HIV infection is associated with differential increase in cytokine response and greater rate of perforation in acute appendicitis

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Introduction

Acute appendicitis is a common surgical emergency and leading cause of morbidity and mortality worldwide. Globally, the incidence has halved over the last half century, but has increased in South Africa.[1,2]

Acute appendicitis is associated with production of cytokines. The profile of cytokines depends on the duration of infection and presence of complications. The profile of early phase of acute appendicitis is predominantly pro-inflammatory where interleukin (IL)-1, IL-6, IL-8, interferon-inducible protein (IP)-10 and macrophage inflammatory protein (MIP)-1β have been described, whereas the profile in late phase or complicated appendicitis features anti-inflammatory cytokines including IL-6,and IL-10. [3,4,5,6,7]

Human Immunodeficiency Virus (HIV) infection is also accompanied by a series of cytokine production. During early stage of HIV infection, the cytokine profile is predominantly pro-inflammatory while during late stage the profile features anti-inflammatory cytokines.[8,9] The high prevalence of HIV infection in South Africa of 13.1% generally, and 20% in women of reproductive age in 2018 is a potential confounding factor.[10]

Material and methods

A prospective observational study was undertaken to compare cytokine levels in patients with and without HIV infection during acute appendicitis.

The study was approved by The Faculty of Health Sciences Research Ethics Committee, University of Pretoria (Reference: 305/2016).

Patients were recruited between November 2016 and May 2017 from Kalafong and Steve Biko Academic Hospitals in Pretoria. Pre-operative serum and intra-operative peritoneal fluid (PF) samples were collected from patients admitted with a clinical diagnosis of acute appendicitis for cytokine measurements by Bio-Plex suspension array system. (Laboratory measurements and statistical methods are in Appendix A.)

Results

One hundred and twelve patients were enrolled with mean age 31.9 years and 24 were excluded because they had non-appendicitis surgical abdominal emergencies or normal appendix. Fifty (56.8%) of the remaining 88 patients were male and seven of these (14%) were HIV+ve. Of the 38 females, 14 (36.8%) were HIV+ve (p=0.02). The overall HIV infection rate was 23.9%. Symptoms and duration of illness were similar in HIV+ve and HIV-ve patients. HIV+ve patients had significantly more *free* appendix perforations 9/21 (42.9%) vs 7/67 (10.5%) (p=0.002). Of the 9 HIV+ve patients with perforation, 6 (66.7%) had low CD4+ T-cell counts (p=0.003). Three patients required intensive care treatment, all were HIV+ve. There were no deaths.

There was a general increase of serum pro-inflammatory cytokines and increased serum and PF anti-inflammatory cytokines in HIV+ve patients. The increase in cytokine response in

serum was exaggerated in both groups compared to PF, but more evident in HIV+ve patients (Table 1).

Table 1: Comparison of selected cytokine and chemokine profiles in HIV positive and HIV negative patients with acute appendicitis.

