Appendix A

Material and methods

The objective of the study was to compare cytokine levels in patients with and without HIV infection during acute appendicitis.

Study design

Prospective observational study

Setting, patient selection, data collection

Serum and peritoneal fluid (PF) cytokine profiles were measured in patients admitted with clinical diagnosis of acute appendicitis. Patients, 18 years or older, were recruited to participate in the study between November 2016 and May 2017 from Kalafong and Steve Biko Academic Hospitals in Pretoria, South Africa. Patients were stratified according to HIV infection status and histological appendicitis severity. The following parameters were recorded: patient demographics, symptoms and signs, duration of symptoms, intensive-care unit (ICU) admission, HIV infection status, CD4+ T-cell count, HIV ribonucleic acid (RNA) viral load (VL) count and histological staging of appendicitis. Exclusion were patients younger than 18 years of age, patients on non-anti-retroviral chronic medication, on immunosuppressive medication including chemotherapy, systemic steroid therapy, anti-allograft rejection therapy, and with any malignancies. Prior written informed consent was obtained from the patients for participation in this study. Pre- and post-test counselling for HIV testing was performed as per routine hospital practice. Open appendectomy was performed in all cases.

Investigations

Diagnostic blood tests, viral load and histology specimens were processed at the National Health Laboratory Service affiliated to the University of Pretoria Departments of Haematology, Virology and Anatomical Pathology respectively. Appendicitis was histologically staged into simple or complicated categories. Histopathologists were blinded to the patients' HIV status.

Pre-operative serum and intra-operative PF samples were collected from patients admitted with suspected acute appendicitis. HIV enzyme-linked immunosorbent assays (ELISAs) were performed. CD4+ T-cell count and HIV VL were determined in HIV+ve patients.

Cytokine determination

Blood samples for analysis of serum cytokine levels were centrifuged at 2683xg for 15min and serum separated within 2 hours of collection. Serum and PF samples were stored at -80°C until use. Samples were thawed at room temperature before analysis. Cytokine concentrations in serum and PF samples were determined using a Bio-Plex suspension array

system (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions based on published protocols.[16]

HIV subgroup analysis

HIV+ve patients were further subdivided into various categories based on CD4+ T-cell count, use of antiviral therapy and HIV viral load thus: Low CD4+ T-cell count (CD4+ T-cell count <350 cells/μL); highly active anti-retroviral therapy (HAART) naïve (CD4+ T-cell count >350 cells/μL); and controlled HIV (patients taking HAART with undetectable viral load and a CD4+ T-cell count > 350 cells/μL).

Statistical analysis

HIV+ve and HIV-ve patients were compared and stratified into simple and complicated appendicitis categories. Data from the cytokine analysis in this two-factor (HIV and Appendicitis) study design was evaluated using a two-way analysis of variance (ANOVA) with interaction and log regression for continuous values to correct for skewed data. Sample size was adequate for this analysis. Cytokine concentrations were expressed as geometric mean and 95% confidence intervals. Categorical data was expressed as number and percentage, and analysed by Fisher's exact or Chi-square tests. Continuous clinical variables were analysed using the t-test. A p-value ≤ 0.05 was considered statistically significant.