

## Poster presentations

Poster numbers marked with \* will also be presented in an Oral Selected Poster Discussion.

## P.01 - Basic Sciences Track

**P1.001 HIGHCD45RO+ EXPRESSION ON CD4 T CELLS IN LATE STAGE OF HIV INFECTION IN INDONESIAN POPULATION: A HOSPITAL-BASED STUDY**

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**Background** Human Immunodeficiency virus (HIV) infection incidence is increasing in Indonesia. CD45RO+ cells are hyper-responsive cells that mediate immunological memory and HIV tends to infect RO+ memory subset of CD4 T cells. CD4+CD45RO+ T cells are major latent virus reservoir in HIV infection. However, up to now, there is no report of this reservoir cells in Indonesian HIV patients at various disease stages.

The aim of this study is to know the relationship between the CD4+CD45RO+ T cells with clinical HIV stage in a hospital-based study.

**Method** This observational cross-sectional study was conducted in 48 HIV patients (35 males; 13 females) with various stages of diseases in HIV clinics of Sardjito Hospital, Yogyakarta, Indonesia. The clinical HIV stages of infections were determined while the CD4 counts and CD45RO expressions were detected from peripheral blood cells using flow cytometer. The differences of CD4+CD45RO+ T cells percentages among patient clinical stages and its correlations with CD4 counts were analysed using ANOVA and Pearson Correlation tests.

**Result** Clinically the most dominant stage was stage 3 (n = 15; 31.3%). The Late stage HIV infection (stage 3 and 4) has significantly higher CD4+CD45RO+ T cells percentages than stage 1 and 2 (p = 0.006). The CD4+CD45RO+ cells percentages positively correlated with clinical HIV stage (r = 0.46; p < 0.05) but negatively correlated with CD4 cells absolute count (r = -0.55; p = 0.001)

**Conclusion** T cells expressing CD4+RO+ were higher in late stage of HIV infection and negatively correlated with CD4 T cells absolute count.

**P1.002 DEVELOPMENT AND PERSISTENCE OF ANTI-CHLAMYDIAL ANTIBODIES IN WOMEN WITH INCIDENT CHLAMYDIA TRACHOMATIS INFECTIONS IN UGANDA AND ZIMBABWE**

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**Background** *Chlamydia trachomatis* (Ct) IgG antibodies could provide evidence of past chlamydial infection for epidemiologic studies. Antibodies to chlamydial heat shock protein 60 (cHSP60) have been associated with complicated infection and infertility. Few studies have prospectively evaluated antibody development and persistence.

**Methods** Chlamydia serology (Medac IgG MOMP and cHSP60) was performed on stored sera from a cohort of 18–35 yr-old women seeking reproductive health services in Uganda and Zimbabwe who participated in a prospective study of HIV infection; study visits (including Ct-PCR testing) occurred on average every 80 days for up to 28 months. We analysed data on 135 women with ≥ 1 incident Ct infections who were IgG-seronegative prior to and had at least one IgG test on or after the date the incident infection was detected (“day zero”).

**Results** Sixty-six (49%) women seroconverted; of 54 tested on day zero, 46 (85%) were positive and 8 (15%) were negative and then positive when next tested (median 90 days). Of 12 seroconverters not tested on day zero, 11 (92%) were seropositive when next tested (median, 157 days). Nineteen (28%) of 69 non-seroconverters had no IgG testing beyond day zero and could not be assessed for delayed seroconversion. Of 52 seroconverters with subsequent testing, 27 (52%) remained persistently IgG-positive through the last test (median 248 days after seroconversion). Persistent IgG-positivity occurred in 61% (22/36) of those who were ever cHSP60-positive and 37% (6/16) of those who were not (NS), and in 56% (19/34) of those with only one Ct-PCR-positive visit and 50% (9/18) of those with more than one Ct-PCR-positive visit (NS).

**Conclusions** Anti-MOMP IgG antibodies developed in half of women with incident Ct infection and persisted in half of them. Although persistence was more common in those who were cHSP60-positive (suggesting complicated infection), the difference was not statistically significant.

