Aim: It has recently been proposed that hyperthermal incubation at 39°C increases cytokine (interferon-gamma, IFN-g) and chemokine (IFN-g induced protein 10; IP-10) secretion in whole blood interferon-gamma release assays specific for tuberculosis increasing test sensitivity in healthy vaccines and active TB patients compared with normothermal incubation at 37°C (1). It is unknown if hyperthermal incubation in addition of co-stimulatory factors have the same impact in patients with persistent infections associated with immune activation and higher background IP-10 levels. The current proof-of-concept study evaluated the cellular IP-10 response to recall antigens in chronically HCV or HCV/HIV co-infected patients dependent on incubation temperature and immune response modifying co-substrates in an in-house whole blood assay.

Methods: 400ul whole blood of 20 HCV pat. (11/20 HCV/HIV co-infected, mean CD4 cell count 620/ul; HCV genotype 11/20 3a, 9/20 gen.1a/b) were stimulated each overnight with optimal peptides covering common HLA-matched epitopes of recall antigens (CMV, EBV and influenza; CEF) in addition with anti-IL10 or IL7 at 37° and 39°C. Negative (no antigen) and positive (phytohaemagglutinin, PHA) controls were included. IP-10 secretion was measured by ELISA. Positive responses were defined by the antigen-specific minus the negative control +3SD IP-10 concentration; paired t-test was used for statistical analyses.

Results: 4/20 patients did not show a response towards recall CEF antigens (all HCV/HIV co-infected). In 16/20 pat. with a detectable CEF response there was no significant difference between normo- and hyperthermal incubation alone (mean IP-10 37°C 11.8 vs. 39°C 11.6 ng/ml, p=.5). Addition of co-stimulatory factors increased IP-10 secretion significantly (anti-IL10: mean IP-10 37°C 12.5 vs. 39°C 13.7 ng/ml, p=.007; or anti-IL10/IL7: mean IP-10 37°C 11.3 vs. 39°C 14.1 ng/ml, p=.002). The same pattern of IP-10 secretion was observed in the positive PHA control reaction.

Conclusion: Hyperthermal incubation alone has no significant impact on IP-10 secretion to recall antigens in a whole blood assay in individuals with persistent chronic infections, probably because of their already elevated IP-10 level. Nevertheless hyperthermal incubation in conjunction with co-stimulatory factors may proof beneficial, possibly increasing whole blood test sensitivities.
22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 31.03.2012 - 03.04.2012, London, UK; O 557 (oral session HIV infection)

Reference
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A Cluster of Invasive Aspergillosis After Solid Organ Transplantation: Identification of Modifiable Risk Factors

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Aim: Between 09/2009 and 01/2010 we observed an unusual incidence of invasive aspergillosis (IA) after solid organ transplantation (SOT). An investigation was initiated with the goal of identifying preventable risk factors.

Methods: We conducted a retrospective case-control study. Cases were matched 1:4 with controls for type of organ transplanted and time of transplantation, by choosing the two preceding and the two following patients transplanted with the same organ of each case. Observation time started at transplantation until occurrence of IA, and was identical between cases and controls. Odds ratios for predefined risk factors were calculated by exact univariable conditional logistic regressions analyses. Risk factors and modifiers analyzed were as follows: Operation time, surgical revisions, days of neutropenia, rejection, type of immunosuppression, graft function, intrahospital transfers, steroid use, active diabetes, colonization with aspergillosis, prophylaxis. Quantification of airborne aspergillosis spores was systematically performed.

Results: Seven patients developed 5 proven and 2 probable IA during this period. The transplanted organs included: heart (n=1), liver (n=2), lung (n=1) and kidney (n=3). The inhospital mortality was 71.4% and 7.1% for cases and controls, respectively. Steroid use and prophylaxis (bacterial, fungal or viral) were very similar in all patients and thus not discriminating. Type of immunosuppression, duration of operation, and number of rejections were not risk factors for IA. A trend for older age (case group: median age 60 y, control group: 45 y) and reduced graft function as risk for IA was observed. The major distinctive risk factors were the time spent in the intensive care unit (OR 5.20, 95% CI 1.24 – +Inf, p=0.008) and the number of intrahospital transfers (OR 1.65, 95% CI 1.10 – 3.50, p=0.005). Most air measurements failed to detect airborne conidia above 10 colony forming units (CFU)/m3 but very rarely revealed levels up to 100 CFU/m3.
Conclusions: In addition to the expected risk factors, our data revealed that intrahospital transfers, usually between wards or for special exams, were a significant risk factor for IA. We hypothesize that our patients may have been transiently exposed to aspergillosis spores during transfers, likely due to construction work. Since enforcing our guidelines to wear a mask when leaving the room no cluster has been observed.
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A rare cause of fever of unknown origin

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Case report: In October 2011, a 68-year old Caucasian Swiss entrepreneur with a history of chronic lymphocytic leukemia (CLL) for 4 years was referred to the University Hospital Zurich because of fever of unknown origin (FUO). He had a four week history of nearly daily episodes of recurrent temperature up to 40°C, chills, night sweats and weight loss. The history of the patient was remarkable for clinically asymptomatic tick bites years ago and extensive travelling all over the world. On admission, the patient was in a reduced general condition, with a temperature of 39.7°C without any hints of infectious foci. Laboratory findings showed leucocytosis of 19.1 G/L (94% neutrophils) and an elevated C-reactive protein of 65 mg/L (Norm < 5 mg/L) and procalcitonin of 20.2 ug/L (< 0.1 ug/L). A progression of CLL or a therapy-associated myelodysplastic syndrome has been excluded by bone marrow biopsy and splenectomy prior to this hospitalisation. Repeated microbiological and immunological analyses as well as various imaging procedures (echocardiography, computed tomography scans of thorax and abdomen incl. positron emission tomography) failed to reveal an infectious, malignant, or autoimmune cause of the fever. Ultimately, bacterial broad-range 16S rRNA gene polymerase chain reaction (PCR) followed by sequence analysis identified Candidatus Neoehrlichia mikurensis in a peripheral blood sample and in a bone marrow biopsy. Antibiotic treatment with doxycycline for six weeks was installed. The patients became afebrile within two days of treatment and clinical symptoms improved. After 27 days of treatment, bacterial broad range PCR of a blood sample was negative for the first time.

Discussion: Here we report the diagnosis of human Neoehrlichiosis in an immunocompromised patient who initially presented with FUO. Candidatus Neoehrlichia mikurensis is an obligate intracellular pathogen which was first described in Rattus norvegicus rats and Ixodes ovatus ticks in Japan in 2004. Until present, six cases of human Neoehrlichiosis in Europe have been published of which one in Switzerland. Transmission by ticks is assumed. Because cultivation has not yet been successful and cross reactivity with Anaplasma and Ehrlichia serological tests is not observed, Candidatus N. mikurensis can only be diagnosed by detection of the pathogen’s DNA. We encourage performing a bacterial broad range PCR of blood samples in cases of FUO in order to detect this novel fastidious pathogen.
Antiretroviral drug-related liver mortality is low in the absence of HBV or HCV co-infection: The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study.

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Background: Liver diseases are leading causes of death in HIV-positive persons since the widespread use of combination antiretroviral treatment (ART). Most of these deaths are due to hepatitis C (HCV) or B (HBV) virus co-infections. Little is known about other causes. Prolonged exposure to some antiretroviral drugs might increase hepatic mortality.

Methods: All patients of the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study without HCV or HBV co-infection were prospectively followed from date of entry, until death, or last follow-up. In patients with liver-related death, clinical charts were reviewed using a structured questionnaire.

Results: We followed 22,910 participants without hepatitis virus co-infection for 114,478 person-years. There were 12 liver-related deaths (incidence, 0.10/1000 person-years); 7 because of severe alcohol use and 5 due to established ART-related toxicity. The rate of ART-related deaths in treatment-experienced persons was 0.05 (95% confidence intervals 0.02, 0.12) with 5 events over 1000 person-years. In patients with liver-related death, time of first HIV diagnosis was earlier and ART exposure was longer, compared to 21,851 patients alive and 1047 deceased from other causes.

Conclusions: We found a low incidence of liver-related deaths in HIV-infected persons without HCV or HBV co-infection. Liver-related mortality because of ART-related toxicity was rare.
APOBEC3 restriction of HIV-1 Vif mutants in a humanized mouse model

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Aims: HIV-1 neutralizes some, but not all APOBEC3 proteins by Vif-mediated proteasomal degradation. Partially active Vif alleles are found in patients, suggesting that complete APOBEC3 neutralization is not necessary for viral replication. We want to show that suboptimal Vif activity is essential for HIV-1 diversification and potentially acts as one of the main driver of the HIV-1 pandemic.

Methods: Here we examined the fitness of wild-type (WT) and Vif-mutant viruses in NSG humanized mice (immunodeficient mice transplanted with human CD34+ cells) over a 3 months period. We selected three Vif-mutant viruses that selectively fail to neutralize APOBEC3G (E45G), APOBEC3F (W11R) or both (144AAA). Plasma viremia (RNA copies/ml) and CD4/CD8 ratios were measured over time and served as surrogate markers for viral replication and pathogenicity. In another experiment, mice were infected with WT virus or mutants E45G, W11R and subsequently treated with the RT inhibitor 3TC to determine which one developed faster resistance to this drug. Statistical analyses were performed (Mann-Whitney tests).

Results: WT virus and the E45G and W11R mutant viruses replicated and caused a profound decrease of CD4 T-cells over time as compared to the uninfected animals. However, the replication of the E45G mutant virus decreased significantly over time as compared to the WT (P=0.0039) and the W11R mutant. In contrast, in the mice infected with the Vif mutant 144AAA, there was no replication and no reduction of the CD4/CD8 ratio. Donor cells supported replication of WT and Vif mutant viruses with the exception of Vif 144AAA which also failed to efficiently spread in vivo. In the experiment in which the animals were treated with 3TC all the viruses replicated at the same rate and the E45G mutant showed a higher increase of the viral load after developing the resistance to the drug as compared to the WT or the W11R mutant, indicating an advantage of this mutant under selective pressure. Experiments to examine the viral diversity associated with this phenotype are on-going.

Conclusions: The humanized mouse model provided us with an in vivo system mirroring the complexity of the lymphoid system in humans. We found that suboptimal neutralization of APOBEC3 affected viral replication in this in vivo HIV infection model. HIV-1 Vif may, thus, act as a virulence factor impacting on HIV/AIDS pathogenesis in humans by modulating viral replication and diversification.
Aspirin plus Ticlopidine Prevents Experimental Endocarditis induced by Continuous Low-Grade Bacteremia: a role in Prophylaxis of Endocarditis Due to Spontaneous Bacteremia in Humans?

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Aim: Platelets are strongly implicated in infective endocarditis (IE) and are key players in vegetations structure. Thus, there is a rational for the use of antiplatelet drugs to prevent IE. Combinations of aspirin and ticlopidine were previously tested to prevent Staphylococcus aureus experimental endocarditis [1], but this strategy was unsuccessful. One of the reasons could be that prophylaxis was overwhelmed by the artificial inoculation of large bacterial numbers resulting in high-grade bacteremia. In humans, IE usually follows cumulative low-grade bacteremia, which often results from routine daily activities such as tooth brushing. Here we tested the prophylactic effect of aspirin plus ticlopidine in such as setting, using a new rat model of experimental endocarditis induced by continuous low-grade bacteremia mimicking cumulative daily events in man [2]. Streptococcus gordonii Challis and S. aureus Newman were used as infecting organisms.

Methods: Four hours after insertion of a catheter across the aortic valve, animals received intravenous saline (controls), aspirin (8 mg/kg), ticlopidine (10 mg/kg) or aspirin plus ticlopidine, twice daily for 48h. Rats were then infected i.v. by continuous low-dose infusion at a pace of 0.0017 ml/min and over 10h with an inoculum of S. gordonii Challis (106 CFU) and S. aureus Newman (105 CFU) infecting >90% of controls [2]. Animals were killed 24h after the end of inoculation, and vegetations weight and infection rate were determined.

Results: The vegetation (veg.) weight and infection rate after prophylaxis with aspirin (ASA) and ticlopidine (TCL) were:

Table (annexe files)

Conclusions: Aspirin plus ticlopidine successfully reduced vegetations weight and infection rate when implemented in a realistic model of low-grade bacteremia experimental endocarditis. These results suggest that aspirin plus ticlopidine could be effective in preventing IE that follows spontaneous low-grade bacteremia in human.

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Broad-range 16S rRNA Gene PCR for Diagnosis of Culture-Negative Bacterial Infections.

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Background:
Broad-range 16S rRNA gene PCR is used for detection and identification of bacterial pathogens in clinical specimens from patients with a high suspicion for infection. However, prospective studies addressing impact and clinical value of broad-range bacterial 16S rRNA gene amplification for diagnosis of acute infectious diseases in non-selected patient populations are largely lacking.

Methods:
We first assessed the diagnostic performance of 16S rRNA gene PCR as compared with routine bacterial culture. Second, we addressed prospectively the impact and clinical value of broad-range PCR for the diagnosis of acute infections using patients’ samples negative by routine bacterial culture; the corresponding patients’ data were evaluated by detailed chart reviews.

Findings:
Results from 394 specimens showed a high concordance of >90% for 16S rRNA gene PCR and routine bacterial culture, indicating that the diagnostic work-up of acute bacterial infections by PCR is comparable to bacterial culture, which is currently considered the gold standard.

In the prospective study two-hundred thirty-one specimens negative for routine bacterial culture were analyzed with PCR and patients clinical data. On basis of these results, broad-range 16S rRNA gene PCR showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 42.9%, 100%, 100% and 80.2% for culture negative bacterial infections.

Conclusions:
This study defines the role of 16S rRNA gene PCR for diagnosis of culture-negative bacterial infections. Our data show that 16S rRNA gene PCR is particularly useful for identification of bacterial pathogens in patients pretreated with antibiotics.

Reference:
Colonization with Vancomycin-Resistant Enterococci (VRE) after Discharge from an Epidemic Ward: Results of Ambulatory Contact Screening by Visiting Nurses

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Background: Nosocomial transmission of vancomycin-resistant Enterococci (VRE) occurs through direct contact with VRE-positive patients or through indirect contact via the hands of health-care workers or environmental contamination. Between November 2010 and August 2011, we experienced a first large outbreak of VRE E. faecium van B in a 900-bed tertiary care hospital including 42 positive patients and 453 contacts (roommates or patients hospitalized in an epidemic ward). As some VRE contacts are susceptible to be readmitted, they could be source of new transmissions and we therefore recommend preemptive contact isolation measures until exclusion of VRE colonization. The objective of this study was to assess the rate of VRE colonization after discharge from an epidemic ward among patients with no or incomplete screening during hospital stay.

