**Adrenal insufficiency associated with advanced HIV may explain the high mortality.**

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**Abstract:**

**Background:**

Sub-Saharan Africa is burdened by a vast HIV-positive population, at risk of life-threatening AIDS defining illnesses. AIDS defining illnesses and disseminated opportunistic infections have been associated with both primary adrenal insufficiency (PAI) and secondary adrenal insufficiency (SAI). We hypothesized that hypoadrenalism may partially account for the high mortality seen with advanced HIV.

**Materials & Methods:**

We undertook a prospective case-finding study of HIV-positive patients presenting with advanced disease who were 18 years or older, with a CD4 count of less than 100 cells/ mm3, and a coexistent opportunistic infection. Exclusion criteria were use of oral, intravenous or inhaled steroids in the previous 3 months. A tetracosactide test was performed in patients with random cortisol concentrations in the morning of less than 500 nmol/L.

**Results:**

**Conclusion:**

**Introduction:**

Adrenal insufficiency is an invariably fatal medical condition, caused by deficiency of glucocorticoids and mineralocorticoids without treatment. Prior to the availability of glucocorticoid therapy, the majority of the primary adrenal insufficiency patients died within 2 years of diagnosis (1). Autoimmune adrenal insufficiency predominates in European populations, (2) and in South Africa, despite the high background prevalence of tuberculosis (TB) (3). Our recent survey suggests that coexistent infections are a significant cause of primary adrenal insufficiency in sub-Saharan Africa with an estimated prevalence of 34% and 29.8% for TB and Acquired Immune Deficiency Syndrome (AIDS), respectively (4).

Patients with HIV may suffer from primary adrenal insufficiency due to tuberculosis, mycobacterium avium Intracellulare (MAI), CMV, toxoplasmosis, remove cyst this Carini histoplasmosis, malignancies such as non-Hodkings lymphoma and Kaposi sarcoma KS, fungal infections such as cryptococcus, blastomycosis, and histoplasmosis. Secondary adrenal insufficiency may be caused by toxoplasmosis and cytomegalovirus infection of the Pituitary gland. Eldrisi & Verghese.

There are varied results describing the prevalence of hypoadrenalism in patients with HIV. In a Pakistan study by Afreen *et al*, of 64 HIV infected, predominantly male patients (84.9%), adrenal insufficiency (AI) was reported in 9 (14.0%), using the of 250 microgram intravenous tetracosactide test and a 60 minutes cortisol concentration of less than 18 microgram/dl (500 nmol/L),(8) whereas in a Nigerian study of 43 newly diagnosed HIV positive patients who had not been initiated on antiretroviral treatment, reported the AI incidence to be 34.8%, using a 1 microgram tetracosactide test (9) and a 30minute cortisol of less than 500 nmol/L.

There is a high background prevalence of 9% (10) of Human immune virus (HIV) infection (7) in sub-Saharan Africa, which is associated with significant mortality estimated in 2001 to be 2.2 million of 3 million deaths worldwide (12). Compared to Europe and North America, early mortality in HIV positive patients is higher in resource limited settings, including South Africa, (12) due to late presentation and inadequate health resources. Although undiagnosed adrenal insufficiency is associated with invariable mortality (13), these patients receiving treatment retain poorer survival due in part to cardiovascular, malignant and infectious diseases, compared to background populations (14) (15). It has also been established that both overtreatment and insufficient replacement with glucocorticoids during infections and stress-related events may also confer a higher mortality. (Reference)

Since adrenal insufficiency is one of the endocrine complications of HIV , we hypothesized that it may also be one of the additional causes of mortality mortality. Moreover, we considered that initiation of appropriate replacement therapy for adrenal insufficiency may confer a positive impact on mortality. Our objectives were to determine the incidence of hypoadrenalism among ill, hospitalized HIV-infected patients. In addition, we wished to explore the positive predictive clinical and biochemical characteristics for adrenal insufficiency, and the predictors for survival.

**Materials and methods:**

Permission to conduct the study (HREC 163/2015) was obtained from the University of Cape Town Faculty of Health Sciences, Human Research and Ethics Committee, which endorses the latest Helsinki declaration of 2013. We undertook a prospective case-finding of HIV positive patients, presenting with advanced disease and an opportunistic infection to an acute tertiary care medical ward. All the participants provided written informed consent. If participants were affected by delirium the research and ethics committee permitted us to take retrospective consent, with the view of limiting bias and providing an opportunity for all patients to receive potentially life-saving treatment.

*Inclusion and exclusion criteria*

Inclusion criteria included age 18 years and older, and a CD4 count of 100 cells per mm3 or fewer and an opportunistic infection. The use of oral or inhaled steroids in the previous three months represented an exclusion criterion.

