25.014
Cellular Antigen Stimulation Test (CAST) as a Novel, Affordable Laboratory Technique to Assess Potential Hypersensitivity to All Available Antiretroviral Compounds
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Introduction: Recently, novel allergometric techniques allow to test inhalants, food, and also drugs by specific in vitro assays. A flow cytometry technique based on the search of sulphidoleukotrienes LCT4, LTD4 and LTE4 released by basophils stimulated in vitro by IL-3 in presence of the examined antigens (cellular antigen stimulation test, or CAST), has a 80–90% sensitivity-specificity rate, and becomes particularly useful when prick tests are not applicable, and allergic reactions are not mediated by allergen-specific IgE immunoglobulins.

Methods-Results: During the past three years, 13 HIV-infected subjects (eight females and five males, aged 37–52 years), underwent a standardized, specific in vitro cellular antigen stimulation test (CAST), due to serious cutaneous (six cases), systemic (two patients), and combined cutaneous-systemic hypersensitivity reactions (five subjects). Apparently not elicited by the introduction of abacavir and nevirapine (which are the antiretroviral agents burdened by the greatest frequency of expected early allergic reactions, mediated by already recognized pathogenetic mechanisms). Based on the results of CAST testing, an allergic intolerance to ritonavir (six cases), lopinavir, nelfinavir, and didanosine (three cases each), saquinavir and lamivudine (two cases), and fosamprenavir, zidovudine, zalcitabine, stavudine, and efavirenz (one case each), was documented: in 10 cases out of 13 (76.9.5%) multiple intolerances were detected. A perfect relationship was documented between the results of CAST testing and the panel of combined antiretroviral compounds recently experienced by each allergic patient, and a CAST-based elimination of in vitro allergenic molecules allowed a rapid introduction of another effective antiretroviral combination.

Conclusions: Adverse events to antiretroviral drugs are quite frequent among HIV-infected patients, compared with the general population. Further, controlled studies are needed to implement in vitro allergometric testing in patients treated for HIV infection, who are exposed to unpredictable drug intolerance reactions. In fact, HIV-infected subjects may suffer from frequent allergic drug reactions which may be difficult to be systematically recognized (due to the frequent, multiple concurrent pharmacotherapy, and the combined antiretroviral therapy itself).

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25.015
HIV Genotypes and Patterns of Drug Resistance in Patients Failing HAART in a University Hospital Clinic
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Background: HAART strategies yield excellent results in drug naive patients starting first-line treatment. Yet, data from clinical cohorts indicate drug resistance emerging as a consequence of toxicity, drug interactions or adherence problems leading to sub-optimal serum drug concentrations.

The Aga Khan University Hospital, Nairobi, has comprehensive HIV care services since 2003 at the one stop clinic. About 500 patients are on current follow-up by standard international protocols.

Methods: Over one year, 38 patients failed ART and were referred to the laboratory for resistance testing performed by population sequencing technique using VIRASEQ (Abbott) by manufacturer’s protocol. All clinical details were recorded during sample collection.

Results: All patients belonged to a high socioeconomic group with corporate health insurance cover and claimed excellent drug adherence.

Patient age ranged from 6 to 57 years with a median of 38 years. CD4 counts ranged from 12 to 450 with a median of 135. Viral loads ranged from 2100 to 13 million with a median of 140,000. 82% (31/38) of patients had multiple hospital admissions indicating 2 to 5 episodes of opportunistic infections of which TB (14 patients), PCP (11 patients), Cryptococcosis (10 patients) and Salmonellosis (8 patients) were most common. 4 patients had 3 simultaneous opportunistic infections. 6 patients had 2 simultaneous opportunistic infections. Only 8 patients were on second-line HAART which included boosted PI. 9 patients used ARVs for over 10 years. A, D and CIFO1 were predominant subtypes. Major NRTI/NNRTI mutations were seen in 80% of patients while major PI mutations were seen in 8% of patients. High-level resistance was seen to Tenofovir in 8% of patients, Nevirapine, Efavirenz, 3TC & FTC in 80% of patients and AZT in 29% of patients.

Conclusions: In the long run, high-level multiple resistance is inevitable in patients on HAART, with serious implications for national ARV programs and prophylactic regimens.

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25.016
Multi-Pronged Strategy Greatly Enhances Enrollment of HIV Positive Children Into HIV Care and Treatment: Experience from Uganda
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Background: Although Uganda had successfully rolled out Antiretroviral therapy (ART) in HIV-infected adults, enrollment of children aged less than 15 years onto ART had generally been very slow. However, the implementation of a multi-pronged strategy for scaling-up pediatric HIV care has started to yield results.
greatly improved enrollment of children into HIV care and ART over the past two years.