Methods: VRE colonization was excluded after 3 negative rectal swabs or stool cultures, each of them done at least 1 week apart. Contact patients discharged home before complete screening were informed by letter and then contacted by phone. From July to December 2011, a mobile team of 5 visiting nurses performed VRE screening at home. Screening swabs were inoculated into an enrichment broth and then grown on specific chromogenic VRE agar.

Results: A total of 272 eligible patients were contacted for home screening: 205 had a complete screening (3 swabs), 24 a partial screening (1 or 2 swabs) and 43 were excluded (27 had wrong addresses, 10 had died, and 6 refused). No patient screened at home was found to be VRE positive. The mobile team needed 554 hours (CHF 27'700) and 2'396 km (CHF 1'677). The cost of the study therefore amounted to a total of CHF 29'377 in addition to lab costs; on the other hand, it prevented isolation days in readmitted contact patients, the cost of which is estimated at CHF 90 per day.

Conclusions: All VRE-contact patients screened after hospital discharge were negative. Further investigations are needed to determine after what exposure level, if at all, preemptive contact isolation measures are really justified when a VRE-contact patient is readmitted.
Concentration of mould fungus spores in a hospital building - impact of potted plants and environment

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Aims: We compared fungal spore counts in the air outside and inside a hospital building, in the absence and presence of potted plants to assess to which extent the soil of indoor plants increases the exposure of immunodeficient patients to fungal spores in a hospital building.

Methods: We monitored outdoor as well as indoor fungal spore concentration of a building of the Cantonal Hospital of Aarau, Switzerland, during one year before and one year after the installation of large potted plants in the staircase. Twice weekly, air was sampled by a MAS100 (Microbiological air sampler, MBV AG, Stäfa, Switzerland) using impaction technique on Sabouraud dextrose agar plates. Six measuring sites were in the building and one outside. Plates were incubated at 28°C and at 35°C to grow all cultivatable moulds and pathogenic Aspergillus species, respectively. Mould colonies were identified macro- and microscopically to genus level.

Results: The indoor concentration of all spores was higher in summer (median 53 spores/m³, inter-quartile range (IQR) 33 - 100) than in winter (11 spores/m³; IQR 8 – 23). The annual median of the spore concentration was 18 spores/m³ (IQR 6 – 64) before and 22 spores/m³ (IQR 22 – 122) after the installation of the potted plants (p < 0.001). However, the median concentration outside the building was 118 spores/m³ (IQR 40 – 253). Pathogenic Aspergillus spores, the main pathogen for invasive mould infections, did not show seasonal variability, and there was no increase in the number of these spores after the installation of the plants. In both monitoring intervals, 2 Aspergillus spores/m³ (IQR 0 – 4) were measured inside the building. The concentration of Aspergillus spores outside was significantly higher (5 spores/m³, IQR 2 – 10, p < 0.001).

Conclusion: Potted plants significantly increase the number of fungal spores within a building and may be a risk factor for fungal infection in severely immunodeficient patients. However, exposure to spores – particularly Aspergillus spores – is significantly higher outside the building. Furthermore, seasonal fluctuation of the spore concentration was documented both outside and inside the building. This finding illustrates the important impact of environmental conditions on the air quality inside hospital buildings.
Economical influence of coding infections due to multi-resistant bacteria in the new SwissDRG system

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Aim
The burden of infections due to multi-resistant micro-organisms is well known (Kock R, et al). The SwissDRG system is applicable by law throughout Switzerland for billing of acute hospital care since January 2012. For this system a new interventional Swiss CHOP code (93.59.5: Complex treatment of colonization or infection due to multi-resistant organisms) was created. We aimed to determine the potential impact of this new code on hospital reimbursement.

Methods
Episodes of care in 2009 during which infections due to MRSA or ESBL-producing bacteria were recorded prospectively by the infection control program using active surveillance. The complete diagnostic and interventional codes used for the previous and at that time applicable AP-DRG billing system were retrieved for these episodes. Using the SwissDRG grouper, DRG and cost-weight results were determined with and without the new CHOP code 93.59.5, and were subsequently compared.

Results
For 175 episodes of acute care, an infection due to MRSA (n=150) or ESBL (n=25) was detected. For 30 episodes with MRSA (20%) and 10 with ESBL (40%), the cost-weight changed when the CHOP code 93.59.5 was included. For these cases the case-mix (sum of cost-weights) increased by 1.3% and 10.9 points. Considering the current base-rate (value per cost-weight point) of over 10’000 CHF, this difference will represent some 100’000 CHF annually for our institution.

Conclusions
The retrospective and additional coding of infection due to multi-drug-resistant organisms (MDRO) increased the cost-weight for more than 23% of infected cases applying the SwissDRG system. Therefore this new system takes into account the additional burden associated with these cases, at least in part.

Thus, exact documentation and exhaustive coding of infections and colonization with MDRO with the new SwissDRG system may increase hospital revenues and may allow better detection of MDRO carriers.

Reference
Ecthyma contagiosa – an ORF(ph)an disease?

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Aim: Ecthyma contagiosum – or orf – is caused by a DNA parapoxvirus (Parapoxvirus ovis) particularly adapted to epidermal cells. It is an uncommon human viral infection usually causing cutaneous lesions upon direct contact with an infected animal. Timely recognition of this usually benign and self-limiting infection avoids overtreatment. Careful patients’ history is crucial for accurate diagnosis.

Case Presentation: A 48 year old woman presented herself with a bloodshot papulo-bulbores cutaneous lesion at her right index finger. Two weeks before she first noticed a fissure and developed after 4 days a singular indolent papule with a progressive accompanying erythema. The patients’ history was significant for having fed lambs, but she could not remember having been injured. The lesion was surgically removed. Histological examination revealed inflammatory cellular infiltrates and eosinophil intracytoplasmatic inclusion bodies in epithelial cells. Parapoxvirus was detected by real-time PCR confirming the diagnosis. Further evaluation of the lamb demonstrated typical mucous eschar, and diagnosis and epidemiological linkage was confirmed by Parapoxvirus rt-PCR positivity. The patients’ lesion healed within 3 weeks without sequelae.

Discussion: Orf disease is endemic in sheep’s and goats, but artiodactyls in general are susceptible. In Switzerland orf seems to be rather sporadic, but reliable surveillance data is lacking. Human infections may be more common than reported given the benign disease course. Transmission occurs usually by direct contact but the virus remains contagious in the environment for years. Ecthyma contagiosa clusters have been described in shepards’ communities. The disease course is usually self-limited with spontaneous healing. Nevertheless more severe cases due to disease dissemination have been described in immune-compromised individuals. Diagnosis is mainly made by the patients’ history and the disease course that is characterised by different stages (erythematous papule – “target” nodule/pustule – crust). Timely recognition of human orf may prove beneficial since complications are mainly due to overtreatment.
Effectiveness of once weekly fluconazole on the incidence of invasive candidiasis (IC) in a reverse-isolation hematological unit

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Objectives: Fluconazole 400mg once daily was recommended as first line prophylactic agent for patients with acute leukemia and for those receiving allogeneic HSCT. Since 2001 our institutional policy for hematological high risk patients has been 400mg fluconazole once weekly despite the fact that doses below 400mg/d have not shown a significant benefit in preventing invasive fungal infections. Our study aimed to evaluate the impact of this policy on incidence of IC and the potential of emergence of resistance to fluconazole.

Methods: Patients received antifungal prophylaxis with fluconazole 400mg weekly if they underwent high-dose chemotherapy, allogeneic or autologous stem cell transplantation. Antifungal prophylaxis was routinely stopped when antifungal treatment for invasive fungal infection (IFI) was initiated. Our hematological unit (HU) is located in a separate wing, equipped with laminar air flow, has restricted access and dedicated staff.

Data from patients were prospectively recorded by standardized case report forms. IC was defined as isolation of Candida spp. from blood or normally sterile compartments.

Results: From January 2003 until December 2010 a total of 1358 patients were treated in the HU, among them, 563 underwent allogeneic or autologous transplantation. 56 Candida spp. were isolated from all submitted specimens, among them 28 were regarded as invasive and 28 as colonization. Identification of Candida spp. from this unit - including also non-IC isolates - remained stable over the whole period of eight years with an average of 1.71 /1000 patientdays (PD) (all candida isolates) and 0.86/1000 PD for IC, respectively (p<0.05). 55 of 56 strains were tested for fluconazole susceptibility. There was no significant increase of non-albicans Candida spp. or an increase of fluconazole resistant isolates of Candida spp. (p<0.05). There was no trend for increased resistance over the study period (figure).

Conclusion: This policy of once weekly fluconazole did not result in emergence of Candida resistance or a shift towards non-albicans Candida spp. and might therefore be considered as alternative to once daily fluconazole.
Figure:

Fluconazole Susceptibility of all Candida spp.
2003-2010

- FLU-R
- FLU-S-DD
- FLU-S
Efficacy of broad spectrum antifungal prophylaxis in adults with acute myeloid leukemia in a Swiss Tertiary Care Hospital

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Aim: Randomized clinical trials have shown reduced incidence of invasive fungal infection (IFI) and reduced mortality in high risk patients receiving prophylaxis with posaconazol (POS). Our aim was to evaluate the efficacy of POS prophylaxis during the first year after its introduction in our hospital.

Methods: All patients with acute myeloid leukemia admitted to the isolation ward for remission-induction chemotherapy received prophylaxis with POS 200mg tid. During periods of severe mucositis POS was replaced by intravenous deoxycholate-amphotericin B (AMB) 0.4mg/kg qd. In cases of suspected breakthrough IFI e.g. persisting neutropenic fever despite broad spectrum antibiotics and imaging findings compatible with IFI, prophylaxis was switched to antifungal (AF) therapy with liposomal AMB. Patients were prospectively monitored. The diagnoses of IFI were reassessed retrospectively by reviewing charts and imaging studies.

Results: During the observation period of 14 months, 44 patients received POS prophylaxis. Median age was 55 years (IQR 42-66); 24 (57%) patients were male. The median duration of neutropenia was 34 days (IQR 19-43). The median total duration of prophylaxis was 26 days,(IQR 18-50); 19 days (IQR 12-30) with POS, 7 days (IQR 3-15) with AMB, the latter was prescribed to 35 (80%) individuals. Among the 21 (48%) patients with suspected break-through infections who were given AF therapy there were 7 patients with no IFI and 10 with possible IFI. Proven or probable IFI was diagnosed in 4 (9.5%) patients, 95%CI 0.8-18.1 (2 in each category). There was one case of invasive pulmonary infection due to Hormographiella aspergillata and one case with positive histology on lung biopsy but negative culture. The median duration of therapy was 29 days (IQR 13-67). The median POS levels were similar in individuals with and without suspected breakthrough IFI (0.61 ug/mL (IQR 0.4-0.93) versus 0.63 ug/mL (IQR 0.27-1.16)).

Conclusions: Despite AF prophylaxis with POS and AMB 48% of our patients received AF therapy because of suspected breakthrough IFI which was retrospectively confirmed as at least possible IFI in two thirds of them. Compared to the 2 years immediately preceding introduction of POS prophylaxis, when 75% of patients received empiric AF therapy, the rate of patients receiving treatment was substantially lower. Our 10% rate of proven or probable break-through infection was higher than in published trials albeit based on a small number of patients.
Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF (Quad) has Noninferior Efficacy and Favorable Safety Compared to Efavirenz/Emtricitabine/Tenofovir DF in Treatment Naïve HIV-1 Infected Subjects

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Aims: The integrase inhibitor elvitegravir (EVG) has been coformulated with the pharmacoenhancer cobicistat (COBI), emtricitabine (FTC), and tenofovir DF (TDF) in a single once daily tablet (Quad). We report the Week 48 results of a prospective, randomized, doubleblind, active-controlled, ongoing Phase 3 trial comparing Quad with co-formulated efavirenz/emtricitabine/tenofovir DF (EFV/FTC/TDF) as initial therapy for HIV infection.

Methods: Treatment-naïve subjects with HIV were randomized 1:1 to blinded Quad or EFV/FTC/TDF once daily plus matching placebos. Eligibility criteria included screening HIV RNA ≥ 5,000 copies/mL (c/mL), CLCr > 70 mL/min and sensitivity to EFV, FTC, and TDF. Randomization was stratified by HIV-1 RNA > or <100,000 c/mL. The 1° endpoint was the proportion of subjects with HIV RNA <50 c/mL at Week 48 per the FDA snapshot algorithm (12% prespecified noninferiority margin).

Results: 700 subjects (89% male, 37% non-white, 33% with VL >100,000 c/mL) were randomized and treated. The 1° endpoint was met; Quad was noninferior to EFV/FTC/TDF with 88% and 84%, respectively, having viral suppression at Week 48 by snapshot algorithm (difference +3.6%, 95% CI -1.6%, +8.8%). Among subjects with baseline HIV RNA >100,000 c/mL, response rates were similar (Quad 84%, EFV/FTC/TDF 82%). Virologic failure rates at Week 48 were 7% in both arms (FDA snapshot). At Week 48, mean CD4 cell increase was 239 cells/µL in Quad and 206 cells/µL in EFV/FTC/TDF (p=0.009). Drug discontinuation rates for adverse events were similar (Quad 3%, EFV/FTC/TDF 5%). Among AEs occurring in >10% of subjects (all grades), nausea was significantly more frequent in Quad than EFV/FTC/TDF (21% vs. 14%) while dizziness (7% vs. 24%), abnormal dreams (15% vs. 27%), insomnia (9% vs. 14%) and rash (6% vs. 12%) were significantly less common in Quad than EFV/FTC/TDF. CLCr decrease occurred by Week 2 of Quad therapy and was significantly greater than with EFV/FTC/TDF (-14.3 vs. -3.0 mL/min
by Week 48). Total cholesterol and LDL increases at Week 48 were significantly lower for Quad than EFV/FTC/TDF.