**P1.003 EXPERIMENTAL STUDY ON PATHOGENIC DIVERSITY OF DIFFERENT CHLAMYDIA TRACHOMATIS SEROVARS IN MOUSE GENITAL INFECTION**

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**Background** *Chlamydia trachomatis* infection increases above gonorrhoea and syphilis, ranking first among the STDs. Molecular epidemiological researches have shown the predominant genotypes vary between regions, periods and population subgroups. However, serovars E, D and F are the most prevalent serovars. It is unclear whether the epidemiological characteristics were contributed to geography or pathogenicity. We explored the pathogenic diversity of different *C. trachomatis* serovars in mouse genital infection.

**Methods** One hundred of female BALB/C mice were divided into serovar E, F, H, J and K groups. The mice in study group treated by medroxyprogesterone acetate were inoculated 10<sup>7</sup> *C. trachomatis* into genital tract. *C. trachomatis* was detected by culture, direct immunofluorescence assay (DFA) and PCR in the cervicovaginal secretion. On the days 7 and 35 after inoculation, inflammation of the cervix, uterus and oviduct were examined by HE stain, and expressions of cHSP60 and CPAF in the uterus and fallopian tube were detected by ELISA.

**Results** The inflammatory of the cervical mucosa was more severe in serovar E group compared with J, K and H groups on day 7 post-inoculation. Accordingly, cHSP60 and CPAF expression increased significantly in E group compared with other experimental and control groups. On day 35 post-inoculation, the histo-pathological changes of the genital tract were obvious in J, K and H groups, characterised with uterine swelling, pyometra and effusion, fallopian expansion, hydrops, fibrosis and stenosis. cHSP60 and CPAF expression in H group was superior to that in other groups. Positive correlation between cHSP60 and CPAF expression was present on day 7 and 35 post-inoculation, respectively.

**Conclusion** There existed pathogenic diversity among different *C. trachomatis* serovars in mouse genital infection. The expression of inflammatory cytokines of cHSP60 and CPAF during Chlamydial infection might partially explain the pathogenic mechanism and the stage of the Chlamydial infection.

**P1.004 SEROVAR D AND E OF SEROGROUP B INDUCE HIGHEST SEROLOGICAL RESPONSES IN UROGENITAL CHLAMYDIA TRACHOMATIS INFECTIONS**

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**Background** Chlamydia trachomatis is the most prevalent bacterial sexually transmitted infection worldwide. A strong link between serogroup/serovar and serological response has been suggested previously. This study aims to elucidate serovar specific serological responses in two independent Dutch patient groups using two serological assays.

**Methods** We performed genotyping of serovars in two patient groups of *C. trachomatis* infected patients (total n = 718). We pooled the two study populations to form one study group and within this group we analysed men and women separately. We used two commercially available ELISA kits (medac Diagnostika) to determine specific serum IgG levels.

**Results** Calculations of the kits to determine IgG concentrations were comparable and could therefore be pooled. We observed very significant differences when comparing the mean IgG titres of three serogroups, B, C, and I. In the female group B vs. C:  $p < 0.0001$  (mean titres B 270.0 vs. C 88.8); B vs. I:  $p < 0.0001$  (270.0 vs. 108.5). Male group B vs. C:  $p = 0.0005$  (190.2 vs. 69.6); B vs. I:  $p = 0.0002$  (190.2 vs. 92.9); C vs. I was not significant. Serovars D and E of serogroup B induce the highest mean IgG titres compared to the other serovars in both men and women: 145.5 and 199.1 vs.  $\leq 107.9$  for men and 305.6 and 262.7 vs.  $\leq 161.5$  for women.

**Conclusions** This study shows a statistically significant higher serological response induced by B group serovars compared to the C and I group serovars *in vivo* in both men and women. This study is currently being extended with a different ethnical population and a different serological test.

**P1.005** **EVALUATION OF CYTOKINES AND MATRIX METALLOPROTEINASES GENES EXPRESSION IN GENITAL ORGANS AFTER VAGINAL EXPOSURE TO CHLAMYDIA MURIDARUM**

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**Background** Although the pathologic consequences of *Chlamydia* genital infection are well-established, the mechanisms leading to tissue damage are not completely understood.