Cytokine	HIV-ve Geometric mean (95% CI)	HIV+ve Geometric mean (95% CI)	Effect of HIV*	p-value
PF pro-inflammatory cytokines				
IL-1β	449.14 (8.76-889.52)	511.63 (-251.05-1274.33)	↑1.14	0.879
IL-7	8.56 (6.29-10.82)	6.91 (3.50-10.31)	↓0.81	0.450
IL-8	1869.12 (457.58-3280.66)	6695.57 (-1069.04-14460.20)	↑3.58	0.059
IL-9	100.41 (74.04-126.78)	52.83 (30.32-75.33)	↓ 0.53	0.013
IL-12	42.39 (24.71-60.07)	11.13 (3.43-18.83)	↓ 0.26	0.002
IL-15	140.03 (108.83-171.22)	337.51 (207.60-467.41)	↑ 2.41	<0.001
IL-17	118.64 (82.08-155.20)	104.70 (52.97-156.42)	↓ 0.88	0.667
GM-CSF	89.91 (47.86-131.96)	11.88 (2.65-21.10)	↓ 0.13	<0.001
IFN-γ	70.29 (45.26-95.32)	47.72 (20.25-75.19)	↓0.68	0.256
IP-10	1006.29 (308.05-1704.52)	896.17 (-167.09-1959.45)	↓0.89	0.869
TNF-α	219.48 (146.44-292.51)	356.88 (170.48-543.29)	↓1.62	0.115
Serum pro-inflammatory cytokines				
IL-1β	9.68 (6.29-13.06)	29.96 (11.11-48.81)	↑ 3.09	0.003
IL-7	10.25 (8.59-11.92)	16.57 (11.92-21.22)	↑ 1.62	0.005
IL-8	263.89 (103.59-424.20)	1091.59 (-218.93-2402.12)	↑ 4.14	0.042
IL-9	162.09 (143.55-180.62)	199.50 (158.14-240.85)	↑ 1.23	0.089
IL-12	29.16 (19.92-38.41)	72.97 (32.58-113.36)	↑ 2.50	0.006
IL-15	66.66 (54.36-78.97)	84.10 (59.87-108.34)	↑1.26	0.189
IL-17	89.76 (74.38-105.13)	156.95 (108.65-205.25)	↑ 1.75	0.003
GM-CSF	35.15 (20.43-49.86)	119.04 (30.75-207.33)	↑ 3.39	0.006
IFN-γ	43.78 (35.77-51.78)	79.49 (53.32-105.65)	↑ 1.82	0.003
IP-10	667.5933 (558.20-776.97)	1464.01 (1033.69-1894.33)	↑ 2.19	<0.001
TNF-α	105.05 (91.25-118.84)	120.67 (92.77-148.56)	↑ 1.15	0.309
PF anti-inflammatory cytokines				
IL-4	4.83 (3.52-6.13)	5.21 (2.99-7.44)	↑1.08	0.758
IL-5	10.12 (4.83-15.41)	92.05 (15.36-168.75)	↑ 9.09	<0.001
IL-6	1189.90 (-0.25-2380.05)	1053.55 (-532.44-2639.54)	↓0.89	0.889
Serum anti-inflammatory cytokines				
IL-4	5.31 (4.81-5.82)	7.33 (6.07-8.59)	↑ 1.38	0.004
IL-5	10.55 (5.44-15.66)	12.86 (-6.26-31.99)	↑1.22	0.804
IL-6	38.99 (21.51-56.46)	297.94 (65.91-529.97)	↑ 7.64	<0.001
PF chemokines				
Eotaxin	48.08 (27.02-69.15)	48.80 (14.57-83.02)	↔1.01	0.972
MCP-1	356.86 (218.92-494.79)	419.82 (158.14-681.51)	↑1.18	0.659
MIP-1β	604.77 (334.93-874.61)	1765.88 (532.55-2999.20)	↑ 2.91	0.012
Serum chemokines				
Eotaxin	77.76 (67.30-88.22)	116.90 (87.74-146.07)	↑ 1.50	0.006
MCP-1	116.88 (85.85-147.92)	300.56 (155.91-445.20)	↑ 2.57	0.001
MIP-1β	1097.06 (833.60-1360.52)	820.64 (473.70-1167.58)	↓ 0.75	0.246

PF= peritoneal fluid, IL = interleukin, GM-CSF = granulocyte-macrophage colony stimulating factor, IFN = interferon, IP = Interferon inducible protein, MCP = monocyte chemoattractant protein, MIP = macrophage inflammatory protein, TNF = tumor necrosis factor

*= ratio of HIV+ve and HIV-ve patients cytokine levels, ↑ = increase, ↓ = decrease

Fig. 1 shows cytokine levels that were differentially greater in the PF of HIV-positive patients with complicated appendicitis. Serum levels did not show this clear differential cytokine production, except for IL-15, where serum levels were also differentially raised in HIV-positive complicated appendicitis, and MIP-1β, which was raised in serum of HIV-positive patients with simple appendicitis.