*Data extraction*

Demographic and clinical data were obtained from history and physical examination. Records of routine biochemistry, haematology and microbiology were extracted from the National Health Laboratory Service (NHLS) repository. Patients who met the inclusion criteria had blood samples taken for serum cortisol and plasma ACTH between 08:00 and 09:00 on the day of enrolment. Where the random serum cortisol was less than 500 nmol/L, a short 250 µg intravenous tetracosactide stimulation test was performed, usually on the following day.

*Biochemical measures of plasma ACTH and serum cortisol*

For the random plasma ACTH (and cortisol, clotted blood and plasma were submitted to Lancet laboratory which analyzed the samples on the Cobas immunoassay platform.

*The tetracosactide test*

The samples for plasma ACTH and cortisol were submitted to the National Health Laboratory Services (NHLS). The method employed by this laboratory is the Roche (Modular Analytics E170). Blood was taken at 0 minutes for serum cortisol, followed by 250 µg of tetracosactide administered intravenously and a 30-minute serum cortisol was taken at conclusion of the test. Adrenal insufficiency was excluded if a 30-minute serum cortisol exceeded 500 nmol/L. In patients with confirmed cortisol deficiency, a plasma ACTH which is twofold the upper limit of the reference range ( 67.7 pg/mL) is consistent with primary adrenal insufficiency, (Bornstein et al 2016) while low or normal ACTH is consistent with secondary adrenal insufficiency. ( Pazderka et al 2017)

*Determination of survival*

Telephonic follow-up was done to determine post discharge survival via direct patient or family contact. A hospital database (Clinicom®) also corroborated survival, by virtue of their attendance at various clinics in the drainage area of our tertiary hospital facility.

**Statistical analysis:**

Categorical variables were presented as frequencies and percentages, and continuous variables were expressed as medians and were compared using the Wilcoxon-Mann-Whitney test. Proportions and categorical variables were compared, using Pearson’s chi-square test or Fisher’s exact test as appropriate. Univariate logistic regression was used to estimate the association between AI and various risk factors. Multivariable logistic regression analysis was used to identify independent predictors of AI. We ranked CD4 counts into tertiles of 0-33; 34-66 and 67-100, respectively to determine if AI was associated. Statistical analyses were performed using STATA Statistical Software version 15 (StataCorp, College Station, Tx, USA, 2017).The significance level was set at *p*<0.005. For analysis of survival data, we described those who were discharged from the hospital within 3 months (early survivors), followed by 6 months (intermediate survivors), and 12 months follow-up (late survivors).

**Results: Awaiting the analysis of the final / complete 559 participant dataset**

A total of 533 patients were recruited, of whom 5 withdrew from participation and 528 participants were included in the final analysis. H. The median age at enrollment was 36 years [interquartile range (IQR) 31.0-42.0) years and when subdivided by gender median age for males was…. (insert interquartile range) females ….( Insert interquartile range) The cohort comprised mainly black Africans 421 (79%), Mixed ancestry in 105 (20%), and White 2 (0.4%) participants. The median (IQR) duration of the presenting illness was 14 (14-21) days.