Conclusions: In this first Phase 3 study directly comparing once daily single tablet regimens for HIV, Quad demonstrated similarly high response rates compared to EFV/FTC/TDF and favorable CNS, rash, and fasting lipid results. These results suggest that Quad could become an important new option for initial HIV therapy.
Emergence of disseminated infections due to the yeast *Blastoschizomyces capitatus* in central Switzerland

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Background: Immunocompromised patients, notably those with hematological malignancies and severe neutropenia, are at risk for invasive fungal infections, most commonly due to *Candida* or *Aspergillus* spp. Among emerging fungal pathogens, *Blastoschizomyces capitatus* (synonyms: *Trichosporon capitatum* and *Geotrichum capitatum*) is a rarely described yeast causing severe and frequently fatal infections, usually restricted to areas with a Mediterranean climate. So far, only one case has been described in Switzerland, in 1983.

Methods: We conducted a multicenter retrospective study in Switzerland from September, 2007 to February, 2012 involving three tertiary care hospitals in the temperate zone north of the Alps to characterize cases of invasive Blastoschizomycesosis. Identification of the yeast was based on standard culture and molecular methods.

Results: Ten cases of disseminated or invasive infection with *B. capitatus* were identified. The majority of cases occurred in patients with hematological malignancies (i.e. acute myeloid leukemia (5 patients) and aggressive lymphoma (1 patient)). The infection developed during the period of profound and prolonged neutropenia. The yeast was easily recovered from blood in all patients. Pulmonary involvement was universal, presenting with a wide clinical spectrum. Dissemination to other organs (e.g. brain, bone marrow, skin) was common. Treatment consisted of voriconazole or liposomal amphotericin B. Mortality was 50%. Two patients under immunosuppression following kidney-transplantation had locally invasive infections (emphysematous cystitis, fungal peritonitis) that responded to antifungal treatment with voriconazole. One patient presented with severe septic shock due to disseminated infection with *B. capitatus* following gastrectomy and splenectomy for relapsing gastrointestinal bleeding. Another patient with advanced pancreatic cancer presented with liver abscesses containing *B. capitatus*.

Conclusion: Our observation should alert clinicians caring for severely immunocompromised patients in temperate areas to consider infections due to unfamiliar fungal pathogens, notably *B. capitatus*. Given the high mortality rate of *B. capitatus* infections, early diagnosis and effective therapy is crucial. Considering the intrinsic lack of activity of echinocandins against this yeast, following the recommended empirical therapy for yeast infections in high-risk patients (caspofungin or lipid-associated amphotericin B preparations) may critically delay adequate treatment.
Epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae in a septic orthopaedic ward

IU Uçkay [1]


Objective Wards cohorting infected orthopaedic patients may be particularly prone to transmitting extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E), due to high antibiotic use and long hospital stays.

Methods We analyze their epidemic pattern by performing molecular typing of ESBL-E isolated from patients and healthcare workers during 20 months from our septic ward.

Results
Between March 2009 and November 2011, 186 patients were admitted from the community and 1335 transferred from other institutions, totalling 12,401 patient-days with an average length of hospital stay of 27 days. Bed occupancy averaged 83%.

Among 565 anal swabs, ESBL-E were detected in 204 samples from 45 patients, suggesting prolonged carriage in affected patients. In six patients two different ESBL-E strains were detected, and 3 patients carried three distinct isolates. Among the 45 positive patients, 29 (64%) were detected during the first three days of admission, the remainder after a median of 13 days of hospitalisation, range 7-52 d). At the time of sampling, 26 patients received antibiotic therapy without clinical activity against their respective ESBL-E; a further 7 patients were treated with antibiotics which their ESBL-E strains were susceptible to in vitro (carbapenems or quinolones). Most positive patients were asymptomatically colonised with ESBL-E. Two patients had arthroplasty infections due to ESBL-E, of which one was acquired on our ward.

We also screened 41 healthcare workers (HCW) on 49 occasions during the study period. Six samples (13%) were positive.

None of the ESBL-E detected in HCW were related to any of the patient isolates.

Among 60 environmental samples taken at the peak of the epidemic (room floors, beds, curtains, tables, doors, offices, computers, telephones, kitchen, physiotherapy material, and toilets), none revealed ESBL-E.

Conclusion
HCW may also be anal carriers, but their strains might be different from the patients.

Second, among 25 cases with identical ESBL-E species and positive epidemiological links, only 9 were really attributable to our service. This underlines that epidemiological attribution of ESBL by simple vicinity, timing, and species identification might grossly overestimate transmission within a given unit.
Evaluation of the immunomodulatory and antiviral effects of the cytokine combination IFN-alpha and IL-7 in the Lymphocytic Choriomeningitis Virus and Friend Retrovirus mouse infection models

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Aim(s)/Methods: Existing therapies for chronic viral infections are still suboptimal or have considerable side effects. Thus new therapeutic strategies need to be developed. One option is to boost the host’s immune responses with cytokines. We have recently shown in an acute ex vivo HIV infection model that co-administration of interferon (IFN)-alpha and interleukin (IL)-7 allows for combining the potent anti-HIV activity of IFN-alpha with the beneficial effects of IL-7 on T cell survival and function. Here we evaluated the effect of combining IFN-alpha and IL-7 on viral replication in vivo in the chronic Lymphocytic Choriomeningitis Virus (LCMV) and acute Friend Retrovirus (FV) infection models.

Results: In the chronic LCMV model, cytokine treatment was started during the early replication phase, i.e., on day 7 post infection (p.i.). Under the experimental conditions used, exogenous IFN-alpha inhibited FV replication, but had no effect on viral replication in the LCMV model. There was no therapeutic benefit of IL-7 either alone or in combination with IFN-alpha in either of the two infection models. In the LCMV model, dose-dependent effects of the cytokine combination on T cell phenotype/function were observed.

Conclusions: It is possible that these effects would translate into an antiviral activity in re-challenged mice. It is also possible that another type of IFN-alpha/beta or induction of endogenous IFN-alpha/beta alone or in combination with IL-7 would have antiviral activity in the LCMV model. Furthermore, we cannot exclude that some effect on viral titers would have been seen at later time points not investigated here, i.e., beyond day 34 p.i.. Finally, IFN-alpha/IL-7 may inhibit the replication of other viruses. Thus, it might be worth testing these cytokines in other in vivo models of chronic viral infections.
Evaluation of two real-time PCR, two antigen tests and culture for the detection of toxigenic Clostridium difficile.

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Objectives.
To define the performance of four commercial tests for the detection of C. difficile (CD) in the stool

Methods.
During the period of September 2010 to February 2011 stools from patients with suspicion of CD infection were collected (n=117). We meant to retrieve an similar number of positive or negative stools. The rapid immunochromatographic tests C.DIFF Quik Chek COMPLETE® detecting toxin A and B plus the presence of CD (Techlab) and RIDA®QUICK Toxin A/B (r-biopharm) were used for antigenic testing. Two multiplex PCR Xpert® C. difficile (Cepheid) and RIDA®GENE CD +ToxB (r-biopharm) after stool extraction on EasyMag (bioMérieux), were used for genomic testing on the same stools. Culture using selected plates after alcohol choc on the stool were used to recover C. difficile but no toxigenic test on cell culture was done on stools or isolated strains. A positive stool was considered when at least 3 tests or the 2 PCR were positive.

Results.
Fifty stools (42.7%) were determined to be positive. PCR tests showed sensitivities of 98% and 100% and specificities of 92.5% and 98.5% with Xpert and RIDA GENE, respectively. Antigenic detection of toxin A and B tests showed sensitivities of 62% and 70% and specificities of 94% and 97% with QUIK CHEK and RIDA QUICK, respectively. We also compared the utility of tests detecting only the presence of C.difficile in stools. Sensitivities of 100%, 90% and 90% and specificities of 89.5%, 85.6% and 85.1% were obtained for RIDA GENE CD, QUIK CHEK antigen and CD culture, respectively.

Both genomic test are highly sensitive and adequate to detect toxigenic strains in stools. Xpert CD showed, however, a slightly poorer specificity. Antigenic tests have low sensitivities missing up to 38% positive stools, with good specificities. The use of a combined test with CD antigenic detection to enhance sensitivity does not improve the overall detection as no further toxigenic strains were detected. Such a strategy lowers specificity (85.6%) leading to unnecessary treatment.

Conclusion.
Genomic amplification tests are more adequate to diagnose CD infections. The high sensitivity enables to give a quick and reliable response in a few hours without need for repeat samples.
Functional impaired influenza-specific cellular immunity after vaccination in HIV positive patients with low CD4 nadir despite absolute CD4 cell recovery under cART

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Aims:
The impact of the CD4 nadir reflecting the timing of ART initiation on the immune functionality to recall antigens in the long-term is not yet well understood. A preserved polyfunctional CD4 T-cell profile characterised by double / triple cytokine secreting CD4 cells is thought to be of advantage.

Methods:
In a pilot study we evaluated the influenza A-specific polyfunctional CD4 T-cell profile (IFN-γ, TNF-α, IL-2) in 3 HIV-positive patients on HAART with a high (>500/ul) and low (<50/ul) CD4 nadir each compared to 3 healthy HIV-negative controls before (BL) and 2 weeks (w2) after seasonal 2010/11 influenza immunisation as a model of recall-antigen exposure. The functional cytokine profile of CD4 T-cell responses (sample minus neg. control +2SD) were evaluated in duplicates by FACS.

Results:
Baseline characteristics (age, time on ART, CD4 cell count at time of immunisation, % CD38+/HLA-DR+ and % PD-1 expressing CD4 T-cells) between HIV-pos. patients with a high or low CD4 nadir were not significantly different, although we observed a trend towards a higher number activated CD4 T-cells (CD38+ or HLA-DR+) in patients with a low CD4 nadir. The mean increase in the number of influenza A-specific CD4 T-cells/10e6 PBMC after immunisation was not different in HIV-pos. compared to HIV-neg. patients (delta% 2.2 versus 1.8, p=0.68). The changes BL-w2 of the percentage of single and multiple cytokine pos. cells / total influenza-specific CD4 T-cells in HIV-neg. controls and HIV-pos. patients with a CD4 nadir >500/ul were similar with a significant decrease of single cytokine pos. in favour of an increase of multiple cytokine pos. CD4 T-cells, with a dominant increase of IL-2 pos. secreting CD4 cells (p=.002 and .036 resp.). In contrast, HIV-pos. patients with a low CD4 nadir showed no quantitative changes of single / multiple cytokine positive CD4 T-cells (p=.65). The patterns of the cytokine expression changes (delta BL-w2) were similar in controls and HIV pos. patients with a high CD4 nadir compared to HIV pos. patients with a low CD4 nadir (see fig.).

Conclusion:
A low CD4 nadir is associated with an functional impaired influenza A-specific CD4 T-cell response after immunisation despite absolute CD4 T-cell recovery under HAART compared to healthy controls and HIV-pos. patients on HAART with a CD4 nadir >500. These preliminary findings need to be verified in a larger cohort, but argue for a substantial benefit of an early HAART initiation.
Greetings from home

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Background: Diagnosis and treatment of brucellosis may be complicated by unspecific symptoms, a limited sensitivity of blood cultures and a significant recurrence rate.

Aim: Describe our experience with a family epidemic secondary to the consumption of infected unpasteurized fresh cheese some 4 weeks earlier.

Methods: Case report and review of the literature.

Results: Both parents and 2 sons were affected. Symptoms included fever, chills, nausea as well as unspecific abdominal and musculoscelettal pain. Labory tests revealed pancytopenia, relative thrombocytosis and hepatitis. Blood cultures were positive in 3 patients, none of whom showed echocardiographic signs of endocarditis. The mother developed a sternoclavicular arthritis. Treatment consisted of oral doxycyclin (2x100mg) and rifampin (2x450mg) for 6 weeks combined with 7 days of gentamicin.

Conclusion: Epidemiological information is key for making an early diagnosis. Combined antibiotic treatment cleared the infection in 4/4 cases.
Harmless insect larvicide? Bacillus thuringiensis sepsis and pneumonia in a neutropenic patient – Case report

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CASE

A 64 year old man was diagnosed with acute myeloid leukemia FAB M4 in July 2011. He had a history of rheumatoid arthritis since 2009, for which he was treated with methotrexate. A first induction chemotherapy cycle was started with Clofarabine / Idarubicin / Cytarabin. He was given prophylaxis against PCP and invasive fungal infection with cotrimoxazole and posaconazole, respectively. Two days after becoming neutropenic on day 7 he developed fever (38.9°C) and neutropenic colitis. Empirical treatment with cefepime and metronidazole was started. In 2/3 blood cultures drawn before antibiotic treatment viridians streptococci were cultivated. The patient initially responded well to antibiotic treatment. On day 14, however, he became febrile once more, CRP rose to a max. of 422mg/l and he required emergency intubation and transfer to the intensive care unit due to septic shock with acute respiratory failure. A CT scan of the lungs showed right sided dense infiltrates consistent with bacterial pneumonia. Cefepime and metronidazole were switched to meropenem; vancomycin and ciprofloxacin were added empirically. In 2/2 blood cultures and in tracheobronchial secretions of day 14 Bacillus thuringiensis (Bt) was cultured (see Graph 1).

Graph 1

<table>
<thead>
<tr>
<th>Kultur allgemein</th>
<th>Keim 1</th>
<th>Bacillus thuringiensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakterien/Pilze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anzahl positive Flaschen</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Resistenzprüfung</td>
<td>Keim 1</td>
<td></td>
</tr>
<tr>
<td>Imipenem MHK (E-Test)</td>
<td></td>
<td>0.094 mg/l</td>
</tr>
<tr>
<td>Meropenem MHK (E-Test)</td>
<td></td>
<td>0.125 mg/l</td>
</tr>
<tr>
<td>Ertapenem MHK (E-Test)</td>
<td></td>
<td>0.094 mg/l</td>
</tr>
<tr>
<td>Vancomycin MHK (E-Test)</td>
<td></td>
<td>2.000 mg/l</td>
</tr>
<tr>
<td>Ciprofloxacin MHK (E-Test)</td>
<td></td>
<td>0.125 mg/l</td>
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</table>

Four days later, the patient’s condition improved, he was extubated and transferred back to the isolation unit. There were no further complications and the patient was discharged on day 27 with continued ciprofloxacin treatment planned until start of the next chemotherapy cycle.

Chest CT scans of the first hospitalization are shown below.

day 14, admission to ICU  day 23
COMMENT
Bt is a soil bacterium with the potential to produce insecticidal crystal proteins. It is well established as insecticide in vector control for mosquitoes and is recommended by the WHO as part of integrated vector management in the fight against malaria, filariasis and onchocerciasis. It is also widely used in agriculture as a biological pesticide and its Bt toxin is incorporated in a variety of transgenic crops. Toxicological studies have documented this insecticide's excellent safety profile for humans.

