Late Breaking News

LB2392 **The structure of the cell wall polysaccharide of *Enterococcus faecalis* is critical for evasion of complement deposition by the lectin pathway**

C. Theilacker*, S. Geiss, P. Sanchez-Carballo, O. Holst, J.A. van Strijp, J. Hübner, S. Rooijakkers (Freiburg, Borstel, DE; Utrecht, NL)

Objective: *Enterococcus faecalis* is a Gram-positive pathogen that may cause invasive infections in immunocompromised patients. *E. faecalis* is resistant to serum but the molecular mechanism underlying complement evasion is not known. The mechanism of increased serum sensitivity of an insertion mutant of the putative glycosyltransferase EF1172 in *E. faecalis* strain V583 was investigated.

Methods: Complement deposition and phagocytosis was quantified by FACS. Killing of enterococci in the presence of serum and PMNs was measured using an opsonophagocytic killing assay. Human pooled serum was preabsorbed with live wild-type *E. faecalis* V583 for all assays. Structural polysaccharide analysis was performed by gas chromatography and NMR spectroscopy.

Results: Incubation of *E. faecalis* V583Delta1172 with 1.5% serum and PMNs for 90 min reduced the number of viable bacteria by >2 logs, while wild type bacteria were resistant to killing under these experimental conditions. After 15 min incubation of live bacteria preopsonized with human pooled serum (HPS), PMNs phagocytosed fivefold more *E. faecalis* V583Delta1172 than wild type bacteria. Also, significantly more C3b was deposited on V583Delta1172 compared to the wild type strain. Measurement of C3b deposition by serum depleted of complement factor C1q or Ca++ excluded a role of the classical and alternative pathway of complement activation by the mutant strain. Furthermore, V583Delta1172 incubation with HPS lead to more binding of C4b – a component of the C3 convertase generated by the classical and lectin pathway – than wild type cells. The role of the lectin pathway in increased susceptibility to complement was confirmed by elevated levels of mannose binding lectin-associated serum protease 2 (MASP-2) bound to the mutant strain. Biochemical characterization of the cell wall polysaccharide of V583Delta1172 revealed an altered composition with a loss of galactosamine, a increased ratio of rhamnose:glucose (~2:1 in *E. faecalis* V583 wild type, ~4:1 in V583Delta1172) and lower phosphate content compared to the wild type strain.

Conclusion: The structure of the wall polysaccharide of *E. faecalis* is critical for resistance to complement activation by the lectin pathway, possibly by altered charge or three-dimensional structure of the polysaccharide.

LB2393 **Different erythromycin-resistant phenotypes of *Streptococcus pyogenes* do not prevent human polymorphonuclear cells from their antibacterial activity**

G. Banche, V. Tullio, V. Allizond, N. Mandras, J. Roana, D. Scalas, F. El Fassi, S. D'Antico*, N. Carlone, A.M. Cuffini (Turin, Italy, IT)

Objectives: Albeit the prevalence of macrolide resistance in *Streptococcus pyogenes* reported by several countries has significantly increased, these alarming *in vitro* results not always demonstrate a relationship with an *in vivo* negative impact on clinical efficacy, since the standard susceptibility testing methods do not take into account several host's defense mechanisms, that play a key role during infection in preventing the triggering and spreading of a bacterial infection process. In order to evaluate the potential immunomodulatory activity of erythromycin upon the binomial antibiotic-resistant bacterium/host, the PMN phagocytic and bactericidal activities against streptococcal strains Ery-S and Ery-R belonging to different phenotypes were assessed in the presence of the macrolide.

Methods: The erythromycin-resistance phenotypes of *S. pyogenes* were determined by the triple-disk diffusion testing. Polymorphonuclear cells (PMNs) were separated from lithium heparinized venous blood pooled from healthy donors, negative for the presence of microbial diseases, by using Ficoll–Paque. The effects of erythromycin on either the phagocytosis of radiolabelled *S. pyogenes* [3H-uracil (specific activity: 1.27 TBq/mmol; NEN Products, Milan, Italy)] or intracellular bacterial killing by PMNs were investigated by incubating the bacteria and the phagocytes (bacterium:PMN ratio was 10:1) at 37ºC for various periods in the presence of the MIC of the drug. Erythromycin-free controls were also included.