**Clinical characteristics**

*Table 1.1: Patient presentation by CD4 count category*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable |  | **0 - 33**, N = 2711 | **34 - 66**, N = 1521 | **67 - 100**, N = 1191 | **p-value**2 |
| **Age at enrolment, median (IQR) (years)** |  | **35.0** (30.5, 42.0) | 37.0 (32.0, 43.3) | 37.0 (33.0, 46.0) | **0.016** |
| **Gender, n(%)** |  |  |  |  | 0.3 |
| Female |  | 137 (50.6%) | 85 (55.9%) | 55 (46.6%) |  |
| Male |  | 134 (49.4%) | 67 (44.1%) | 63 (53.4%) |  |
| **Ethnicity, n(%)** |  |  |  |  | 0.3 |
| Black African |  | 220 (81.2%) | 117 (77.0%) | 89 (74.8%) |  |
| Other |  | 51 (18.8%) | 35 (23.0%) | 30 (25.2%) |  |
| **Duration of current illness, median (IQR) (days)** |  | 14.0 (14.0, 21.0) | **14.0** **(12.5, 22.5)** | 14.0 (7.0, 21.0) | **0.036** |
| **Weight loss** |  | 231 (87.5%) | 129 (87.2%) | 92 (82.9%) | 0.5 |
| **log10 viral load** |  | **11.6 (9.3, 12.8)** | 8.9 (6.5, 12.2) | 7.3 (3.9, 10.6) | **<0.001** |
| **Tuberculosis** |  | 230 (84.9%) | 131 (86.2%) | 98 (82.4%) | 0.7 |
| **Cryptococcus neoformans** |  | 16 (5.9%) | 5 (3.3%) | 9 (7.6%) | 0.3 |
| **Pneumonia** |  | 30 (11.1%) | 19 (12.5%) | 13 (10.9%) | 0.9 |
| **Staph aureus** |  | 1 (0.4%) | 0 (0.0%) | 0 (0.0%) | >0.9 |
| **Kaposis sarcoma** |  | 6 (2.2%) | 0 (0.0%) | 0 (0.0%) | **0.055** |
| **Cytomegalovirus** |  | 0 (0.0%) | 1 (0.7%) | 0 (0.0%) | 0.5 |
| **HSV** |  | 3 (1.1%) | 4 (2.6%) | 1 (0.8%) | 0.4 |
| **HepB** |  | 9 (3.3%) | 8 (5.3%) | 1 (0.8%) | 0.13 |
| **Candida** |  | 21 (7.7%) | 11 (7.2%) | 2 (1.7%) | 0.064 |
| **GE/c diff** |  | 12 (4.4%) | 7 (4.6%) | 4 (3.4%) | 0.9 |
| **Parvo B19** |  | 0 (0.0%) | 1 (0.7%) | 0 (0.0%) | 0.5 |
| **Syphilis** |  | 8 (3.0%) | 7 (4.6%) | 1 (0.8%) | 0.2 |
| **B menigitis** |  | 3 (1.1%) | 1 (0.7%) | 2 (1.7%) | 0.8 |
| **UTI / Leptospirosis** |  | 4 (1.5%) | 0 (0.0%) | 3 (2.5%) | 0.13 |
| **PCP** |  | 4 (1.5%) | 1 (0.7%) | 0 (0.0%) | 0.5 |
| **COVID-19** |  | 1 (0.4%) | 1 (0.7%) | 0 (0.0%) | >0.9 |
| **Neurocysticercosis** |  | 1 (0.4%) | 1 (0.7%) | 0 (0.0%) | >0.9 |
| **White cell count X109** |  | **5.0 (2.9, 7.4)** | 5.8 (4.2, 8.4) | 6.8 (4.5, 9.7) | **<0.001** |
| **Lymphocyte count X109** |  | 0**.6 (0.3, 1.3)** | 0.9 (0.5, 5.8) | 1.3 (0.8, 3.0) | **0.011** |
| **Neutrophils** |  | **2.4 (1.0, 5.9)** | 6.6 (2.8, 15.7) | 5.1 (2.9, 11.5) | **0.002** |
|  |
|  |

The majority of the patients were female. The duration of illness was 14 days and the major complaint was weight loss with suppressed CD4 counts, white cell counts, lymphocytes and neutrophils. Most of the patient had tuberculosis 84% followed by pneumonia 11%, then cryptococcus 5.7% and candida fungal infection 5.5%, GIT infection 4% and syphilis 3.2%.

Table 1.2: Baseline patient characteristics on admission

|  |  |
| --- | --- |
| Variable | **N = 549**1 |
| **Age at enrolment, median (IQR) (years)** | 36.0 (31.5, 43.0) |
| **Female gender, n (%)** | 280 (51.1%) |
| **Black African Ethnicity, n (%)** | 430 (78.3%) |
| **Duration of current illness, median (IQR) (days)** | 14.0 (12.0, 21.0) |
| **Weight loss** | 452 (86.1%) |
| **Viral load, log10 copies/ml** | 10.8 (7.3, 12.3) |
| **Tuberculosis** | 461 (84.0%) |
| **Cryptococcus neoformans** | 30 (5.5%) |
| **Pneumonia** | 62 (11.3%) |
| **Staph aureus** | 1 (0.2%) |
| **Kaposis sarcoma** | 6 (1.1%) |
| **Cytomegalovirus** | 1 (0.2%) |
| **HSV** | 8 (1.5%) |
| **HepB** | 18 (3.3%) |
| **Candida** | 34 (6.2%) |
| **GE/c diff** | 23 (4.2%) |
| **Parvo B19** | 1 (0.2%) |
| **Syphilis** | 17 (3.1%) |
| **B menigitis** | 6 (1.1%) |
| **UTI / Leptospirosis** | 7 (1.3%) |
| **PCP** | 5 (0.9%) |
| **COVID-19** | 2 (0.4%) |
| **Neurocysticercosis** | 2 (0.4%) |
| **Total CD4 count** | 33.5 (15.0, 62.0) |
| **White cell count x109** | 5.5 (3.7, 8.2) |
| **Lymphocyte count x109** | 0.8 (0.4, 1.9) |
| **Neutrophils** | 3.6 (1.5, 8.3) |
| **ART exposure** | 207 (37.7%) |
| **Kidney medication** | 52 (9.5%) |