There are only rare case reports of human infections. Cases of eye infections (corneal ulcer, periorbital cellulitis) and infections in immunocompromised hosts have been reported such as nosocomial blood stream infections, infections in patients with severe burn wounds and a fatal case of Bt pneumonia in a girl with chemotherapy-induced neutropenia.

CONCLUSION
We describe a rare infection with an ubiquitous germ in a severely immunocompromised patient. In keeping with the literature this indicates that B. thuringiensis must be regarded as a potential opportunistic pathogen in neutropenic patients.

1 www.who.int/malaria/publications
3 Samples JR et al., J Infect Dis. 1983;148(3):614
5 Kuroki R et al., Intern Med. 2009;48(10):791
7 Ghelardi E et al., Microb Infect 2007;9:591
Hidden under a cauliflower-like skin tumour

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Case report
An 80-year-old man presented with a recurrent skin tumour on the amputation stump of the left index finger, two years after the excision of a tumour that had been diagnosed as squamous cell carcinoma. Traumatic amputation had occurred 30 years earlier. His personal history revealed an aortic valve stenosis, chronic renal failure and suspected pulmonary metastases of an unknown primary tumour. The histology of the excised tissue showed pseudoepitheliomatous hyperplasia and the presence of numerous chest-nut pigmented, round to oval, thick-walled, occasionally septated fungal elements - so-called sclerotic bodies, forming small clusters and being diagnostic for chromoblastomycosis. The fungus invaded skin, subcutaneous tissue and bone. The patient had no systemic symptoms, no immunosuppressive treatment. Originating from the northern part of Italy, he had lived in Switzerland over 40 years and had not travelled. He remembered no traumatic skin lesion except for minor scratches from gardening. At follow-up one month after the second excision, the left index was overwarmed and again showed a black spot on the amputation stump. Surgical revision with radical bone debridement was planned but unfortunately the patient died shortly before from cardiogenic shock.

Discussion
In non-tropical regions chromoblastomycosis is an uncommon chronic fungal infection, with Fonsecaea pedrosoi being the culprit in 86-96%. In 80-85% chromoblastomycosis involves the lower extremities. Our case is remarkable because the patient had a negative tropical travel history, infection involved a finger and invaded the bone. Immunosuppression associated with age, chronic renal failure and the suspected metastatic tumour disease as well as the missing compacta in the partially amputated phalanx of the index may have enabled the high grade of invasiveness. Clinically and histopathologically chromoblastomycosis can be confounded with squamous cell cancer; delays in diagnosis are not unusual. On the other hand long standing infection can result in malignant transformation into squamous cell carcinoma. Eradication of chromoblastomycosis is difficult, especially with bone invasion. A combination of local physical, surgical as well as systemic antifungal treatment is most effective.

Conclusions
The localisation, the grade of invasiveness and the lack of a tropical travel history are remarkable, especially as infection almost surely occurred in Switzerland.
HIV-associated multicentric Castleman disease – complete remission in two patients treated with combination antiretroviral therapy (cART) without chemotherapy

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Objective: HHV-8 plays a pivotal role in the pathogenesis of multicentric Castelman disease (MCD). Chemotherapy may limit immune recovery and control of HHV-8. We present 2 cases of HIV-associated MCD treated with cART without chemotherapy and complete remission.

Methods: Description of two patients with MCD with clinical and virological follow up (2 and 5 years). Stored samples were from the Swiss HIV Cohort Study.

Results: Patient 1, a 32y old man, HIV-1 seropositive since 2008, presented with episodes of fever, nasal obstruction, lymphadenopathy and thrombocytopenia. cART with tenofovir, emtricitabine and efavirenz was started at CD4 counts of 248/ml (table). After initial improvement he developed IRIS. Histopathological and immunohistochemical examination of an axillary lymph node demonstrated simultaneous occurrence of MCD and Kaposi sarcoma. HHV-8 PCR in plasma was positive prior to and at time of diagnosis (table). CD4 counts increased to 899/ml and HIV-1 VL was consistently suppressed after 12 months to below 200 copies/mL. Initial transient increases of HHV-8 VL were treated with valganciclovir. Thereafter, HHV-8 was low at approx. 3 log10. The patient remained in complete remission up to 2 years now. Patient 2, a 71y old man, HIV-1 seropositive since 1984, presented with fever, weight loss, night sweats and lymphadenopathy. MCD was diagnosed in 2005 from a supraclavicular lymph node. Symptoms correlated with HHV-8 viraemia. In 2005 (CD4 262/ml, HIV-1 2.34 log10) T-20 was added to cART consisting of tenofovir, emtricitabine, fosamprenavir/r. HIV-1 was suppressed since then. HHV-8 PCR in plasma was positive prior to and at time of diagnosis. HHV-8 decreased stably by approximately 3 log10. The patient remained in complete remission with a follow up of 5 years.

Conclusion: In these two patients with HIV-associated MCD complete remission could be achieved by control of HIV with subsequent immune reconstitution and significant reduction of HHV-8 replication.
Improved hepatitis C-specific cellular immune diagnostic by interleukin 10 blockade

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Aim: Hepatitis C (HCV)-specific cellular immune responses play a major role in HCV containment and are associated with superior HCV treatment responses. Nevertheless the measurement of HCV-specific cellular immune responses is notoriously difficult rendering immeasurable responses in up to half of chronically HCV-infected patients. We propose a simple modification in the cellular assay systems to improve test sensitivity by blockage of inhibitory cytokine effects during HCV-specific T-cell stimulation.

Methods: HCV-specific interferon-gamma (IFN-g) Enzyme Linked Immuno Spot (ELISpot) assays were performed in 21 chronically HCV infected or HCV/HIV co-infected individuals. 400,000 PBMCs were incubated immediately after separation overnight with overlapping genotype-specific pooled peptides spanning the HCV core antigen with or without anti-IL10 antibodies in duplicates. Negative (no antigen; triplicates) and positive (phytohaemagglutinine, PHA) controls were included. IFN-g spot-forming units (SFU) were measured with a standardised ELISpot reader. Test positivity was determined by the mean HCV-core specific SFU minus the mean SFU in the negative wells + 3SD SFU.

Results: 11/21 individuals with chronic HCV infection (12/21 genotype 3a, 8/21 gen. 1a/b) were HIV-co-infected (mean CD4 cell count 620/ul, range 380-1’000). 13/21 individuals had no HCV-core specific detectable IFN-g responses (mean SFU 135/10e6 PBMCs; undetectable responses in 4/10 HCV and 4/11 HIV co-infected individuals). Addition of anti-IL10 antibodies ex vivo to the ELISpot assay increased overall assay positivity to 18/21 (mean SFU 175/10e6 PBMCs, p=0.06 McNemar 2-tailed; undetectable responses in 1/10 HCV and 2/11 HCV/HIV-positive individuals). The mean increase of SFU in previously negative assays was 65 SFU/10e6 PBMCs (overall 40 SFU/10e6 PBMCs) after addition of anti-IL10.

Conclusion: Addition of anti-IL10 antibodies to HCV-specific ELISpot assays increases test sensitivity. Ameliorated HCV-specific cell based assays may prove beneficial in the risk stratification before HCV treatment initiation and/or the evaluation of the immunogenicity of HCV vaccine candidates in the future.
In Vitro Synergism Between Cpl-1 and Daptomycin or Vancomycin Against Streptococcus pneumoniae.

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Background. Several studies have reported anti-bacterial effect of Cpl-1 (the pneumococcal Cp-1 bacteriophage lysis) against Streptococcus pneumoniae (S. pneumoniae), but only few have investigated synergism between Cpl-1 and currently available antibiotics. Here we tested the potential for synergy between Cpl-1 and daptomycin (DAP) or vancomycin (VAN) against drug-susceptible and drug–resistant S. pneumoniae strains in vitro.

Methods. Both penicillin-susceptible virulent isolate D39, and avirulent isolate R6 and the penicillin-resistant isolate WB4, were used. Synergy of Cpl-1 and daptomycin or vancomycin was tested in time-kill assays, at concentrations ranging from 0.25x to 1x the Minimal Inhibitory Concentration (MIC) for each organism. Bactericidal activity was defined as a ≥3-log10 decrease in cell counts below the starting inoculum 2 or 6h after exposure to the drugs. Synergism was defined as a ≥2 log10 increased killing at 2 or 6h by the drug combination compared to the more active drug tested alone.

Results. MICs of Cpl-1 for strains D39, R6 and WB4 were 128, 512 and 128 mg/ml, respectively. MICs of DAP were 1 mg/ml for the three strains. MICs of VAN were 0.5, 0.25 and 0.5 mg/ml, respectively. In time-kill curves, Cpl-1, DAP or VAN alone at concentrations of 0.25x to 1x the MIC, did not displayed significant bactericidal effects, except against WB4. In contrast, combinations of Cpl-1 with either DAP or VAN displayed synergistic bactericidal activity 2 or 6h after exposure for all tested strains.

Conclusions. In vitro, combinations of Cpl-1 and DAP or VAN were synergistic and bactericidal against S. pneumoniae, including penicillin-susceptible and penicillin-resistant strains. Testing of Cpl-1/DAP or Cpl-1/VAN combinations for the treatment of pneumococcal infections in experimental animal models is currently in progress to assess the potential clinical benefit of these combinations in patients who failed conventional therapy.
Infections Associated with Deep Brain Stimulators: Review of 8 cases over a 10 year-period

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Background: Treatment of deep brain stimulator (DBS) infections is not standardized. Complete device removal with delayed reimplantation is generally recommended. Partial or complete retention combined with debridement and antimicrobial treatment against biofilms is an attractive alternative, but data on the outcome are lacking. We analyzed DBS-associated infections treated at our institution.

Methods: All DBS revisions for infection performed at our institution between 1/2001 and 12/2010 were retrospectively reviewed. DBS-associated infection was diagnosed if a sinus tract communicating with the device was present or if local signs of infection (redness, swelling, secretion) were associated with positive deep cultures (≥2 positive samples were required for low virulent organisms).

Results: We identified 8 cases of DBS infection. Seven infections occurred after implantation at our institution (179 DBS implantations during the 10-year period, incidence 3.9%). In all 8 cases of infection, DBS was implanted for treatment of Parkinson’s disease. Infection occurred after a median of 132 days after implantation (range, 10-4678) and 128 days after last revision (range, 3-266). The causative pathogens were identified in 7 cases including S. aureus (n = 5), P. acnes (n = 1) and mixed infection in 1 case (S. marcescens and S. aureus). As initial procedure, device retention was performed in 7 cases (5 partial and 2 complete); removal of the whole device was performed in 1 case. Median duration of antibiotic treatment was 41 days (range, 14-168 days), 5 patients received rifampin-containing regimes. After a median of 41 months after completion of treatment, 5 cases (62.5%) showed no signs of infection, 2 patients had a relapse 225 and 820 days after completion of antibiotic treatment and 1 patient had persistent infection. All 3 failures were treated with complete removal of the device. Rifampin-resistant S. aureus was cultivated in 2 cases of failure.

Conclusions: DBS-associated infection was observed in 3.9% of implanted devices with S. aureus as the predominant pathogen. Debridement and device retention was performed in 7 of 8 patients, of whom 3 developed a relapse or persistent infection. The role of device retention and rifampin in the treatment of DBS-associated infections need to be further investigated.
Infectious gastroenteritis: Evaluation of two multiplex-PCR assays

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AIM
Two newly developed multiplex PCR assays allow the simultaneous detection of a broad range of pathogens. For the use in a diagnostic laboratory, the accuracy and usefulness of these assays was evaluated by comparison with conventional methods.

METHODS
126 routine stool specimens from patients with gastroenteritis were examined. Conventional detection consisted in culture on selective media and in specific EIA. For Noroviruses, RT-PCR on LightCycler was used.

Two commercially available multiplex-PCR assays were tested. The Seeplex Diarrhea ACE assay consists in three parallel amplification reactions by dual priming oligonucleotides and detection by auto-capillary electrophoresis. The xTAG GPP assay is based on a single one-step (RT)-PCR followed by target specific primer extension, hybridisation to specific xTAG beads and laser detection (Luminex technology).

RESULTS
Positive results were found in 42 of 126 routine stool specimens. The pathogens found reflect the normal spectrum found to cause infectious gastroenteritis in Switzerland: Campylobacter spp., Clostridium difficile Toxin A/B, Salmonella spp., Shigella spp., E. coli Verotoxin, Yersinia enterocolitica, Norovirus, Rotavirus. An equal result was obtained with all three methods in 30 stool samples, 6 cases were detected by two methods, and 6 specimens were positive with just one method. The two multiplex assays gave identical results in 116/126 samples. No pathogen was found in 84 specimens.

CONCLUSIONS
Multiplex PCR allows a rapid diagnosis of a wide range of infectious gastroenteritis causing pathogens within one assay. The definition of a higher cut-off than proposed by the manufacturer allowed a better correlation compared to conventional methods. For some bacteria, additional culture remains necessary for susceptibility testing and proper identification.
Interferon alpha improves whole blood and neutrophils killing of group A streptococcus

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Aim
Group A streptococcus (GAS) is a Gram positive human pathogen causing mild as well as life threatening infections such as toxic shock syndrome and bacteraemia. When bacteraemia occurs, timely and accurate recognition and elimination of the invading bacteria is necessary in order to avoid dissemination. We showed that Toll like receptor 9 (TLR9) is important for the recognition and timely elimination of GAS infections1. Upon contact with GAS murine macrophages as well as plasmacytoid dendritic cells secrete interferon alpha (IFN-α) in a TLR9 dependent manner. We hypothesized that this cytokine secretion might further aid innate immune cells to phagocyte and kill the invading bacteria efficiently.

Methods
To test if INF-α enhances bacterial killing by whole blood and neutrophils, whole blood or neutrophils were pre-incubated with different INF-α concentrations. GAS were then added and the surviving bacteria enumerated by colony counting after 30 min of co-incubation.

Results
Addition of exogenous INF-α to whole blood and neutrophils enhanced GAS clearance of both whole blood as well as neutrophils.

Conclusion:
We found that addition of INF-α improves bacterial killing mediated by whole blood and neutrophils. Our data show a possible mechanism by which TLR9 recognition of GAS and consequent increase of INF-α levels could lead to a more efficient bacterial clearance by the host innate immune cells.