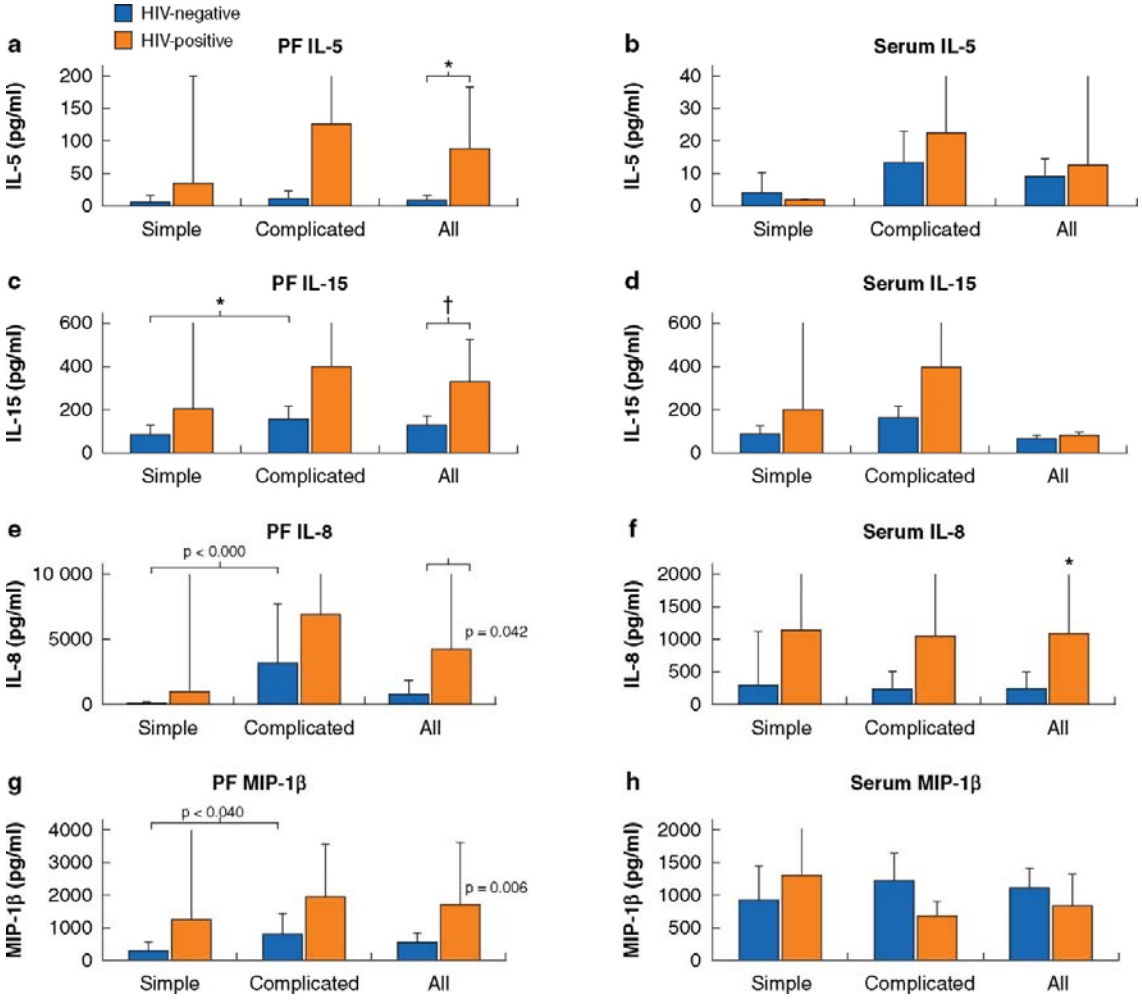

Figure 1. Selected cytokine/chemokine levels in peritoneal fluid and serum where peritoneal fluid levels were more raised in human immunodeficiency virus-infected patients with complicated appendicitis

a Peritoneal fluid (PF) interleukin (IL) 5 (*P < 0.001, *t* test); **b** serum IL-5; **c** PF IL-15 (*P = 0.026, †P < 0.001, *t* test); **d** serum IL-15; **e** PF IL-8 (*P < 0.001, †P = 0.042, *t* test); **f** serum IL-8 (*P = 0.035, *t* test); **g** PF macrophage inflammatory protein (MIP) 1β (*P = 0.040, †P = 0.006, *t* test); **h** serum MIP-1β in human immunodeficiency virus (HIV)-negative and HIV-positive patients, overall and in simple and complicated appendicitis. Values are mean (95 per c.i.).

Fig. 2 shows cytokine levels that were differentially more raised in PF but not in serum of HIV-negative patients with complicated appendicitis.