Results: Our results showed that when erythromycin, PMNs and streptococci, both Ery-S and Ery-R (cMLSB, M, iMLSB-A, -B, -C phenotypes), were present in the culture medium, the phagocytic activity of PMNs overlapped that of controls without drug. In contrast, the results emphasized a significant high increase of intracellular killing by PMNs in the presence of erythromycin for Ery-S and Ery-R *S. pyogenes*, with high (cMLSB, iMLSB-A and iMLSB-B phenotypes) and moderate (M and iMLSB-C phenotypes) level of Ery-resistance compared to controls without drug.

Conclusion: These results indicate that erythromycin has a significant immunomodulating activity on host-bacterium interaction being able to determine a significant increase of microbicidal activity in human PMNs against all *S. pyogenes* strains, either Ery-S or Ery-R, with high and moderate resistant level, confirming that the antibiotic resistance detected *in vitro* does not always imply a failure of antimicrobial treatment.

LB2394 **Three simple ESCMID severity criteria predict poor cure rate and slower resolution of diarrhea in *Clostridium difficile* infection**

M.A. Miller*, E.J. Kuijper, D.N. Gerding, S. Gorbach (Montreal, CA; Leiden, NL; Chicago, Boston, US)

Objectives: The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has recently published a Clostridium Difficile Infection (CDI) "treatment guidance document". Of their 14 severity criteria, 3 had been routinely collected in a recently-completed phase 3 randomized clinical trial (RCT) of fidaxomicin vs vancomycin in the treatment of CDI. We examined the prevalence of these 3 criteria and their relationship to cure, recurrence, global cure and time to resolution of diarrhea (TTROD).

Methods: The only 3 ESCMID severity criteria which had been collected routinely and could be used were: temperature, leukocyte count, and acutely elevated serum creatinine. They were available in all subjects in the "modified Intent to Treat" (mIT) group analysis (n = 596). Baseline serum creatinine was not available so the surrogate of initial creatinine $\geqslant 1.5$ mg/dL was used instead. Per ESCMID guidelines, patients with $\geqslant 1$ criteria could be classified as severe CDI. Standard definitions of cure were applied. Global cure = initial cure without recurrence. TTROD was counted from onset of treatment to $\leqslant 3$ unformed bowel movements. Analyses were performed on mITT subjects; the "Per Protocol" group analyses were very similar. The 2 treatment groups were combined for this analysis.

Results: 155 subjects (26%) had $\geqslant 1$ of the 3 severity criteria ("severe group"): 8 (1.3%) with T > 38.5ºC; 84 (14.1%) with WBC >15×10^9/L; and 86 (14.4%) with creatinine >1.5 mg/dL. The cure rate for severe cases (77%) was lower than for non-severe cases (90%) (P < 0.001). Recurrence rates were not statistically different in the 2 groups. Median

TTROD was longer in the severe group compared to the non-severe group (103 h vs 59 h, respectively; P = 0.002).

Conclusions: To serve as a useful tool, a severity score should be easy to use and correlate with outcomes. In the fidaxo vs. vanco RCT, 26% of subjects could be classified as severe by 3 simple ESCMID criteria. The classification of severe was significantly correlated with a lower cure rate at end of therapy and with prolonged TTROD. These 3 simple severity markers were useful in predicting clinical outcomes of CDI. If such criteria are validated prospectively, they may prove invaluable for categorizing patients in RCTs of CDI and for guiding therapy in individual CDI patients.

LB2395 **Identification of common fungal pathogens in a single sample**

K.M. McCarney*, K. Dempsey, J. Redden, A. Ricketts, W. Ewen Smith, P. Lewis White, M.D. Perry, R.A. Barnes (Glasgow, Cardiff, UK)

Objectives: The objective was to develop a highly sensitive and specific single test for the direct detection of *Aspergillus* and *Candida* species within clinical specimens.