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Irregularities in the M1 protein structure are responsible for enhanced virulence of Group A streptococcus

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AIMS The M1 protein of group A streptococcus (GAS) is a major virulence factor that increases GAS virulence, invasiveness and resistance to clearance. M1 presents an irregular coiled-coil structure responsible for autoimmune reactions in the host. Recent data showed how ‘idealization’ of the M1 structure (M1id), giving rise to a regular coiled-coil, influences fibrinogen (Fg) binding in vitro. To investigate the importance of this structural change in live GAS, we created a GAS M1T1 5448 strain expressing M1id and looked at differences in bacterial virulence in in vitro and in vivo models.

METHODS GAS-M1id was created by precise allelic exchange mutagenesis. Fg binding assays were performed by FACS analysis on GAS coated with fluorescently labeled Fg. EA926 cells were used to assess adherence and invasion. Whole blood and neutrophil killing assays were performed by incubating bacteria with whole blood or neutrophils and by enumerating survivors. In vivo experiments were carried out on wt and TLR9-deficient mice. Bacteria were injected subcutaneously, the lesion size was measured for 4 days, mice were sacrificed and the bacterial count in the skin was assessed.

RESULTS We show that Fg binding to the surface of GAS-M1id is reduced, confirming previous data obtained using recombinant M1wt and id proteins. We also demonstrate that GAS-M1id possesses a lower ability to adhere invade host cells and that adherence and invasion abilities are greatly influenced by the presence of Fg. In the presence of Fg, the difference in whole blood and neutrophil killing between M1wt and M1id strains was also exacerbated, with the M1id strain presenting a markedly lower resistance to killing. In vivo experiments carried out in mice showed reduced virulence, persistence and invasiveness associated with the GAS-M1id.

CONCLUSIONS Our experiments clearly indicate the importance of studying host-pathogen interactions occurring during the infection process. Both the host protein Fg and the virulence factor M1 protein are in fact required to interact to enhance GAS virulence. We found that enhanced GAS virulence is strictly dependent on Fg binding to M1. We also demonstrate how M1 is the main Fg binding protein expressed on the surface of GAS and how a change of its structure causes a dramatic decrease in Fg binding, leading to a decrease in invasiveness of strains expressing M1id. This clearly highlights the importance of the M1 protein structure for infection in live GAS.
Manifold Sources of Artificial in vitro Recombinants of HIV-1 Variants Generated During the Procedure of RT-PCR

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Background: Next-generation sequencing provides a powerful tool to investigate the diversity of HIV-1 in vivo. Variant analyses based on each substitution separately is mainly limited by the error rate of the procedure, which is currently estimated to be in the range of 0.01 – 1% in regard to the 454 pyrosequencing technology. Haplotype analyses based on the complete sequence struggles in addition with artificial in vitro recombination during the procedure of reverse transcription and PCR. Here, we investigated the different possible sources of artificial in vitro recombinants.

Method: Five clonal virus stocks were mixed in equal amounts followed by RNA isolation, reverse transcription using the same RNA copy number in each RT-reaction and three different RT enzymes (RT-RNaseHplus, RT-RNaseHplus/high-fidelity, and RT-RNaseHminus), 20-cycle PCR, nested PCR using the same copy number in each PCR reaction (40 cycles), and 454 pyrosequencing. Standard and recombination-decreasing PCR conditions (e.g., increased dNTP and primer concentrations, expanded elongation time) were chosen to amplify the viral protease. Recombination and haplotype analyses were assessed using Recco (http://bioinf.mpi-inf.mpg.de/recco/) and ShoRAH (http://www.bsse.ethz.ch/cbg/software/shorah), respectively. All experiments were carried out in triplicates.

Result: Using a standard RT-PCR protocol, 25-37% of the sequences were recombinants; no differences were observed between RT-RNaseHplus vs. RT-RNaseHplus/high-fidelity enzymes. A reduction was achieved by improving the PCR condition. Here, the estimated recombination rates were 14-17%. The RT-RNaseHminus enzyme in combination with the improved protocol reduced the recombination rates even further to 1.7-2.9%.

Certain recombinants appear in almost all experiments suggesting hotspots for in vitro recombination. None of them has been observed in control experiments in which each virus stock was pyrosequenced separately.

Conclusion: Our data suggest that not only the PCR but also the step of cDNA synthesis is one possible source of in vitro recombinants generated during the procedure of RT-PCR. A RT enzyme lacking RNaseH activity reduces the recombination rate maybe due to an enhanced processivity. Artificial recombinants need to be considered in haplotype analyses of HIV-1 variants.
Nasopharyngeal pneumococcal colonization density as a marker for disease severity in adult pneumonia

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Aims: High genomic load of pneumococcus from blood or cerebrospinal fluid has been associated with increased mortality. We aimed to analyze whether nasopharyngeal (NP) colonization density in pneumonia patients is associated with markers of disease severity or poor outcome.

Methods: Quantitative lytA rtPCR was performed on NP swabs in adults hospitalized for pneumonia at Chris Hani Baragwanath Hospital, Soweto, South Africa. Pneumonia etiology was considered pneumococcal if any of sputum culture or Gram stain, urinary pneumococcal C-polysaccharide-based antigen, blood culture or whole blood lytA real-time (rt) PCR revealed pneumococcus. Infection- and prognosis-related biomarkers were measured using commercially available assays (KRYPTOR) for midregional proadrenomedullin (MR-proADM), midregional proatrial natriuretic peptide (MR-proANP) and copeptin and for procalcitonin (PCT Lia sensitive).

Results: Among patients with X-ray-confirmed pneumonia, mean NP colonization densities increased with increasing CURB65 scores (p=0.02). In HIV-infected patients with pneumococcal pneumonia, NP colonization density was higher among non-survivors (n=8) than survivors (n=76; 7.7 vs. 6.1 log cfu/ml, respectively; p=0.02) and among those who had pneumococcus identified from blood cultures and/or by whole blood lytA rtPCR than those with non-bacteremic pneumococcal pneumonia (6.6 vs. 5.6 log cfu/ml, p=0.04). NP colonization density correlated positively with PCT (Spearman correlation coefficient r=0.37, p<0.0001), ProADM (r=0.40, p=0.008), copeptin (r=0.30, p=0.01), but not with ProANP or CRP.

Conclusions: NP colonization density serves not only as a diagnostic but also as a severity marker for pneumococcal pneumonia in adults. It correlates with mortality, clinical severity scores, prognostic biomarkers and biomarkers which indicate the likelihood of a bacterial infection.
Acknowledgment: This work was supported by Centers for AIDS Research (CFAR) Grant National Institutes of Health (NIH) P30 A1050409.
Outcome after medical thoracoscopy - infection or just SIRS?

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Infection rates after medical thoracoscopy are not well studied and range from 1% to 10%. We sought to determine the outcome of patients undergoing thoracoscopy in our institution and to evaluate factors discerning real infection from unspecific systemic inflammatory response syndrome (SIRS).

We retrospectively reviewed the charts of all patients which underwent medical thoracoscopy at our tertiary care center between 01/2009 and 06/2011. Patients were evaluated for infection mortality, ICU-stay and rehospitalisation. For patients with antibiotic treatment 30 days before and/or after intervention, the postinterventional infection rate (surgical site or respiratory tract) was determined according to the CDC-criteria. Furthermore, we compared characteristics of patients receiving postinterventional antibiotics with and without confirmed infection.

Of the 108 included patients 67% were male. Median age was 62 years (IQR 57-75) and 67% received talc poudrage. Within 30 days after intervention (median follow-up 25), 2 (2%) patients needed intensive care, 7 (7%) were rehospitalised after discharge and 6 (6%) died. None of the deaths were related to the intervention nor to infection.

27/108 (25%) patients received periinterventional antibiotics. In 18/108 (17%) patients, a new antibiotic was initiated after the intervention. We found 2 infections directly attributable to the intervention (2 empyemas due to S. marcescens and S. aureus). Additionally, 5 patients had nosocomial pneumonia (1 with secondary P. aeruginosa bacteremia, 1 with C. freundii in the BAL, 3 diagnosed clinically) and 1 had a LRTI (K. pneumoniae/ E. cloacae in the BAL). Median onset of infection was on day 10 (IQR 5-15) after intervention.

Patients with talc poudrage had a significantly higher CRP within the first 3 days compared to those without poudrage. Patients with infection tended to have a second CRP increase after day 7 (see Figure).

Our postinterventional infection rate lies between 2% (only empyema) and 7% (including pneumonia and LRTI), which is within the expected range. However, the diagnosis of infection in patients after medical thoracoscopy, especially with talc poudrage, is complicated due to poudrage-induced SIRS. Whether the CRP course or other biomarkers (i.e. PCT) are helpful in distinguishing real infection from unspecific SIRS and whether the use of prophylactic antibiotics can reduce the infection rate has to be investigated in further studies.
Pneumonic tularemia with concomitant bacteremia caused by *Francisella tularensis* subsp. *holarctica*

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Introduction
Tularemia is a potentially severe zoonosis occurring in the northern hemisphere. It is caused by *Francisella tularensis*, an extremely infectious small gram-negative rod. Infections in humans usually present as (ulcero-)glandular tularemia. A rare case of a tularemic pneumonia with bacteremia is reported.

Case Presentation
A 79-year-old male presented with non-productive cough, progressive dyspnea and generalized weakness. On admission, the patient was febrile, with a slightly elevated blood pressure and tachycardia. Respiratory rate was 30/min, oxygen saturation 91% on ambient air. Chest examination revealed crackles over the right hemithorax. Conventional radiography confirmed an infiltration in the right lung. Leucocyte count was normal with left shift and toxic signs. There was thrombocytopenia and elevated CRP 334mg/l. Community-acquired pneumonia was diagnosed. An antimicrobial therapy with AmoxiClav 2.2g iv three times a day combined with clarithromycin 500mg twice a day for two days was started. After seven days the patient was afebrile. One week later laboratory levels returned to normal.

After seven days, one blood culture grew a gram-negative coccobacillus. No identification was possible with automated identification system and MALDI-TOF. 16s RNA gene sequencing identified *F. tularensis* subsp. *holarctica*. Pneumonic tularemia was diagnosed; this was confirmed by serology.

In a follow-up interview the patient reported having cut the lawn in his cottage in a rural area with his lawnmower 12 days before admission. Inhalation of contaminated dust was the most probable infection mechanism.

As relapses are described in literature a therapy with doxycyclin for 14 days was initiated even in the already asymptomatic patient.

Conclusion
There are two main subspecies of *F. tularensis*. While *F. tularensis* subsp. *tularensis*, which is endemic in North America, has a high aerosol-related infection rate, low infectious dose, and the ability to induce fatal disease, *F. tularensis* subsp. *holarctica* is endemic in Europe, considerably less virulent and virtually nonlethal in humans, even if appropriate treatment is not effected.

Diagnosis can be made by culture, however identification can be difficult by automated identifications systems and MALDI-TOF because *Francisella* might not be included in the product database. In these cases DNA sequencing should be performed. Alternatively the diagnosis can be confirmed by serology or by PCR on tissue biopsies.
Postraumatic septic arthritis: caveat Gram-negative pathogens

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Objective
Trauma-related septic arthritis is a rare and serious infection. Little is known about its microbiologic spectrum, clinical pattern and outcome.

Method
Review of the literature from 1945 to 2010 with an emphasis on post-traumatic cases.

Results
We retrieved 14 large-scale epidemiological surveys without detailed stratification regarding the origin of septic arthritis (3340 episodes) and 131 case reports. Post-traumatic septic arthritis is witnessed predominantly in young healthy males (75%; median age, 31 years) and involves the knee in 54% of cases. Four distinct origins differ in pathogenesis and microbiology: bites; thorn punctures; and trauma sustained in terrestrial or aquatic environments. Overall, causative microorganisms in post-traumatic arthritis are predominantly Gram-negative (51%), in contrast to primary native joint arthritis where Staphylococcus aureus prevails. Variability for the choice of antimicrobial agents is larger than in primary native arthritis, but the duration of antibiotic therapy and surgical therapy remain similar. Mortality equals zero and microbiological cure is achieved in 96% of cases. Severe functional mechanical sequelae, such as ankylosis or amputation, occur in 19% of cases.

Conclusion
Post-traumatic septic arthritis has a different clinical and microbiological pattern than primary native joint arthritis. In the case of empirical antibiotic treatment, a broader spectrum covering Gram-negative rods is more appropriate than simple anti-Gram-positive therapy.
Prevalence of anti-HEV IgG in blood donors from southwest Switzerland: a comparison of three EIA screening kits and one confirmatory immunodot assay

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Background
Hepatitis E virus (HEV) seroprevalence varies widely between industrialized countries. Gold standard diagnostic algorithms are missing. We explored the sensitivity and specificity of three different enzyme immunoassay (EIA) kits in blood donor samples.

Methods
We tested 550 consecutive anonymized blood donor samples collected in the region of Lausanne, Switzerland, for the presence of IgG anti-HEV using three EIA screening kits (MP Diagnostics, Dia.Pro and Fortress). Samples testing positive with at least one kit underwent confirmatory testing by an immunodot assay (Microgen Diagnostik recomLine HEV IgG/IgM).

Results
124/550 samples were positive with ≥1 EIA. Depending on the combination of tests (one EIA with or without confirmatory immunodot), seroprevalence varied from 3.3% to 21.8%. Of 124 EIA positive samples, 67 were negative on immunodot, 51 were positive and six were indeterminate. MP Diagnostics and the Dia.Pro kits had identical sensitivity (35.3%) and similar specificity (98.2% and 99% respectively); the Fortress kit had higher sensitivity (100%) and lower specificity (86.2%). Taking only positive EIA samples confirmed with immunodot as positive tests, seroprevalences were 3.3% using the MP Diagnostics and Dia.Pro kit, and 9.3% using the Fortress kit.

Conclusions
Taking the recomLine immunodot as a reliable confirmatory test of existing EIA kits, and assuming that no true positive sample tested negative with all three EIAs, we estimate HEV seroprevalence in this population to be 9-10%, twice that previously reported for this region.
Prosthetic valve endocarditis caused by Propionibacterium acnes successfully treated with rifampin in combination with intravenous penicillin: report of two cases.

