a history of confirmed or suspected contact with HCV without confirmed HCV infection (seronegative): 3 needle-stick injuries (1 "donor" HCV seropositive); 1 newborn of an HCV seropositive mother; 1 wife of a man with liver cirrhosis; 1 i.v. drug-abuser; 1 nurse with a prior blood transfusion, an indeterminate HCV Western Blot, and "indeterminate HCV results" in a blood donation center.

Conclusion: Based on our experience to date, borderline results and/or discordant replicates obtained with the Amplicor HCV Test Version 2.0 are indicative of very low-level viremia (<100 copies/ml) due to either an HCV infection (seropositive patients) or transient contact with the virus with or without subsequent HCV infection (patients will be seronegative and may remain so). Follow up of such patients is mandatory.

HCV genotypes and age distribution in patients of Vienna and surrounding areas

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Background: HCV infection will progress in 75% or more to chronic hepatitis C (CHC) with an almost normal prognosis, which can also progress to cirrhosis (20%) and hepatocellular carcinoma.

Objective: To determine HCV genotype (GT) / subtype (ST) and age distribution, HCV sources, and associations between GT/ST and the patients' liver situation.

Study design: Retrospective investigation of 250 consecutive patients diagnosed as CHC.

Results: Frequency of GTs 1, 2, 3, 4 and 5 was 74.8%, 2.8%, 16%, 5.2% and 0.4%, respectively. Main STs were 1b (54%), 1a (15.6%) and 3a (15.6%). Patients with GT 1 (53.8±18.3 years) and 2 (51.0±12.2 years) were older than patients with GT 3 (37.2±9.6 years; P<0.0001, P<0.01) and GT 4 (37.2±10.7 years; P<0.001, P<0.05). ST 1b patients (58.1±17.5 years) were older than 1a (40.8±14.3 years; P<0.0001) and 3a patients (37.5±9.5 years; P<0.0001). HCV sources (main GT/ST): 21.6% blood and blood products (GT 1 83.4% [1a 13%, 1b 66.7%]), 30.0% intravenous drugs (GT 1 53.3% [1a 21.3%; 1b 26.7%], GT 3 40% [3a 38.7%]), 44.8% unknown. 13.6% of patients (n=34; CHC in 70.6% >10 years) showed cirrhosis (GT 1 97.1% [1b 76.5%, 1a 17.6%] and GT 3 2.9%) and were significantly older compared to asymptomatic patients.

Conclusion: The younger age of patients infected with STs 1a, 3a and GT 4 (n=91, 36.4%) and the shorter duration of their HCV infection (40.7% <10 years, 35.2% 10 to 20 years) with unclear long-term consequences will become a serious challenge for the public health system.

CMV Diagnosis in Renal and Bone Marrow Transplant Recipients: The Impact of Molecular Biological Based Assays

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Background: Cytomegalovirus (CMV) infections are a major threat in transplant patients. In the last years new tests for routine CMV diagnosis, based on molecular biological techniques, have become available.

Objective: To evaluate the impact of molecular biological based assays for CMV diagnosis.

Study design: 53 patients (32 RTX, 21 BMT) were screened for CMV infection from October 1999 to January 2000. Serological test (AxSYM CMV IgG and recombinant CMV IgM assay, Abbott), antigenemia, CMV DNA (qualitative in house PCR; quantitative PCR, Amplicor, Roche) and CMV mRNA (NASBA, Organo Teknika) tests were performed.

Results: In 9/21 BMT and 10/32 RTX patients there was no evidence of active CMV infection. 18 RTX and 8 BMT patients were CMV-IgM positive, 10 RTX and 1 BMT patients were antigenemia positive. There were more BMT patients CMV-DNA positive in serum (8/21) than antigenemia positive (1/21). CMV mRNA was positive in 2 BMT patients (one patient with no other evidence of CMV infection, the other one with positive CMV-DNA and negative antigenemia). The antigenemia positive patient was negative for CMV mRNA, but positive in all other tests. 8 RTX patients were positive for CMV mRNA. 6/8 patients were also antigenemia positive and 5 were positive for CMV-IgM. One CMV mRNA positive patient was CMV-IgM positive but antigenemia negative, the other CMV mRNA positive patient was negative in all other tests. Two antigenemia positive patients were negative for mRNA and CMV-IgM.

Conclusion: Antigenemia seems to be a good screening test for CMV infection in solid organ transplant recipients, whereas in BMT patients, tests based on molecular biological techniques seem to be better.

Diagnosis of HIV-1 Infection: Role of Nucleic Acid Amplification Techniques and Fourth Generation Screening Enzyme Immunoassays

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Since their introduction in 1985, the performance of human immunodeficiency virus (HIV) screening assays has continued to improve. The time interval between infection and antibody detection has substantially been shortened by using

third generation antigen-sandwich assays. The window interval between the presence of HIV-1 RNA in plasma and antibody seroconversion varies between 10.2 and 27.4 days, depending on the route of infection. HIV infection is detected between 9.4 and 17.4 days earlier by p24 antigen testing than with current third generation assays. Additional screening for HIV antigen has not been introduced world wide in blood banks for reasons of cost effectiveness. Although the prevalence and incidence of HIV infection in the general population in industrialized countries are relatively low, the residual risk of HIV transmission by blood donation (mostly by viremic but antibody negative donors) is 1/493,000 per unit in USA. By additional screening for p24 antigen the risk of HIV infection may be reduced to 1/676,000 per unit.