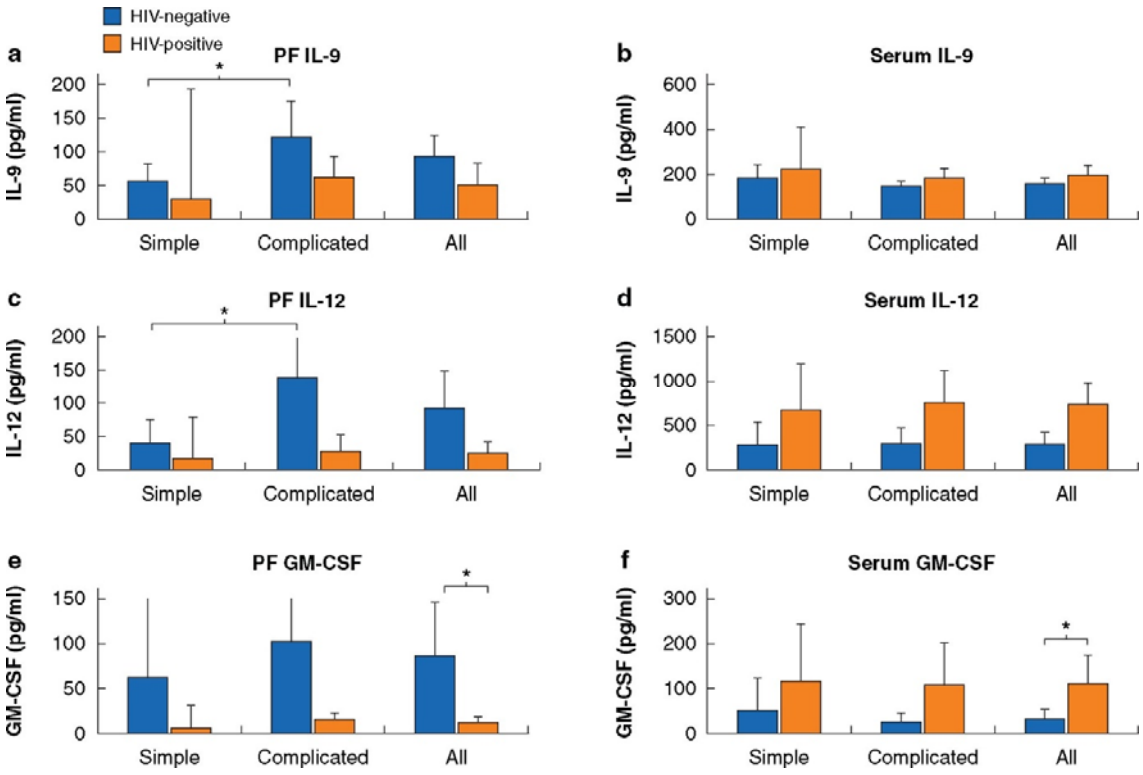

Figure 2. Selected cytokine/chemokine levels in peritoneal fluid and serum where peritoneal fluid levels were more raised in human immunodeficiency virus-uninfected patients

a Peritoneal fluid (PF) interleukin (IL) 9 (**P* = 0.009, *t* test); **b** serum IL-9; **c** PF IL-12 (**P* = 0.026, *t* test); **d** serum IL-12; **e** PF granulocyte–macrophage colony-stimulating factor (GM-CSF) (**P* < 0.001, *t* test); **f** serum GM-CSF (**P* = 0.026, *t* test) in human immunodeficiency virus (HIV)-negative and HIV-positive patients, overall and in simple and complicated appendicitis. Values are mean (95 per c.i.).

Fig. 3 shows cytokine levels that were differentially raised in PF but not in serum of both HIV-positive and HIV-negative patients with complicated appendicitis, except for IL-6, interferon (IFN) γ, and monocyte chemoattractant protein 1, which were also raised in serum of HIV-positive patients with complicated appendicitis. Serum IL-1β and IL-17 levels were raised in HIV-positive patients with simple appendicitis.