**Methods** All the experiments were approved by the Ethical Committee of the University of Bologna. Animals used were 24 female Balb/c mice, 7 weeks old. All animals received medroxyprogesterone acetate 9 and 2 days prior the infection.

Twelve mice were infected by placing 15  $\mu$ l of sucrose-phosphate-glutamic acid (SPG) buffer containing  $10^6$  inclusion forming units (IFUs) of *C. muridarum* into the vaginal vault. Nine animals were inoculated with 15  $\mu$ l of SPG containing heat-inactivated  $10^6$  IFUs of *C. muridarum*. As controls of inflammation, 3 animals were challenged with 15  $\mu$ l of SPG.

At 3, 10, and 20 days post-infection 4 infected animals, 3 animals inoculated with heat-inactivated bacteria and 1 control were sacrificed.

Genital tracts were divided into the cervical-vaginal region, uterine horns, and oviducts.

Right uterine horns and oviducts were stored in formalin and later processed for histological examinations. The remaining parts of the organs were used for RNA extraction, by using Trizol Reagent (Invitrogen), in combination with RNeasy Mini Kit (Qiagen).

cDNA was synthesised with SuperScript III RT (Invitrogen). Real-time RT-PCR was performed with SYBR Green Fast Start kit (Roche Diagnostics). Primers used to assess INF- $\gamma$ , TNF- $\alpha$ , MMP-2, MMP-9 and GAPDH levels were from SuperArray (SABiosciences).

**Results** At histological examination no controls showed inflammation. On the contrary, scores of inflammation in all the organs from infected animals peaked at day 10, whereas only a single animal inoculated with inactivated bacteria showed a very mild inflammation at day 10 in its uterus.

At day 10, organs from infected animals showed significantly higher MMP-2 and MMP-9 genes expression than organs obtained from non-infected mice.

**Conclusions** Our study confirms the pivotal role of MMPs in the development of tissue damage.

**P1.006** **THE EFFECTS OF SYPHILIS ON CD4 CELL COUNTS AND PLASMA HIV-1 VIRAL LOADS AMONG PATIENTS WITH HIV-SYPHILIS CO-INFECTION**

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**Background** Concomitant syphilis and human immunodeficiency virus (HIV) infection is increasingly frequent in industrialised countries. We examined the effect of active syphilis on CD4 counts and plasma HIV-1 viral loads

**Methods** All patients with syphilis-HIV coinfection treated at the Sheba medical centre between 2007 and 2012 were included in the study. Patients were divided to those with early or secondary syphilis and to those with latent syphilis (early and late). CD4 cell counts and plasma HIV-1 viral loads were measured before, during and following syphilis treatment.

**Results** 17 patients were included: all were men having sex with men, 11 (65%) were treated with ART: 5 with primary syphilis, 4 patients with secondary syphilis, 2 patient with early latent and 6 patients with latent syphilis of unknown duration (rpr > 1:32), one of them with neurosyphilis. Median CD4 cell count significantly dropped during syphilis by 25%: from 575 (246–828) before syphilis diagnosis to 415 (195–630) cells/mm<sup>3</sup> during syphilis ( $p = 0.0002$ ). After penicillin treatment it rose back to 621 (250–1379) ( $p = 0.01$ ).

Plasma VL in 6 of the patients that did not receive ART increased during syphilis, although this rise was not statistically significant.

**Conclusions** Syphilis was associated with a transient decrease in the CD4 cell count and with an increase in VL in HIV-infected men; This increase in VL, although statistically non significant, may explain at least partially the increased risk of HIV transmission in HIV patients not treated by ART that are co infected with syphilis.

**P1.007** **IMMUNE-RESPONSE IN LESIONAL SKIN OF SECONDARY SYPHILIS**

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**Background** It is well known that tissue immune response plays an important role in the pathogenetic mechanisms of several bacterial and viral infections. Several studies focused on the cutaneous host immune-response during infections by mycobacteria, HIV, HHV8, HCV and HSV, while few data are known about cell-mediated immunity in skin syphilitic lesions.

**Methods** By an immunohistochemistry technique, we characterised the cutaneous inflammatory infiltrate in 5 cases of secondary syphilis, using a large panel of monoclonal antibodies (MAbs) and a specific polyclonal antibody against *Treponema pallidum* (TP) antigen.