Fourth generation assays, which permit the simultaneous detection of HIV antigen and antibody, reduce the diagnostics on average by 4 days in comparison to antibody screening assays. The detection limit for p24 Ag (> 20 pg p24 Ag/ml) is higher than that of current antigen assays (3 - 15 pg/ p24 Ag/ml). Since fourth generation EIAs combine two different test principles in one assay, the potential for nonspecific reactivity (0.2 - 0.4%) is higher than with third generation antibody assays (< 0.1%).

Current commercially available nucleic acid amplification protocols are not adapted to large scale screening of blood donations, and false-negative reactions have been reported in patients with low HIV-1 RNA or cDNA copy number irrespective of the HIV-1 subtype. Detection of HIV-1 RNA in plasma pools with nucleic acid amplification techniques represents a time saving and cost-effective alternative for screening of large numbers of samples. However, fourth generation assays are more sensitive than any other single test, including RT-PCR, since HIV positive patients with a low viral load will have no detectable HIV-1 RNA, even if ultrasensitive PCR technology is used.

Molecular Detection of HPV in Women with Minor-Grade Cytological Abnormalities

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Background: Human papilloma virus (HPV) of high-risk type is the major pathogen connected with cervical neoplasia. Cervical cytology (Pap smear testing) has been widely used as a first-line screening method. Today, however, there are new standardized molecular methods available for direct detection of HPV DNA in cervical samples.

Objective: To evaluate the specificity of cytology regarding HPV detection in reactive and mild to moderate dysplastic changes including cellular patterns suggestive of HPV infection.

Study population: 466 women aged 17-58 years who attended STD Clinic at the University Hospital for Infectious Diseases in Zagreb in period from 1997-1999 for routine cervical examination and whose Pap tests revealed "HPV cellular changes" with or without mild and moderate cervical intraepithelial neoplasia (CIN1, CIN2). Pap smears were examined by two senior cytologists and reported as follows: (1) reactive changes (44 patients), (2) CIN1 (250 patients), or (3) CIN2 (172 patients).

Molecular HPV testing: Additional cervical swabs were obtained for molecular testing. The Digene HPV test Hybrid Capture II® was used according to the instructions of the manufacturer. This test can differentiate between low and high-risk HPV DNA groups.

Results: HPV was detected in 289 (63%) patients. It was the high-risk type in 90% of all positive cases. The detection in various cytology groups was as follows: 11/44 (25%) in reactive changes, 138/250 (55%) in CIN1, and 140/172 (81%) in CIN2.

Conclusion: Cytology is not an accurate method for detection of HPV infection in patients with minor-grade abnormalities. Many nonspecific inflammatory changes can mimic the cellular changes suggestive of HPV. Therefore, HPV DNA testing should be performed obligatory as an adjunct to cytology in order to identify patients who are at risk for developing high-grade abnormalities.

Preparation of Recombinant Nucleocapsid Protein of Hantavirus and Development of Their Monoconal Antibodies

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Hantavirus (HTV), also named hemorrhagic fever with renal syndrome virus, is the major pathogen of hemorrhagic fever with renal syndrome (HFRS). The small blood vessels and capillaries of the infectious patients are seriously destroyed. The mortality of the patients is more than 10%. Antibodies appear in the early period of infection. IgM can be detected from the second day of the fever onwards and will reach the peak within 7 to 10 days. IgG appears on the third to fourth day and reaches the peak within 10 to 14 days. So it is greatly promising to survey anti-HFRS IgM for early diagnosis of HFRS.

A small fragment of HTV RNA (position 76 to 118) was amplified by reverse transcription polymerase chain reaction (RT-PCR) and cloned into the expression vector pDS 56/RBS II-(O)-6His to express recombinant nucleocapsid protein (rNP), which was induced by IPTG and purified by affinity chromatography on a nickel-chelate resin, in *E. coli*. The characteristics of rNP were analysed by SDS-PAGE, immunoblot and ELISA.

The results demonstrated that both, the rNP and the viral NP have the same antigenicity, molecular weight (49.6 ku), and specific combination with sera from patients with HFRS. Using rNP as antigen, we immunized the Balb/c mice and took the spleen cells of immunized mice to fuse with the mice myeloma cells (SP2/O). After the procedure of four times fusions (clonal and sub-clonal), we got two hybridomas secreting anti-NP monoclonal antibodies persistently and stably.

HFRS is a serious syndrome and it is also an epidemic disease that has a high mortality in our country. So the early and exact diagnosis of this syndrome is a crucial step for adequate treatment. A u-capture ELISA was set up to detect IgM antibodies of HTV. This is a creative method compared with the IFA method that is usually used for diagnosis of HFRS and it was testified that this method utilizing purified