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Infectious Diseases Service, Department of internal medicine, University Hospital and University of Lausanne
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Cases 1:
In September 2011 a 70-year-old woman with a history of biologic aortic valve replacement in June 2011 was admitted to our hospital with 7 days of fever. Physical examination showed a new 3/6 holostytolic cardiac murmur and splinter hemorrhagia of the right thumb. Two sets blood cultures revealed growth of Propionibacterium acnes. Transoesophageal echocardiography (TEE) showed multiple vegetations of the biologic aortic valve and a paravalvular abscess. Antibiotic treatment was started with intravenous penicillin and oral rifampin. Emergent bioprosthetic valve replacement was performed because of a complete heart block. After six weeks we decided to prolong the antibiotic treatment with ciprofloxacin and rifampin due to still elevated CRP. After a total course of 12 weeks treatment we stopped the antibiotic therapy. Two months after completion of the antibiotic treatment there were no signs of relapse of the endocarditis.

Case 2:
In August 2011 a 58-year-old man was admitted to our stroke unit with sudden onset aphasia. His medical history was remarkable for a biologic aortic valve replacement two years before. CT-Scan of the head revealed ischemia in the basin of the right media artery and thrombolysis was performed. Because of concomitant fever TEE was performed showing vegetations at the biological aortic valve. Two sets of blood cultures revealed growth of Propionibacterium acnes. We started intravenous penicillin G in combination with oral rifampin. Urgent replacement of the aortic root and the bioprosthesis was performed due to uncontrolled infection and new paravalvular aortic abscess. After 8 weeks the treatment was stopped without evidence of persistent infection. Two months later neither clinical nor laboratory results raised any concerns of relapse of the endocarditis.

Discussion
Currently there is no consensus on how to treat an endocarditis caused by Propionibacterium sp. Most authors suggest a course of six weeks with a betalactam antibiotic, mostly penicillin, in combination with an aminoglycoside [1]. Other regimens reported in literature contain vancomycin [2], clindamycin [3-5], daptomycin [5] and rifampin [6-10]. Trampuz et al. recently published data about the efficacy of rifampin to treat foreign-body associated infections caused by P. acnes. In this study rifampin showed the highest rate of biofilm eradication of P. acnes in vitro and in vivo [11]. We chose rifampin in combination with penicillin due to the promising activity of
rifampin against planktonic and biofilm *P. acnes*, the very low minimal inhibitory concentration and the excellent oral bioavailability. A rifampin-containing treatment should be considered in case of *P. acnes* prosthetic endocarditis.

**References**

Recurrent prosthetic valve endocarditis with rifampicin and gentamicin-resistant coagulase-negative staphylococci with increasing minimal inhibitory concentrations to glycopeptides and daptomycin - Case report

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CASE
This 61-year-old male had received a first aortic valve replacement for aortic insufficiency and a dual-chamber implant in 1995. He also had hairy cell leukemia treated with weekly interferon. In May 2011, he was diagnosed with “possible” prosthetic valve endocarditis (PVE) with coagulase-negative staphylococci (CoNS) based on 1 major (3/3 positive blood cultures) and 2 minor Duke criteria (fever and predisposition). The CoNS were resistant to oxacillin and gentamicin. Transoesophageal echocardiography showed no vegetations on valve or pacemaker leads. Treatment with vancomycin 15mg/kg bid (target trough 15-20 mg/l) and rifampicin 450mg bid was given for 5 days and switched to daptomycin 10mg/kg qd and rifampicin for a total of 6 weeks. Five days after discontinuation of treatment the patient presented with fever and chills. Again, CoNS were cultured in (4/4) blood cultures, and treatment with vancomycin and rifampicin was re-started. The transoesophageal echocardiography revealed 2 vegetations on the prosthetic valve and a paraavalvular abscess with paraavalvular leakage. The prosthetic valve and the pacemaker leads were replaced 10 days after the fever recurred. The blood cultures and the culture of the vegetations grew CoNS with additional resistance to rifampicin and increased MICs (by 2 to 6-fold) for glycopeptides and daptomycin (Table 1).

MICROBIOLOGY RESULTS

Table 1: increasing MIC under treatment with daptomycin

<table>
<thead>
<tr>
<th>MIC (mg/l) May, 2011</th>
<th>interpretation</th>
<th>MIC (mg/l) July, 2011</th>
<th>interpretation</th>
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<tr>
<td>Vancomycin</td>
<td>2.00</td>
<td>S</td>
<td>4.00</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>0.25</td>
<td>S</td>
<td>1.50</td>
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<tr>
<td>Teicoplanin</td>
<td>2.00</td>
<td>S</td>
<td>12.00</td>
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MICs determined by E-Test; interpretation according to CLSI

OUTCOME
The clinical failure of daptomycin could be explained by the increased MIC of the isolate. The patient was treated with moxifloxacin 400mg qd and clindamycin 600mg tid for 3 months, (2 weeks i.v. and 10 weeks p.o.), which was well tolerated. The MICs and killing curves for moxifloxacin and clindamycin are shown in Figure 1.
Three months after the end of treatment the patient is fine without signs of a relapse of PVE.

*Figure 1: killing curves for July 2011 isolates*

CONCLUSION
Resistance to glycopeptides and daptomycin in gram-positive bacteria is an emerging phenomenon; so far, CoNS have been reported to be nearly universally susceptible to daptomycin\(^1, 2\), including glycopeptide-intermediate CoNS\(^3, 4\). Daptomycin resistance has been described in patients with methicillin-resistant *Staphylococcus aureus*, often in association with intermediate resistance to vancomycin (VISA). Increased cell wall thickness is thought to explain this observation\(^5\). Treatment options may include substances which target bacterial structures other than the cell wall.

In this report we present a successful treatment option with the combination of moxifloxacin and clindamycin for prosthetic valve endocarditis caused by a highly resistant strain of CoNS.

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Sepsis in a patient with perforated sigmoid diverticulitis caused by Eggerthella lenta

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We present the case of a 39 year old Caucasian white male with a sepsis caused by a perforated sigmoid diverticulitis, in which blood cultures revealed bacteremia with Eggerthella lenta. To our best knowledge this is the first reported case of sepsis by E. lenta in a formerly healthy patient without relevant underlying pathologies.

Our patient presented to the emergency department in February 2012 with hypotension, fever, malaise and progressive left-sided abdominal pain. Laboratory findings included leukocytosis, elevated C-reactive protein and impaired renal function. Due to a history of mitral valve reconstruction in September 2011 a transthoracic echocardiography was performed initially to exclude an acute endocarditis. The following PET-CT revealed a perforated sigmoid diverticulitis as focus of infection. Blood cultures were obtained and later showed E. lenta in all anaerobic specimens taken before initiation of empirical treatment. The patient was successfully treated, first empirically with piperacillin/tazobactam (4.5g three times a day i.v.) and later with oral high-dose amoxicillin/clavulanic acid (1g three times a day p.o.). He recovered completely and is planned to have a colonoscopy when the infection has resolved.

Eggerthella lenta (formerly named Eubacterium lentum) is a Gram positive, non-sporulating, anaerobic rod bacterium. It belongs to the family of Coriobacteriaceae and is part of the human gut microflora. Although reported bacteremia with E. lenta in literature is rare, when bacteremia occurs it is clinically significant. Underlying gastrointestinal disorders, malignancy, surgery, immunodeficiency and foreign bodies are described as the most relevant factors for E. lenta bacteremia. Organisms are often unrecognized in clinical specimens because they have to be transported under anaerobic conditions; furthermore, E. lenta can be overlooked if facultative anaerobic bacteria in the same sample overgrowth. If the bacteria can be subcultured, the microbiological identification of E. lenta is feasible with commercial enzyme-based identification systems. Isolation of E. lenta in the laboratory should always stipulate the search of an intestinal leak.
Serotype-specific disease capacity for pneumococcal pneumonia in HIV-infected South African adults

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Aims: The serotype-specific disease-potential for non-bacteremic pneumococcal pneumonia in adults is unknown. The aim of this study was to assess if critical colonization density differentiating pneumonia from asymptomatic colonization in adults is serotype-specific.

Methods: Etiology was considered pneumococcal in South African HIV-infected adults if any of sputum culture or Gram stain, urinary antigen, blood culture, whole blood lytA real-time (rt) PCR revealed pneumococcus or if nasopharyngeal (NP) quantitative lytA rtPCR was >8000 cfu/ml. Quantitative serotype-specific rtPCR was performed on NP swabs for the serotypes of the 13-valent pneumococcal conjugate vaccine in pneumonia patients and asymptomatic HIV-infected controls.

Results: There was no asymptomatic pneumococcal colonization with serotypes 1, 5, 7F, 18C. For most remaining PCV13-serotypes, serotype-specific NPS colonization densities of >1000-5000 cfu/ml had sensitivities of >90% and specificities of 50-75% for pneumococcal pneumonia versus asymptomatic colonization. Several serotypes (4, 5, 14, 18C, 19A, 23F) were never or rarely found in pneumonia patients whose etiology was considered non-pneumococcal. Colonization densities in pneumococcal CAP were lowest for serotypes 5 and 18C, which were similar or even lower compared to the density in asymptomatic carriage of other serotypes (6A, 19A, 19F).

Conclusions: Mean NP colonization densities varied between serotypes, but were invariably higher in pneumonia than in asymptomatic carriage, particularly for “invasive” serotypes. Colonization density in pneumococcal pneumonia was lowest for serotypes which have higher invasive potential. We propose NP identification of some serotypes (1, 5, 7F, 18C) and identification of critical NP colonization densities for other serotypes as novel means to diagnose pneumococcal pneumonia.

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Social contacts of adolescents that are relevant in case of an invasive meningococcal disease in Switzerland

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Aim: In Switzerland current recommendations entail the administration of an antimicrobial chemoprophylaxis to contacts of the primary case in order to prevent secondary cases. Indications for chemoprophylaxis are family members, classmates and teachers. In order to evaluate the sensitivity of the current recommendations in terms of the capture of relevant close contacts (CC), a study on the social networks of adolescents at school, their working place and during leisure time was undertaken.

Methods: Students aged 16-18 attending either of two public schools, one full-time (FTS), the other part-time (PTS), were asked to document their CC over a period of one week. Overall 653 students were eligible for participation. A close contact was defined as any person with whom the subject interacted for more than 5 minutes at a distance of less than one meter. Contact distributions were generated depending on duration and other contact qualities.

Results: Overall, 58 (18%) students from the FTS and 98 (30%) students from the PTS returned the diary, of which 52 (16%) and 81 (24%) fulfilled the quality standards and were included in the study. In total, 9849 contact events were reported. Most of the participants reported between 20 and 50 distinct contacts over the whole observation period (median 41 contacts in FTS and 33 contacts in PTS). However, most participants documented only short and non-recurrent CC. Not surprisingly, the number of CC substantially dropped with increasing contact duration. At the FTS, contacts at school and during leisure time were almost equally distributed. Almost all CC at school occurred among classmates. Teachers and students from other classes did not play an important role. At the PTS, only 24% of contacts were recorded at school, while 31% were documented at work and 40% during leisure time. An analysis of the chemoprophylaxis coverage of relevant CC based on the current recommendations revealed that in half of the cases more than 70% of CC at the FTS would be covered while this percentage would correspond to only 40% at the PTS. Conclusion: The current recommendations indicating chemoprophylaxis for family members and classmates cover a substantial proportion of relevant CC for FTS students, while an insufficient proportion would be protected in case of parttime students. Coverage of relevant contacts can be increased to 90% or more, if close contacts at the work place and recurrent contacts during leisure time could be included.
Statin potentiate the anti-cytomegalovirus activity of ganciclovir.

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Introduction. A growing body of evidence has implicated human cytomegalovirus (HCMV) in the development of atherosclerosis, through the induction of a smoldering inflammatory state of the endothelium. Statins, the main cholesterol-lowering class of drugs, have been demonstrated to have antiviral activities against HIV-1, HCV or poliovirus. Here, we investigated whether statins interfere with HCMV replication in human endothelial cells (EC) and may potentiate the antiviral activity of ganciclovir (GCV).

Methods. EC were treated for 24-hours with atorvastatin, fluvastatin, pravastatin or simvastatin at 3 doses: a subinhibitory dose (SD) and two doses (IC20, IC50) inhibiting 20% and 50% of cell proliferation, respectively. Cells were subsequently infected with HCMV at an MOI of 1 and kept in cultures under continuous statin treatment.

Results. Atorvastatin, pravastatin and simvastatin exhibited a dose-dependent reduction of HCMV titres of 1 log with the IC20 doses and at least 1,5 log with the IC50 doses at 6 days post infection (dpi). Statin treatment did not affect the virus entry as assessed by RT-PCR at 2hpi. IC50 doses dramatically reduced the immediate early (IE), early (E) and late (L) antigens expression up to 6dpi with strong inhibition of viral DNA replication at 5dpi. In contrast, fluvastatin showed a limited antiviral activity: At IC50, the HCMV titre at 6dpi was only reduced by 1 log, the expression of IE, E and L antigens increased after 4dpi and viral DNA replication at 5dpi was almost not affected.

When EC were co-treated with the IC50 doses and mevalonate, geranylgeranyl pyrophosphate (GPP) or cholesterol, cholesterol failed to reverse the inhibitory activity of statins on HCMV titres, whereas mevalonate completely abolished the effects of all statins. Interestingly, GPP partially reversed the effects of simvastatin but not the other statins. Finally, whereas low dose of GCV alone reduced HCMV infectivity by 1 log, co-treatment with the IC20 dose of pravastatin reduced HCMV titre by 2,5 log, indicating an additive effect. Additionally, co-treatment of GCV with the IC20 dose of atorvastatin or simvastatin as well as with the IC50 dose of fluvastatin resulted in a synergistic activity of the drugs.

Conclusions. These findings demonstrate that statins exhibit a potent cholesterol-independent antiviral activity against HCMV, acting via a different mode of action compared to GCV. This may provide new opportunities for antiviral therapy.
Stenotrophomonas maltophilia and ESBL-Escherichia coli – epidemiology, risk factors, infection control measures and outcome in a tertiary-care hospital in Eastern Switzerland

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Aims:
In 2009, we observed nearly a quadruplication of patients with newly diagnosed S. maltophilia (SM) at our tertiary-care hospital in Eastern Switzerland, while the numbers for ESBL-E. coli (ESBL-EC) remained stable. This prompted us to compare these two multiresistant gramnegative germs with respect to epidemiology, risk factors, infection control measures and outcome.

Methods:
During a 2-year-period (2008-2009), all patients treated at the Cantonal Hospital St. Gallen (700 beds) and newly diagnosed with either SM or ESBL-EC were included (cystic fibrosis and ophthalmologic patients excluded). The same risk stratified isolation strategy (standard hygiene, patient contact with gloves, contact isolation) was applied for both germs. Clinical data were collected retrospectively by chart review.