Methods: Surface enhanced resonance Raman scattering (SERRS) allows more analytes to be detected at lower concentration than fluorescence. Multiplex assays were developed to detect a broad range of pathogenic fungal species, present in clinical samples, in low copy number. Genus specific polymerase chain reaction (PCR) primers and species specific probes were designed to detect multiple targets from spiked serum/whole blood samples. Each probe has a unique spectrum allowing identification of multiple targets in a mixture. One primer from each set was modified with biotin to capture amplified DNA on microbeads. The specific probes were hybridised to the PCR products and captured on microbeads. The probe is released from the microbeads and analysed by SERRS for rapid detection of multiple pathogenic fungal targets.

Results: The assay was tested successfully against blind panels and is capable of detecting most clinically relevant species of *Candida* and *Aspergillus*. It is designed to differentiate azole resistant species and can detect multiple aetiologies in one specimen. The detection limit is 2–3 copies input per reaction and can reproducibly detect 101 organisms within a simulated clinical specimen.

Conclusions: In this preliminary study, the assay successfully identifies the most common fungal pathogens with high efficiency and demonstrates sensitivity down to 2–3 PCR input copies. This assay is ready to go into pre-clinical testing with the addition of software designed to automate the analysis and the detection range will be increased to cover additional fungal agents. This is the first clinical application developed using D3 Technologies' powerful detection platform. The flexibility of this technology will enable ready adaptation to other clinical applications.

LB2396 **Direct detection of triazole resistance in *A. fumigatus* from airway secretions**

S. Park, M. Fraczek, M. Kirwan, S.A. Follett, K. Radej, A. Moody, D.S. Perlin, D.W. Denning* (Newark, US; Manchester, UK)

Objectives: *Aspergillus fumigatus* causes significant morbidity in immunocompromised patients, asthma, cystic fibrosis and chronic pulmonary aspergillosis (CPA). Culture positive rates in invasive disease and CPA are typically ~30%. Rates of culture positivity in allergic aspergillosis (ABPA) are typically ~60%. Increasing rates of azole resistance, determined by MIC on positive cultures, have been noted since 2004. Culture negative cases do not currently allow susceptibility to be determined.

Methods: Patients were recruited from the National Aspergillosis Centre, Manchester if they provided a fresh sputum sample. Samples were split for microscopy and fungal culture or PCR. DNA extraction was performed with the MycXtra kit and real-time PCR using the MycAssay Aspergillus assay. Only those samples that were PCR positive,

culture negative were subjected to a nested PCR approach. The CYP51A gene was amplified using Invitrogen Platinum Taq polymerase in two fragments. Fragment 1 (876 bp) covered the promoter tandem repeat region to codon 98. The second amplicon (748 bp) covered codons 54 to 266. PCR products were cleaned up and each was used as a template to probe with molecular beacon assays for known azole resistance SNPs.

Results: 30 patients were PCR positive and culture negative for *Aspergillus* species. No G54 or M138 mutations were found. Four samples had M220 mutations. Sixteen of 29 (52%) had both a tandem repeat (TR) with an H98L mutation. Of particular interest, two samples had a M220 mutation with a TR+ H98L mutation and the TR was found without the H98L mutation in 3 and the H98L mutation without the tandem repeat in 2. One sample did not amplify fragment 2. Overall, therefore 18 of 30 (60%) samples had evidence of azole resistance. Amongst these patients, 6/8 (75%) had ABPA, 11/20 (55%) had CPA and 1/2 (50%) had bronchiectasis with documented aspergillosis. Four had never received triazole therapy and two had known pan-azole resistant CPA (M220K and unknown mechanism). Four were taking itraconazole (2 clearly failing Rx, one non-compliant, one worsening after response, three were taking voriconazole (2 clearly failed Rx, one stable with toxicity) and 5 were taking posaconazole (3 responders, 2 primary Rx).

Conclusions: Using a commercial real-time assay for *Aspergillus*, residual DNA can be used directly to determine azole resistance in *A. fumigatus*. In this small single centre sample, it would appear that resistance is common.

