College of Obstetricians and Gynecologists (ACOG), the American Cancer Society (ACS) recommend testing for "high risk" HPV types to increase the efficacy of cancer screening.

HPV cannot be grown in cell cultures and serological assays have only limited accuracy and are not commercially available.

The diagnosis of HPV infection relies on viral nucleic acid (DNA and/or mRNA) detection. HPV-DNA can be detected in different samples such as cervical smears, urine, biopsy specimens by various methods. Results of HPV detection are strongly influenced by the technique, and comparison between assays is not always possible.
The first protocols for detect HPV were described about 20 years ago, using L1 consensus primers PCR systems, particularly MY09/11 and GP5+/6+ [2]. Nowadays, several kits such as Amplicor HPV test and Linear array HPV Genotyping test (Roche Diagnostics, Switzerland), Innolipa HPV Genotyping Extra (Innogenetics, Belgium), Biopat kit (Biotools, Spain) or Clart Papillomavirus 2 (Genó mica, Spain) are commercially available which allow for the detection of the virus or the detection and typification of the most relevant HPVs. The major diagnostic techniques for HPV detection and genotyping are target amplification, signal amplification, and probe amplification.

Hybrid Capture Technology (HC)

HC developed by the Digene Corporation, detects nucleic acid targets directly, using signal amplification to provide sensitivity comparable to target amplification methods.
Digene has developed two products for the detection of HPV: the first-generation Hybrid Capture Tube (HCT) test and the more recent Hybrid Capture II (HCII) [3].

Reverse Line Blot

The reverse line blot assay from Roche Molecular Systems (Alameda, CA) was one of the first widely used prototype methods. The line blot assay uses L1 consensus primer-based PCR with PGMY09/11 primers. Probes for multiple HPV types are fixed on a membrane strip, and the PCR product is hybridized to the strip, followed by visual detection. The kit detects a 27 different HPV types.

The Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN) is able to identify 37 types of HPV (14 high-risk genotypes) and the kit is CE-Marked for *in vitro* diagnostic use in Europe [4]. Another commercially available kit based on principles of reverse line blot hybridization is INNO-LiPA HPV Genotyping Extra (Innogenetics, Ghent, Belgium). The INNO-LiPA test amplifies HPV DNA with SPF10 primers at the L1 region. The probes are fixed to membrane strips in sequence-specific lines and visualized as purple/brown bands. The test can detect 24 HPV types. The kit is CE-Marked for use in Europe [5].

Real-Time PCR

Real-time PCR is a highly sensitive target amplification technique available for HPV-DNA detection.

The Abbott Real-Time HR-HPV test is a novel assay based on concurrent individual genotyping for HPV-16/18 and pooled detection of 12 HPV genotypes: -31, -33,-35, -39, -45, -51, -52, -56, -58, -59, -66 and -68 [6]
COBAS 4800 HPV test

This test features automated sample preparation combined with Real Time PCR technology to detect 14 HR HPV. The PCR amplification and detection occur in a single tube, 1-HPV -16, 2-HPV -18, 3-12 HR (31,33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) as a pool, and 4-ß-globin as the control for extraction and amplification adequacy [7].

In summary, HPV infections require a close monitoring, especially in certain groups of individuals (women older than 30 years old, patients with positive Pap test). The accuracy of methods, that using nowadays has emerged as good methods in the control of HPV infection. Moreover, the search is ongoing for safer: more precise markers which may allow for a better control of the infection. These markers include genome quantification by real-time PCR, viral integration into the human genome via E2-E1/E6-E7 genes ratio or the search of viral variants by sequencing.

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