Results:
29 new SM and 63 new ESBL-EC patients were detected in 2008-2009 by clinical samples (no active screening). While ESBL-EC cases remained stable (ca. 30/year), the number of SM patients increased (6 to 23). 7% (2/29) SM were co-trimoxazole (TMP-SMX) resistant. Susceptibility rates for ESBL-EC were: imipenem and fosfomycin 100%, ciprofloxacin 21%, TMP-SMX 31%, nitrofurantoin 93%, tobramycin 38%.

Male gender, winter, prolonged hospitalisation and antibiotic treatment, imipenem, ICU, COPD, steroids, visceral surgery, wound, tracheostoma and intubation turned out to be risk factors for newly detected SM. SM was mainly found as a colonisation of the respiratory tract or wounds. ESBL-EC patients typically had urinary tract infection and ciprofloxacin exposure.

Risk factors requiring isolation measures (wound, tracheostoma, urinary catheter, severe cough if respiratory tract was colonised, panresistance, ICU) were more prevalent in SM patients, and mortality was higher compared to ESBL-EC patients (24% vs 6%). After detection, appropriate antibiotic treatment (against the multiresistant germ) was given to 59% of ESBL-EC, but only 10% of SM patients. However, only a minority of SM patients was considered to have infection.

Conclusion:
Although, patient contact with gloves and contact isolation was more frequent in SM than ESBL-EC patients, the number of new ESBL-EC cases was stable, whereas SM cases increased.
Our results raise several questions: Can imipenem restriction stop this trend?, Is SM detection only a surrogate of more severely ill patients or does the higher mortality result from untreated infections misinterpreted as mere colonisation?

<table>
<thead>
<tr>
<th></th>
<th>ESBL-EC (n=63)</th>
<th>SM (n=29)</th>
<th>p¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>age at diagnosis (years) (median, IQR)</td>
<td>60 (39-73)</td>
<td>66 (55-75)</td>
<td>0.115*</td>
</tr>
<tr>
<td>male gender</td>
<td>35% (22/63)</td>
<td>59% (17/29)</td>
<td>0.03*</td>
</tr>
<tr>
<td>detection in winter (Oct-Mar)</td>
<td>43% (27/63)</td>
<td>76% (22/29)</td>
<td>0.003*</td>
</tr>
<tr>
<td>if inpatient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- hospitalisation prior to diagnosis (days) (median, IQR)</td>
<td>5 (1-13)</td>
<td>10 (5-15)</td>
<td>0.088*</td>
</tr>
<tr>
<td>- total duration of hospitalisation (days) (median, IQR)</td>
<td>16 (3-25)</td>
<td>25 (10-41)</td>
<td>0.028*</td>
</tr>
<tr>
<td>antibiotic treatment within 30d prior to diagnosis (median, IQR)</td>
<td>2 (0-10)</td>
<td>10 (3-13)</td>
<td>0.012*</td>
</tr>
<tr>
<td>imipenem within 30d prior to diagnosis</td>
<td>6% (4/63)</td>
<td>41% (12/29)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ciprofloxacin within 30d prior to diagnosis</td>
<td>30% (19/63)</td>
<td>10% (3/29)</td>
<td>0.038*</td>
</tr>
<tr>
<td>ICU stay prior to diagnosis</td>
<td>19% (12/63)</td>
<td>38% (11/29)</td>
<td>0.052*</td>
</tr>
<tr>
<td>if ICU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ICU days prior to diagnosis (median, IQR)</td>
<td>2 (1-7)</td>
<td>7 (3-14)</td>
<td>0.076*</td>
</tr>
<tr>
<td>COPD</td>
<td>8% (5/62)</td>
<td>35% (10/29)</td>
<td>0.005§</td>
</tr>
<tr>
<td>steroids within 30d prior to diagnosis</td>
<td>11% (7/62)</td>
<td>30% (8/27)</td>
<td>0.076§</td>
</tr>
<tr>
<td>visceral surgery within 30d prior to diagnosis</td>
<td>16% (10/62)</td>
<td>35% (10/29)</td>
<td>0.049§</td>
</tr>
<tr>
<td>wound</td>
<td>24% (15/62)</td>
<td>48% (14/29)</td>
<td>0.022*</td>
</tr>
<tr>
<td>tracheostoma</td>
<td>0% (0/63)</td>
<td>14% (4/29)</td>
<td>0.017§</td>
</tr>
<tr>
<td>intubated at diagnosis</td>
<td>3% (2/63)</td>
<td>21% (6/29)</td>
<td>0.022§</td>
</tr>
<tr>
<td>urinary catheter</td>
<td>37% (23/63)</td>
<td>43% (12/28)</td>
<td>0.566*</td>
</tr>
<tr>
<td>panresistance²</td>
<td>0% (0/63)</td>
<td>7% (2/29)</td>
<td>0.194§</td>
</tr>
<tr>
<td>infection control measures:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- standard hygiene</td>
<td>76% (48/63)</td>
<td>41% (12/29)</td>
<td>0.001§</td>
</tr>
<tr>
<td>- patient contact with gloves</td>
<td>19% (12/63)</td>
<td>38% (11/29)</td>
<td>0.052§</td>
</tr>
<tr>
<td>- contact isolation</td>
<td>5% (3/63)</td>
<td>21% (6/29)</td>
<td>0.051§</td>
</tr>
<tr>
<td>infection</td>
<td>54% (34/63)</td>
<td>10% (3/29)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>urinary tract infection</td>
<td>40% (25/63)</td>
<td>0% (0/29)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>mortality</td>
<td>6% (4/63)</td>
<td>24% (7/29)</td>
<td>0.042§</td>
</tr>
<tr>
<td>albumin &lt;20 g/l at diagnosis</td>
<td>13% (6/46)</td>
<td>30% (7/23)</td>
<td>0.162§</td>
</tr>
<tr>
<td>appropriate antibiotic treatment³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- all</td>
<td>59% (37/63)</td>
<td>10% (3/29)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>- if infected</td>
<td>71% (24/34)</td>
<td>33% (1/3)</td>
<td>0.481§</td>
</tr>
</tbody>
</table>

ESBL-EC = ESBL-Escherichia coli, SM = Stenotrophomonas maltophilia, IQR = interquartile range, Oct = October, Mar = March, d = days

¹ significant results are highlighted with red colour, ² panresistant ESBL-EC = resistance to carbapenems, chinolones and aminoglycosides, panresistant SM = resistance to co-trimoxazole (TMP-SMX), ³ directed against the multiresistant germ
*Mann-Whitney U, #Chi Square, §Fisher Exact
Stent preserving treatment of an infected peripheral vascular stentgraft

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Introduction
Infection of vascular stents is a rare event and treatment recommendations for combined surgical and antibiotic treatment of these infections rely on little evidence, but they cause important morbidity and costs. Only very few case reports of antibiotic treatment without surgery have been published so far.

Case
We report a 68-year-old man who was diagnosed with a dilative atherosclerosis with aneurysms of both femoral and popliteal arteries. Four weeks after endovascular exclusion of these aneurysms with a 25 cm long Viabahn vascular stentgraft, he presented with severe popliteal pain and inflammatory signs. A MRI of the leg showed an edema of the poplitea without abscess formation and patency of the endoprosthesis.

Blood cultures grew staphylococcus lugdunensis and treatment was started with ceftriaxon followed by penicillin and gentamycin iv with rapid resolution of pain and inflammatory signs. Treatment was then continued orally with rifampicin and ciprofloxacin.

One month after the end of therapy, the patient was asymptomatic and had no signs of inflammation. The findings on the MRI had practically normalized.

Conclusion
Infection of peripheral vascular stents can be treated successfully with antibiotics alone if a susceptible germ is identified and if there is no extensive tissue damage. Antibiotics should be bactericidal and active against biofilm producing bacteria on foreign bodies.

More information on the best management of these rare infections is urgently required and a registry of cases is desirable.
Streptococcus tigurinus, a new species causing invasive infections.

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Aims: Viridans streptococci are known as commensals of the oral flora but may cause invasive infections, e.g., infective endocarditis. We recently described a novel species within the Streptococcus mitis group, Streptococcus tigurinus sp. nov. 1, which was demonstrated the causative agent of severe infections.

Methods: By retrospective analysis of the 16S rRNA gene sequence database (SmartGene™, Zug, Switzerland) of the Institute of Medical Microbiology University of Zurich from October 2003 to March 2011, we identified 15 clinical isolates which showed sequence homology of ≥99.6% to S. tigurinus (GenBank accession number JN004270). Clinical data were retrieved from patients’ medical records.

Results: The clinical 15 isolates were subsequently identified as S. tigurinus by molecular analyses of the 16S rRNA gene. The isolates were derived from normally sterile body sites, including blood (n=9), surgically resected aortic (n=1) or mitral (n=2) valves, cerebrospinal fluid (n=1), periarticular hip biopsy (n=1) and vertebral body biopsy (n=1), and were obtained from 12 different patients. Nine patients were male, and 3 patients were female; the mean age was 48.8 years (range 29 to 74). Serious invasive infection was present in all patients. Four patients were diagnosed with definite infective endocarditis according to the modified Duke criteria; one patient developed bacterial meningitis; 3 patients presented with spondylodiscitis; 1 patient with prosthetic joint infection and 1 patient with thoracic empyema. Two patients had bacteremia alone. Both immunocompromised patients as well as immunocompetent patients were affected. All patients recovered after appropriate antimicrobial therapy, with (n=5) or without surgery (n=7).

Conclusions: Streptococcus tigurinus, a novel bacterium belonging to the viridans streptococci, bears a high potential to cause invasive infections. Its natural distribution and pathogenic determinants have to be investigated in the future.

1 Reference: A. Zbinden et al., Streptococcus tigurinus sp. nov., isolated from blood of patients with endocarditis, meningitis and spondylodiscitis, International Journal of Systematics and Evolutionary Microbiology, in press.
The AID modular line probe assay for rapid detection of drug resistances against isoniazid, rifampicin, amikacin, capreomycin, streptomycin, and fluoroquinolones in Mycobacterium tuberculosis.

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Background:
Despite improvements in gene amplification technologies, line probe assays remain the method of choice for detection of drug resistance mutations in Mycobacterium tuberculosis strains in the near future. Not the least because of the complexity involved, i.e. different mutations in multiple genes are involved in drug resistance.

Methods:
Based on epidemiological analyses of mutations involved in drug resistance (1) a TB Resistance line probe assay (reverse hybridization) was designed (AID Autoimmun Diagnostika GmbH, Germany) to cover resistance mutations for the following drugs: isoniazid (inhA -16, -15, -8 and KatG 315), rifampicin (RpoB 516, 526 and 531), streptomycin (rpsL A43G, A88G, A88C; rrs C522T, D523C, G524C and C526T), kanamycin / amikacin and capreomycin (rrs A1400G, C1401T and G1483C/T), fluoroquinolones (GyrA A90V, S91P, D94A, D94N, D94Y, D94G). A modular test design was developed with one module for screening of resistances against isoniazid and rifampicin (first line drugs) and an additional two modules for subsequent detection of resistances against aminoglycosides and fluoroquinolones.

Results:
Following optimization of the assay design the TB Resistance assay was evaluated against a series of clinical drug resistant M. tuberculosis culture isolates (n=62). All isolates had been characterized in detail for antibiotic susceptibility by phenotypic testing and sequence analyses. As control group 13 wt M. tuberculosis isolates were tested. The line probe assay detected all mutations in the clinical isolates with 100% accuracy. To implement the genetic resistance kit in a diagnostic work-flow we evaluated its use to rapidly detect resistance in early positive MGIT cultures. All positive clinical M. tuberculosis cultures obtained (n=50) during January-April 2011 were subjected to screening using the three module AID resistance kit. 3/50 strains showed mutations using module 1 of the AID line probe assay. The predicted antibiotic resistances were confirmed by phenotypic susceptibility testing.

Conclusion:
The TB Resistance line probe assay (AID) is a rapid tool for the accurate detection of drug resistance mutations resulting in drug resistance in Mycobacterium tuberculosis. The assay is easy to use and can be readily implemented in the diagnostic laboratory.

References:
Virologic Suppression is Maintained in Virologically Suppressed HIV-1 Infected Subjects Switching from Efavirenz/Emtricitabine/Tenofovir (EFV/FTC/TDF) Single-Tablet Regimen (STR) to Emtricitabine/Rilpivirine/Tenofovir (FTC/RPV/TDF) STR: Week-24 Results of GS-111

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[5] Central Texas Clinical Research, Austin, TX, United States
[6] Gilead Sciences, Inc., Foster City, CA, United States

Aims: The implication of temporarily reduced rilpivirine (RPV) exposures when switching virologically suppressed HIV-infected patients from efavirenz (EFV; a CYP inducer) to RPV has not been established. At W12, 100% of subjects switching from EFV/FTC/TDF (ATRIPLA®) to FTC/RPV/TDF (EVIPERA®) maintained HIV-1 RNA (VL) <50c/mL (primary endpoint). Furthermore, EFV concentrations were above the 90% inhibitory concentration (IC90) for several wks after EFV discontinuation and RPV exposures were in the range of historical values observed in the ECHO and THRIVE studies starting ~2 wks post-switch. Longer-term W24 follow-up of these switch subjects are presented.

Methods: This 48-week, open label, multicenter study enrolled 50 subjects on EFV/FTC/TDF as their first antiretroviral regimen for ≥3 months with HIV-1 RNA (VL) <50c/mL at screening. Subjects were interested in receiving the alternate single-tablet regimen (STR) of FTC/RPV/TDF due to EFV intolerance. None had known resistance to any study drug according to historical genotype. Secondary endpoints included evaluation of W24 efficacy, safety, and tolerability.

Results: Subjects received EFV/FTC/TDF for a median 2.5 yrs [IQR: 1.4, 3.6] before switching to FTC/RPV/TDF. One subject withdrew consent before dosing. 100% of subjects (49/49) maintained VL <50c/mL through W24 following switch from FTC/RPV/TDF. Median [IQR] change from baseline for CD4 count was +33 [-62, +92] cells/mm3. FTC/RPV/TDF was well tolerated with no discontinuations due to adverse events (AE). 24% (12/49) experienced a treatment-related AE, mostly Grade 1 (9/12) with no Grade 3 or 4 treatment-related AEs reported. Treatment-related AEs included depression (n=1), hyperbilirubinemia without jaundice/scleral icterus (n=1), and rash (n=2). Mean changes in serum creatinine (10.6 micromol/L) and direct bilirubin (2 micromol/L) were not clinically relevant. Median changes from baseline were significant for fasting total cholesterol (-0.44 mmol/L) and LDL (-0.62 mmol/L); p<0.001 for both. 92% subjects reported >95% adherence (pill count). W24 RPV exposures remained in the historical range.