LB2397 **C-reactive protein serum levels as an early predictor of outcome in patients with pandemic H1N1 influenza A virus infection**

O. Zimmerman*, O. Rogowski, G. Aviram, M. Mizrahi, D. Zeltser, D. Justo, E. Dahan, R. Arad, O. Touvia, L. Tau, J. Tarabeia, S. Berliner, Y. Paran (Tel Aviv, IL)

Objective: To retrospectively examine whether the admission serum C-reactive protein (CRP) levels of patients with pandemic influenza A (H1N1) infection can serve as a predictor of illness severity.

Methods: Included were consecutive adult patients who presented to our emergency department (ED) between May and December 2009, with a flu-like illness, a confirmed diagnosis of pandemic influenza A (H1N1) infection and a serum CRP level that was measured within 24 hours of presentation. Patients with a proven additional concurrent acute illness were excluded. Outcome measures were intensive care unit (ICU) admission, initiation of mechanical ventilation and death.

Results: Seventeen of the 191 enrolled patients (9%) were admitted to the ICU, of whom eight required mechanical ventilation and three died. The median admission serum CRP levels were significantly higher among patients who subsequently required ICU care and those who required mechanical ventilation compared to patients who did not (123 vs. 40 mg/L, p < 0.001 and 112 vs. 43 mg/L, p = .017, respectively). Admission serum CRP level and auscultatory finding over the lungs (bronchial breath sounds or crackles) were independent prognostic factors for the need of ICU care. The CRP hazard ratio (95% CI) for being transferred to the ICU was 1.09 (1.06–1.12) for each increase of 10 mg/L in CRP level. The admission serum CRP level was the only independent prognostic factor for mechanical ventilation. The CRP hazard ratio (95% CI) for mechanical ventilation was 1.09 (1.05–1.13) for each increase of 10 mg/L in CRP level.

A Kaplan–Meier estimate of the two outcome measures, i.e., the need for ICU admission and for mechanical ventilation, was performed using tertiles of serum CRP levels (i.e., <28, 28–69 and ⩾70 mg/L). At the 30-day time point since their presentation to the ED, none of the patients with a serum CRP level <28 mg/L needed to be admitted to the ICU or required mechanical ventilation (Figure 1). At the same time point, 19% of the patients with a serum CRP level ⩾70 mg/L needed to be admitted to the ICU and 8% required mechanical ventilation. The differences in the rates between the lower vs. upper tertile groups were

significant (Log-Rank p < 0.001 for ICU and p < 0.024 for mechanical ventilation).

Conclusions: Serum CRP levels in patients with pandemic H1N1 influenza A infection can serve as a useful measurement to predict disease course and assist in patient management as early as ED admission.
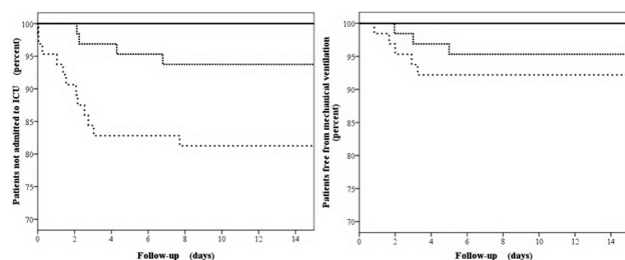

Figure 1. Kaplan–Meier analysis displaying the cumulative probability of not reaching the outcome measure of intensive care unit admission (left box) or mechanical ventilation (right box) for patients according to tertiles of serum CRP levels at admission into the emergency department. The solid line represents <28 mg/L, the dense dotted line 28–69 mg/L, and the thin dotted line ⩾70 mg/L.

| LB2398 | **Mass spectrometry-based high throughput resequencing of 2009 influenza A (H1N1) virus** |

J. Gooskens*, C. Posthuma, J.C. Zevenhoven-Dobbe, E. Claas, A. Kroes, E. Snijder (Leiden, NL)

Objectives: The 2009 pandemic influenza A (H1N1) virus contains novel gene segments from zoonotic-origin and lacks numerous genetic markers associated with increased virulence and antiviral resistance. The use of mass spectrometry (MS)-based comparative sequence analysis was investigated for high throughput determination of viral sequences and detection of mutations.