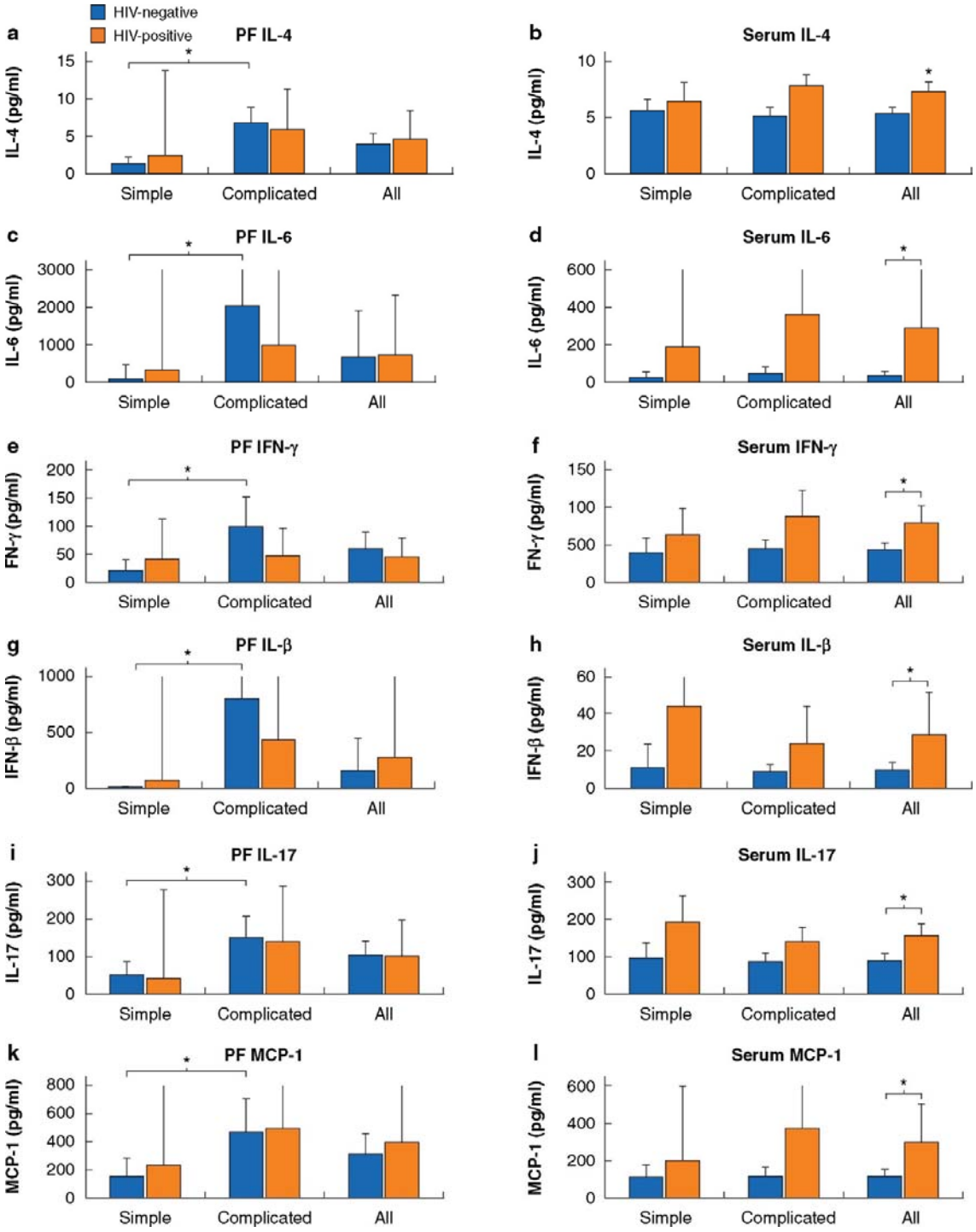

Figure 3. Selected cytokine/chemokine levels in peritoneal fluid and serum where peritoneal fluid levels were raised to a similar extent in human immunodeficiency virus-infected and -uninfected patients

a Peritoneal fluid (PF) interleukin (IL) 4 (*P < 0.001, *t* test); **b** serum IL-4 (*P = 0.005, *t* test); **c** PF IL-6 (*P = 0.004, *t* test); **d** serum IL-6 (*P < 0.001, *t* test); **e** PF interferon (IFN) γ (*P < 0.001, *t* test); **f** serum IFN-γ (*P = 0.002, *t* test); **g** PF IL-1β (*P < 0.001, *t* test); **h** serum IL-1β (*P < 0.001, *t* test); **i** PF IL-17 (*P < 0.001, *t* test); **j** serum IL-17 (*P = 0.004, *t* test); **k** PF monocyte chemoattractant protein (MCP) 1 (*P = 0.003, *t* test); **l** serum MCP-1 (*P = 0.002, *t* test) in human immunodeficiency virus (HIV)-negative and HIV-positive patients, overall and in simple and complicated appendicitis. Values are mean (95 per c.i.).