Methods: The activities for the multi-pronged strategy were developed by the national Pediatric ART Committee that is comprised of major stakeholders in pediatric HIV care and they include:

- Development of a national strategy for scaling up pediatric HIV care including a capacity-building plan for pediatric HIV care and treatment.
- Advocacy, Community mobilization and education on pediatric HIV care, treatment and prevention.
- Establishment of a nationwide system for Early Infant HIV diagnosis using DNA PCR.
- Strengthening of linkages between PMTCT services with HIV care clinics, Postnatal clinics and Maternal and Child Health services.
- Countrywide refresher training in pediatric HIV care including Child counselling with follow-up of trained health workers.
- Training of specialized pediatric HIV counselors.
- Provision of family-centred, comprehensive pediatric HIV services that include community follow-up and nutritional support.
- Establishment of regional supervision and mentoring in pediatric HIV care.
- Use of pediatric dosing wheels and job aides.
- Use of simple pediatric ART formulations.

Results: The number of children on ART has risen from 5000 by end of December 2005 to 12,000 by the end December 2007. The number of health facilities providing ART to children less than 15 years of age has increased from 47 sites to 174 sites out of the total 303 ART sites in the same period.

Conclusion: Scaling-up pediatric HIV care requires a comprehensive and multi-pronged strategy with innovative activities that involve all stakeholders in pediatric HIV care.

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Bacterial Infections (Poster Presentation)

40.001

A Role for Chlamydia pneumoniae in Inducing IL-10, IL-13, IL-18 and the Chemokines CCL3-alpha, CCL3-beta and CCL5 in Atherosclerotic Patients

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The present work explored the spontaneous induction of the inflammatory cytokine interleukin (IL)-18 and the anti-inflammatory cytokine IL-10 and IL-13, and the chemokines CCL3 alpha and beta (macrophage-inflammatory protein-1alpha and beta, CCR1/5 ligand), (CCL5, regulated upon activation, normal T cell expressed and secreted (RANTES, CCR5 ligand) in atherosclerotic patients. In addition, the effects of the chlamydial antigen HSP60 and LPS on the induction of these mediators were examined. Intracellular detection of cytokines and chemokines was assessed by immunohistochemistry. The results of these experiments showed significantly high levels of spontaneously produced IL-18, IL-13 and IL-10 in patients compared to healthy controls. Cells stimulated with CHSP60 showed significantly high production of IL-18, but not IL-13 or IL-10. However, LPS stimulation resulted in increased levels of IL-18 and IL-10, but not IL-13 compared to non-stimulated cells. The examined chemokines were detected at significantly high levels on atherosclerotic patients compared to healthy controls. Stimulation with HSP60 and LPS showed increased levels of CCL3alpha, CCL3beta and CCL5 in patients compared to healthy controls. Thus, we demonstrate for the first time the induction of these inflammatory and anti-inflammatory mediators in atherosclerotic patients and that chlamydial antigens play a role in the immunopathological events in this disease by generating more inflammatory mediators rather than anti-inflammatory response.

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40.002

Cloning of a Cysteine Protease of Avian Staphylococcus aureus and its Expression in E. coli

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Background: Staphylococcus aureus infections are one of the major causes of economic loss in poultry industry and cysteine protease may be one of the major virulence factors of avian S. aureus. All avian species appear to be susceptible to staphylococcosis, which is common worldwide wherever poultry are reared. There are zoonotic risks to humans that S. aureus can cause food poisoning. In order to provide a rational research basis for detection of pathogenicity of S. aureus and study on the virulence factors and expression system, this study on cysteine protease of avian S. aureus was conducted.

Methods: The fragment of protease gene of S. aureus from a standard avian strain was amplified by PCR and cloned into prokaryotic expression plasmid vector pET33b+ with restriction endonuclease to construct recombinant pET-protease, which was verified, by restriction endonuclease and DNA sequencing. The recombinant plasmid was transformed into E. coli BL21/DE3 to express the protease gene. The production of protease was induced by IPTG. Construction of recombinant plasmid pET33b+ with the protease gene inserted downstream of an N-terminal his-tag and transformation into heterologous expression host E. coli BL21/DE3 resulted in the strain BL21/DE3 pET33b+. The presence of protease gene in recombinant cells was confirmed by colony PCR and restriction digestion with appropriate enzymes and sequencing.

Clones of this strain were tested for production of protease before and after induction with IPTG and immunoblotting with pooled polyclonal sera of chicken known to be infected with S. aureus and monoclonal anti his-tag antibodies.

Results: The result showed protease DNA fragment was proved correct through digestion with restriction endonuclease and DNA sequencing. Its sequence was 99% homologous to that published in Gene Bank (gi:19570341). A 36 and 40kDa fusion protein, which was induced by IPTG, was detected by SDS-PAGE. In immunoblots, the recombinant