Methods: During the first 2009 pandemic wave (June 15 through August 31), respiratory specimens obtained from febrile patients with acute respiratory tract infection were tested by influenza A real-time PCR. Novel influenza A (H1N1) virus sequences were monitored for specific mutations or single nucleotide polymorphisms (SNPs) associated with antiviral resistance (NA gene positions 119, 136, 155, 248/266, 274, 294), potential virulence factors (PB2 positions 271, 627, 701; PB1F2 position 66; NS1 position 92), and protein truncation (PB1-F2 positions 12, 58, 88; NS1 position 220) using MS-based comparative sequence analysis (Sequenom MassARRAY®/iSEQ™) and regular cycle sequencing. In addition, follow-up specimens from three immunocompromised patients with an acquired H275Y mutation in the NA gene were tested. MS-derived sequences were compared to regular cycle sequencing results.

Results: Influenza A virus was detected in 84/453 (19%) specimens. Primers for novel influenza A (H1N1) were specific, as no product could be amplified from occasional isolates of seasonal influenza A (H1N1) or A (H3N2). A high concordance was observed between MS-based and regular cycle sequencing results (nucleotide sequences >99%, SNPs >93%, total specimens ~90%). No mutations associated with antiviral resistance, potential virulence factors and protein truncation were detected during the first pandemic wave. MS-based resequencing detected an H275Y mutation in only 4 of 9 (44%) follow-up specimens in two of three patients with an established acquired H275Y mutation by regular cycle sequencing.

Conclusion: High throughput MS-based resequencing provided reliable sequence results on specimens obtained during onset of infection. Detecting mutants in clinical follow-up specimens could be limited by a lower sensitivity of this method but this should be further evaluated.

| LB2399 | **Panobacumab adjunctive immuno-therapy for *Pseudomonas aeruginosa* hospital-acquired pneumonia versus a cohort group** |

Q. Lu, A. Dugard, P. Eggimann, P.F. Laterre, E. Mercier, M. Wolff, J. Chastre, E.J. Giamarellos-Bourboulis, V.C. Gafner*, H. Lazar, E. Mus, A. Perez, M. Tamm (Paris, Limoges, FR; Lausanne, CH; Brussels, BE; Tours, FR; Athens, GR; Berne, Basel, CH)

Objectives: To assess the efficacy of Panobacumab, a fully human IgM monoclonal antibody against *P. aeruginosa* serotype O11, by comparing a phase IIa trial with a standard care cohort trial both in hospital acquired pneumonia (HAP) caused by *P. aeruginosa* O11.

Methods: Demographics, outcome and survival of HAP including Ventilator Associated Pneumonia (VAP) in patients either treated with standard antimicrobial therapy in a retrospective cohort trial (CT) or with adjunctive Panobacumab therapy during an open phase IIa trial were compared. Both trials applied the same inclusion exclusion criteria and the same trial period of 30 days.

Results: 17 patients with VAP/HAP (14/3) caused by *P. aeruginosa* O11 were enrolled in a phase IIa trial (ITT population) and treated with Panobacumab, 13 of them received the full treatment course of 3 infusions (PP population, 12 VAP, 1 HAP) and 4 patients received only one infusion. In the cohort trial 14 patients (VAP/HAP: 12/2) treated with standard antibiotic therapy were included.

The mean age and weight were 65.8 y (years) (SD 17.2) and 78.0 kg (SD 22.1) in the PP, 67.8 y (SD 15.4) and 77.1 kg (SD 20.2) in the ITT population and 51.8 y (SD 22.3) and 67.1 kg (SD 13.0) in the CT.

At the time of suspicion of pneumonia a mean APACHE II and CPIS of 19.4 (13–33) and 8.7 (7–11) in the PP, 18.9 (13–33) and 8.5 (7–11) in the ITT and 14.5 (2–24) and 7.5 (3–12) in the CT population were observed.

Tracheostomy was present in 53.8% and 52.9% in the PP and ITT populations and 38.4% in the CT.