Discussion

The female preponderance found in HIV+ve patients in this study reflects the reported HIV infection rate of 20% in women of reproductive age compared to 9.9% in males in South Africa.[10] Perforated appendicitis rate was 4 times higher in HIV+ve patients (42.9% vs 10.5%). Higher perforation rates have been previously reported for similar groups.[11,12]

HIV infection results in chronic immune activation with dysregulation of cytokine response.[13] Notably, HIV infection results in a decrease in the expression of T-helper (Th)-1 cytokines including IFN-γ, IL-2 and IL-12 and upregulation in Th-2 cytokines, IL-4 and IL-10 which contribute towards down-regulation of Th-1 inflammatory responses.[13] The present study has confirmed some of these changes, principally in PF. These include an increase in PF levels of IL-15.

Higher concentrations of MIP-1β and IL-8 observed in HIV+ve patients' PF suggest heightened regional response of these chemoattractants in HIV+ve patients who had more complicated appendicitis.[4,6] This in turn could result in excessive infiltration of neutrophils and monocytes/macrophages which could lead to more severe collateral tissue damage. Increased PF IL-8 levels have been reported in complicated appendicitis, as have elevated serum IL-8 levels with advanced HIV infection.[6,9] In the current study IL-8 was differentially increased in PF of HIV+ve patients with complicated appendicitis compared to similar elevation in serum of both simple and complicated appendicitis in the HIV+ve patients. In contrast PF granulocyte-macrophage colony-stimulating factor (GM-CSF) levels were significantly lower in the HIV+ve group compared to the raised serum levels of these patients. This would tend to result in less mobilization of neutrophils, macrophages and other cells to the area of infection with deleterious effect.[6]

PF IL-5 levels were elevated in the HIV+ve patients and greater in complicated appendicitis. IL-5 plays a role in recruitment and activation of eosinophils, an important cell type in the immune response in the gut mucosa.[14]

IL-12 is critical in the enhancement of natural killer (NK) cells and Th-1 cell functions. Elevated serum IL-12, IFN- γ, IL-6 and IL-8 levels have previously been reported in patients with perforated appendicitis[4,6] and these were even more elevated in the HIV+ve patients in the current study indicating a heightened pro-inflammatory response.[7] Suppression of IL-12 in HIV infection has been documented,[13] therefore the decrease in PF IL-12 in HIV+ve patients even with complicated appendicitis is to be expected.

The increased serum IL-6 is consistent with previous reports in both severe appendicitis and HIV infection.[9,15] Interestingly the increase in the level of IL-6 in PF was more in HIV-ve patients with complicated appendicitis, while in serum it was greater in HIV+ve patients and more so with complicated appendicitis. This dichotomy could be due to high baseline levels of serum IL-6 in HIV+ve patients, as previously reported.[9,15] IL-6 has both pro- and anti-

inflammatory activities.[16,17] It is anti-inflammatory in chronic infections and inflammatory states such as HIV infection.[18]

IL-17 induces other pro-inflammatory cytokines e.g. IL-6, granulocyte-colony stimulating factor (G-CSF), GM-CSF and IL-8.[7,19] PF IL-17 was more raised in complicated appendicitis and to a similar extent in both HIV+ve and HIV-ve patients. It can be speculated that this resulted in the exaggerated increase of the pro-inflammatory cytokines leading to the complications.

Increased serum IL-4 levels have been documented in appendicitis and in HIV infection.[3,7,9,13] It's anti-inflammatory activity may play a deleterious role leading to disease progression in HIV+ve individuals.

In summary, there is a complex interplay of cytokine production in HIV+ve patients that tends towards a more pro-inflammatory cytokine profile compared to HIV-ve patients. There was accompanying increase of anti-inflammatory cytokines, IL-4, IL-5, which would tend to dampen the pro-inflammatory cytokine avalanche. This was associated with more perforated appendicitis. This outcome may indicate the dual effect of cytokines in septic patients.[20] The baseline cytokine release in HIV infection renders these unreliable as diagnostic or prognostic biomarkers for acute appendicitis. A larger study with more HIV+ve patients is necessary to elucidate the role of cytokines as possible diagnostic or prognostic biomarkers.

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