Conclusion: After switching from EFV/FTC/TDF to FTC/RPV/TDF, virologic suppression is maintained through W24. Transient metabolic induction of CYP enzymes by EFV is not clinically relevant in virologic suppressed, HIV-1 infected
subjects switched to FTC/RPV/TDF. FTC/RPV/TDF provided a well-tolerated, alternative STR treatment option in this study population.
Week 48 Results of an Ongoing Global Phase 3 Study Comparing Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF (Quad) with Ritonavir-boosted Atazanavir plus Emtricitabine/Tenofovir DF in Treatment Naïve HIV-1 Infected Subjects Showing Efficacy, Safety, and Pharmacokinetics

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Aims:
Quad is a single tablet regimen (STR) in development composed of an integrase inhibitor elvitegravir (EVG), a pharmacoenhancer cobicistat, emtricitabine (FTC) and tenofovir DF (TDF). Described are Week 48 results of a Phase 3 study comparing Quad with ritonavir-boosted atazanavir (ATV/r) plus fixed dose FTC/TDF in treatment naive HIV-infected subjects.

Methods:
Subjects with HIV RNA ≥ 5000 c/mL, CLCr >70 mL/min, no prior HIV therapy, and no resistance to ATV, FTC, or TDF were randomized 1:1 to receive either Quad or ATV/r+FTC/TDF (stratified by baseline HIV RNA ≥ and < 100,000 c/mL) in an ongoing, blinded, active-controlled study. ¹° objectives included the proportion of subjects with HIV RNA <50 c/mL at Week 48 (FDA snapshot algorithm, 12% prespecified noninferiority margin) in the intent to treat population and assessment of safety. ²° objectives included pharmacokinetic/dynamic (PK-PD) and bone mineral density (BMD) analyses.

Results:
708 subjects were randomized: 90% male, 26% non-white, 39% with VL ≥100,000 c/mL. The primary objective was met; Quad was noninferior to ATV/r+FTC/TDF with 90% and 87%, respectively, having HIV RNA of <50 c/mL at Week 48 (difference +3.0%, 95% CI [-1.9%, +7.8%]). Among subjects with HIV RNA > 100,000 copies/mL, response rates were similar (Quad 85%, ATV/r+FTC/TDF 82%). Virologic failure (FDA snapshot algorithm) was infrequent, 5%, in both arms. Median CD4 increases were similar (Quad 207 cells/µL, ATV/r+FTC/TDF 211
cells/µL). Discontinuation rates for adverse events (AE) were similar (Quad 4%, ATV/r+FTC/TDF 5%). Among AEs occurring in ≥ 5% of subjects, AEs associated with elevated bilirubin levels were significantly higher in ATV/r+FTC/TDF and no AEs were significantly higher in Quad.

Median change in CLCr from baseline was -12.7 mL/min in Quad and -9.5 mL/min in ATV/r+FTC/TDF (p < 0.001). Median triglyceride increases were 11mg/dL in Quad and 29 mg/dL in ATV/r+FTC/TDF (p = 0.006). PK-PD analyses showed ~ ≥ 90% efficacy across all quartiles or octiles for EVG Ctrough. Median BMD changes for hip were: Quad vs. ATV/r+FTC/TDF (-2.45%, -3.46%) and for spine (2.87%, -3.59%) (p > 0.05 for both).

Conclusions:
Quad demonstrated noninferior efficacy and was well tolerated at 48 weeks in this Phase 3 blinded active-controlled study in treatment naïve HIV infected subjects. The efficacy of Quad was confirmed by robust PK analyses. These data support the use of Quad as a potential new STR option for initial HIV treatment.
A Transfusion-transmitted HBV Infection in Newborns

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Background:
Since September 2009 Hepatitis B Virus (HBV) nucleic acid testing (NAT) screening with a sensitivity limit of 25 IU/ml in the individual donation has been mandatory in Switzerland. Beforehand HBsAg testing was the only specific mandatory marker for HBV. The presented case illustrates the importance of the introduction of very sensitive NAT screening methods for HBV.

Methods:
The implicated donor and recipients of transfusion-transmitted HBV infections were followed up. Multiple samples were tested for HBV serological and molecular markers and if possible full genome sequencing was performed to compare sequences. A case follow-up of a transfusion-transmitted HBV infection from a donor with a low HBV viral load to 2 newborns that occurred before HBV NAT had been declared mandatory.

Results:
The mother of one baby presented 15 month after the birth of her daughter at her doctor's surgery suffering from an acute Hepatitis B infection. A HBV infection was confirmed by laboratory parameters. Based on these data a family investigation was initiated. The 15 month old baby was positive for HBsAg, anti-HBc IgG and had a viral load of $1.4 \times 10^9$ IU/ml. Since four months after birth this child had received a blood transfusion, it was thus assumed that a transfusion-transmitted HBV infection had occurred. Thus an intensive look-back procedure was immediately implemented. The implicated donor, a 65 year male repeat donor (31 donations), had never showed any clinical symptoms of active HBV infection. An archival sample from the implicated donation was found to be HBV DNA positive with a viral load of 253 IU/ml. Serological tests were not performed as no further material was available. Further investigations showed that a second baby was also infected with HBV (HBsAg, HBeAg, anti-HBc and a viral load of $3.96 \times 10^8$ IU/ml), whereas the third baby was not. The mother of the third baby had been previously vaccinated against HBV, but the mothers of the 2 infected babies were not. Unfortunately there was not enough material to sequence the HBV strain of the donor, but the genome sequence of the HBV from the 2 infected babies and the infected mother were identical. After birth none of the 3 babies nor their families had contact with each other, in fact they all resided in different regions of Switzerland.

Discussion:
The HBV transfusion-transmitted infection of 2 of the 3 babies was shown to have occurred from a transfusion with 3 baby splits. The case came to light by chance when the mother of one baby was infected through close contact with her daughter 15 months after the original transfusion had occurred. The present case highlights two important points. Firstly the importance of an effective look-back procedure for transfusion transmitted infections and secondly the value of a highly sensitive HBV
NAT screening strategy to prevent similar HBV infections from donors with low viral loads.
Increased macrophage migratory inhibitory factor (MIF) plasma levels in acute HIV-1 infection

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Background: The cytokine macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine implicated in the pathogenesis of inflammatory and autoimmune diseases. Accumulating data support a role of MIF in viral infections. Yet, there is little information on its role in HIV infection. Aim: To prospectively measure plasma MIF levels in a cohort of acute versus chronic HIV-infected Caucasians and to delineate the impact of ART on MIF levels.

Method: Patients enrolled in the Swiss HIV cohort study (SHCS) were recruited according to four defined groups: group 1 (n=6): acute HIV infection (AHI) (positive P24 antigen and <3 + bands on Western blot); group 2 (n=28): chronic HIV infection naïve to ART; group 3 (n=37): chronic infection on effective ART (viral load (VL) <50 copies/ml); group 4 (n=18): chronic infection on failing ART (2 consecutive VL >50 copies/ml). Healthy HIV-negative volunteers were enrolled as controls (n=40). MIF concentrations were measured by ELISA.

Results: MIF levels were 2.7-fold higher in HIV-infected patients than in HIV-negative controls (13.7±5.4 versus 5±1.9 ng/ml, means±SD, P<0.0001). Median MIF levels in AHI (20.6 ng/ml) were significantly higher than those chronic infection naïve to ART (16.3 ng/ml), chronic infection on effective ART (11.6 ng/ml) or chronic infection on failing ART (11.9 ng/ml). MIF levels from patients on effective ART were 1.4-fold lower than those from patients naïve to ART (P=0.027). MIF levels decreased 2-folds in AHI ≥ 12 months post-HIV diagnosis (P=0.028).

Conclusion: MIF levels are elevated during the course of HIV infection and the highest levels are detected in patients with AHI. Furthermore, MIF levels decreased upon initiation of ART. Our results suggest that MIF is an integral component of the cytokine storm characteristic of AHI.
Expression pattern of sirtuins and influence of sirtuin 1 and 2 on innate immune responses

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Background: The family of histone deacetylases comprises 18 members in mammals, among which seven sirtuins (Sirt1-7). Sirtuins are NADP-dependent deacetylases that have been involved in the control of cell metabolism, proliferation and survival. The expression pattern of sirtuins and their influence on host response to microbial infection remain largely unknown.

Aim: To analyze the expression of Sirt1-7 and to address the effects of Sirt1-2 inhibition on innate immune responses in vitro and in vivo.

Methods: In vitro: bone marrow derived macrophages (BMDMs) and dendritic cells (BMDCs) and RAW 264.7 and J774.1 macrophage cell lines were stimulated for 0, 1, 4, 8 and 24 hours with LPS, Pam3CSK4 lipopeptide and CpG ODN. RAW 264.7 macrophages were transduced with Sirt1 and Sirt2 shRNA lentiviruses. Sirt1-7 expression was quantified by real-time PCR, and TNF production was measured by ELISA. In vivo: BALB/c mice were challenged with LPS (350 ug i.p.) with or without a Sirt1-2 inhibitor. Blood and organs were collected after 0, 1, 4, 8 and 24 hours to quantify Sirt1-7 and cytokines by real-time PCR and ELISA. Mortality was assessed daily.

Results: BMDMs, BMDCs and RAW 264.7 and J774.1 macrophages express, in order of abundance, Sirt2 >> Sirt1, Sirt3, Sirt6 > Sirt4, Sirt5 and Sirt7. Microbial products decrease the expression of all sirtuins except Sirt6 in a time dependent manner in BMDMs (0-24h). Sirt2 is the most expressed sirtuin also in the liver, kidney (together with Sirt3) and spleen. Upon LPS challenge, expression levels of Sirt1, Sirt3, Sirt4 and Sirt7 decrease in the liver (from 4h to 24h), whereas Sirt1-7 decrease within 1h in both the kidney and the spleen. Pharmacological inhibition of Sirt1-2 and shRNA silencing of Sirt1 and Sirt2 decrease TNF production by macrophages stimulated with LPS, Pam3CSK4 and CpG ODN. In agreement, prophylactic treatment with a Sirt1-2 inhibitor increases the survival of mice challenged with LPS (n=12, P=0.03).

Conclusions: Sirtuins are expressed in innate immune cells. Inhibition of Sirt1-2 expression or activity decreases cytokine production by macrophages and protects from endotoxemia, suggesting that sirtuin inhibitors may represent novel adjunctive therapy for treating inflammatory disorders such as sepsis.

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In Experimental Pneumococcal Meningitis Transplanted Neural Stem- and Precursor-Cells Migrate to, and Differentiate at the Injured Hippocampus

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Background: In pneumococcal meningitis (PM), up to 50% of survivors suffer from long-term sequelae including impairment in learning and memory function. The neurofunctional deficits are associated with the occurrence of apoptotic brain injury to stem- and precursor-cells in the hippocampal dentate gyrus in experimental PM.

Aim: To assess survival, migration and differentiation of transplanted fetal neural stem and precursor cells (NPCs) after transplantation into injured hippocampus in vitro and in vivo in an infant rat model of PM.

Methods: In vitro, NPCs from fetal rat hippocampus constitutively expressing green fluorescence protein (GFP) were grafted into the hilus of dentate gyrus organotypic hippocampal slice cultures which were previously injured by challenge with live Streptococcus pneumoniae (serogroup 3, n ≥ 9). The migration and differentiation of grafted stem- and precursor cells were assessed by immunohistochemistry.

In vivo, NPCs were stereotaxically transplanted into the hilus of the hippocampus of rats 1 week after cured PM. At 1, 2 and 4 weeks following transplantation, survival, migration and differentiation of transplanted NPCs were evaluated by immunohistomorphometry.

Results: NPCs grafted in hippocampal slices injured by pneumococcal challenge, migrated to, and differentiated at the site of injury in the granular layer of the dentate gyrus. In rats after cured PM (n=14), GFP-expressing NPCs migrated from the injection site in the hilus to the injured granular layer of the hippocampal dentate gyrus and expressed markers of neuronal differentiation at 1 (n = 5) and 2 (n = 3) and 4 weeks after transplantation (n = 6).

Conclusions: Fetal NPCs transplanted into the hippocampus after PM survived and migrated to the area of brain damage in the granular layer of the dentate gyrus where they differentiated into neurons in vitro and in vivo. The transplantation of NPCs may hold promise for cell replacement therapies aimed at repair of brain damage after PM.
HIV-1 Derived Small Noncoding RNAs Inhibit Virus Replication

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Small noncoding RNAs (sncRNAs) are key players in the regulatory pathway called RNA interference. HIV-1 derived sncRNAs are expressed at very low levels in infected cells, therefore, a new method, based on a sequence targeted enrichment strategy, was developed in our laboratory. SncRNA libraries from in vitro HIV-1 infected primary macrophages and CD4\(^+\) T cells were generated, which led to 216 unique HIV-1 specific sncRNA clones distributed throughout the whole HIV-1 genome, 21 of them in antisense orientation. The impact of these viral sncRNAs (vsRNAs) on the viral life cycle was assessed by monitoring HIV-1 infection in vsRNA transfected primary cells, macrophages and CD4\(^+\) T cells. Exemplary vsRNAs have been chosen based on their frequencies and molecular characteristics.

Individual sense/antisense vsRNA hybrids from the env region, which potentially act like small-interfering RNAs, showed strong and persistent inhibition of HIV-1 replication of up to 90% in macrophages and of up to 75% in primary CD4\(^+\) T cells from two different donors. They act in a sequence-specific manner, since replication of another virus isolate containing mutations in the potential target sites of these sense/antisense vsRNA hybrids could not be inhibited. Tested separately as single-stranded vsRNAs, they showed no or little effect on HIV-1 replication in primary cells. We have tested several identified vsRNAs for their potential to inhibit virus replication. Although, single molecules of single-stranded vsRNAs seem not to have HIV-1 inhibitory potential, hybrids of sense and antisense single-stranded vsRNAs show strong inhibition of HIV-1 replication in primary macrophages and CD4\(^+\) T cells. These potent vsRNAs might play a role as intrinsic factors to regulate viral replication.