The pneumonia was polymicrobial in 69.2%, 70.6% and 85.7% in the PP, ITT and CT respectively.

Stay at ICU and hospital before diagnosis of pneumonia were similar in the 3 groups.

All 13 patients that received 3 doses of Panobacumab achieved resolution of pneumonia with only two relapsing during the study. Hence 85% achieved resolution and 15% recurrence at day 30. In the ITT group 64.7% of the pneumonia resolved, 11.8% recurred and 23.5% continued while in the CT 57% resolved, 7% recurred and 34% continued.

Resolution of pneumonia occurred markedly earlier in the Panobacumab trial (8.9 days, SD: 3.3) than in the cohort trial (15.3 days, SD: 9.5).

The expected mortality derived from APACHE II score was 31% and 32% in the PP and ITT population and 22% in the cohort group. All patients who received 3 doses of Panobacumab survived, 18% died in the ITT group while in the CT 21% mortality matched the predicted mortality.

Conclusions: Treatment of VAP/HAP caused by *P. aeruginosa* O11 with 3 doses of Panobacumab resulted in 100% survival, with highest pneumonia resolution (85%), and in a shorter time when compared with patients under standard therapy. The results indicate that Panobacumab may be effective in such life-threatening indication and warrants larger controlled trials.

| LB2400 | **Detection and characterization of mixtures of hepatitis C virus (HCV) types** |

M. Holodniy*, R.S. Diaz, R.L. Mathis, P.M. Feorino, C. Loveday, Z. Grossman, R. Kantor, Y.W. Tang, R.M. Lloyd Jr. (Palo Alto, US; Sao Paulo, BR; Buford, US; Buckinghamshire, UK; Ramat Gan, IL; Providence, Nashville, US)

Background: Response to approved treatments for Hepatitis C Virus infection is worse with HCV genotype one (the most common genotype) compared with other HCV genotypes. Genotype determination is routine for clinical trials. Detection of mixtures is not currently available; it may

be desirable to detect minor populations of HCV genotypes, since this may impact response to therapy.

Methods: A 15-member virus panel was constructed from patient-derived virus containing genotypes 1b, 2b & 3a, mixed at ratios of 10:90, 30:70, 50:50; $\leqslant$30,000 HCV RNA copies/mL each. Ratio targets were confirmed by clonal analysis (n = 50 each). The panel was blinded, randomized, genotyped and reported using the TRUGENE® 5′NC genotyping kit. TRUGENE® FASTA files were downloaded into a novel reporting module, MixuTYPE™ and compared to the original TRUGENE® genotype. A second MixuTYPE™ analysis was performed on 63 patient genotypes previously genotyped by Vanderbilt University Clinic. FASTA sequences obtained from testing with the TRUGENE® 5′NC genotyping assay were downloaded into the MixuTYPE™ reporting module and subsequent results were compared between the two methods. A confirmatory test was also run on these specimens using either HCV Inno-LiPA or HCV Invader assays at Vanderbilt. Specimens containing confirmed mixtures of two different HCV TYPES were cloned (n = 50) for complete characterization.

Results: The HCV TRUGENE 5′NC assay using the integrated OpenGene® reporting system were correctly identified mixtures in 3 of 15 (20%) of the characterized mixture panel while MixuTYPE™ correctly identified 15 of 15 from TRUGENE® FASTA sequences. The correctly identified genotypes were all from the 1b/3a mixture panel set at 30:70, 50:50 and 70:30 ratios. Sixty-three retrospective patient specimen genotypes obtained by HCV TRUGENE® 5′NC assay were sent for re-analysis of the original genotype using MixuTYPE™. No mixtures were identified during the original testing while MixuTYPE™ identified mixtures in 5 of 63 (7%). Confirmatory testing and subsequent clonal analysis confirmed MixuTYPE™ results in 5 of 5 samples.

Conclusions: HCV-infected patients may have mixed genotypes, reflecting double infections. The ability to detect HCV genotype mixtures may be of importance for treatment strategies (i.e. duration of treatment, prognosis) and if future antivirals present differential response rates according to genotypes.