*1* Median (IQR); n (%)

|  |  |
| --- | --- |
|  |  |
| **Table 1.2: Baseline patient characteristics on admission (Remake as 549)** |  |
| Variable | **N = 528** |
| **Age at enrolment, median (IQR) (years)** | 36.0 (31.0, 43.0) |
| **Gender, n (%)** |  |
| Female | 269 (50.9%) |
| Male | 256 (48.5%) |
| Unknown | 3 (0.6%) |
| **Ethnicity, n (%)** |  |
| Black African | 421 (79.7%) |
| Mixed race | 105 (19.9%) |
| Duration of current illness, median (IQR) (days) | 14.0 (12.0, 21.0) |
| Weight loss | 435 (82.4%) |
| **Serologic severity of HIV** |  |
| log10 viral load (Log10 Copies/ml) | 10.7 (7.4, 12.3) |
| Total CD4 count (Cells per mm3) | 33.0 (15.0, 61.0) |
| **Opportunist infections**  Tuberculosis | 445 (84.3%) |
| Cryptococcus neoformans | 30 (5.7%) |
| Pneumonia | 62 (11.7%) |
| Staph aureus | 1 (0.2%) |
| Kaposi sarcoma | 6 (1.1%) |
| Cytomegalovirus | 1 (0.2%) |
| Herpes simplex virus (HSV) | 8 (1.5%) |
| HBV | 18 (3.4%) |
| Candida | 29 (5.5%) |
| Gastroenteristis | 18 (3.4%) |
| Parvo B19 | 1 (0.2%) |
| Syphilis | 17 (3.2%) |
| B meningitis | 5 (0.9%) |
| Urinary tract infection | 7 (1.3%) |
| Pneumoctis Jirovechiae Pneumonia | 4 (0.8%) |
| COVID-19 | 2 (0.4%) |
| Neurocysticercosis | 2 (0.4%) |
| **Isolated hematological parameters** |  |
| White cell count X109 | 5.4 (3.6, 8.1) |
| Lymphocyte count X 109 | 0.8 (0.4, 1.8) |
| Neutrophils x 109 | 3.2 (1.5, 8.3) |

Table 1.3: age

When subdividing the cohorts by gender, female patients were younger than males *p*=0.017. There was no statistically significant distribution in the viral loads, CD4 counts and opportunistic infections when comparing males and females.

***Table 2: Distribution of patient characteristics by ARV therapy status***

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | **No**, N = 3411 | **Yes**, N = 2071 | **p-value**2 |
| **Age at enrolment** | 36.0 (31.0, 43.0) | 36.0 (32.0, 43.0) | 0.7 |
| **Male gender, n(%)** | 172 (50.4%) | 96 (46.4%) | 0.4 |
| **Black African Ethnicity, n(%)** | 258 (75.7%) | 172 (83.1%) | **0.040** |
| **Duration of current illness, median (IQR) (days)** | 14.0 (12.0, 21.0) | 14.0 (10.0, 21.0) | 0.6 |
| **Weight loss** | 279 (86.6%) | 172 (85.1%) | 0.6 |
| **Viral load;** *log10 copies/ml* | 10.6 (6.2, 12.3) | 10.8 (7.8, 12.3) | 0.5 |
| **Tuberculosis** | 282 (82.7%) | 179 (86.5%) | 0.2 |
| **Cryptococcus neoformans** | 24 (7.0%) | 6 (2.9%) | **0.039** |
| **Pneumonia** | 38 (11.1%) | 24 (11.6%) | 0.9 |
| **Staph aureus** | 1 (0.3%) | 0 (0.0%) | >0.9 |
| **Kaposis sarcoma** | 3 (0.9%) | 3 (1.4%) | 0.7 |
| **Cytomegalovirus** | 1 (0.3%) | 0 (0.0%) | >0.9 |
| **HSV** | 3 (0.9%) | 5 (2.4%) | 0.2 |
| **HepB** | 10 (2.9%) | 8 (3.9%) | 0.6 |
| **Candida** | 23 (6.7%) | 11 (5.3%) | 0.5 |
| **GE/c diff** | 11 (3.2%) | 12 (5.8%) | 0.15 |
| **Parvo B19** | 0 (0.0%) | 1 (0.5%) | 0.4 |
| **Syphilis** | 14 (4.1%) | 3 (1.4%) | 0.082 |
| **B menigitis** | 4 (1.2%) | 2 (1.0%) | >0.9 |
| **UTI / Leptospirosis** | 4 (1.2%) | 3 (1.4%) | >0.9 |
| **PCP** | 5 (1.5%) | 0 (0.0%) | 0.2 |
| **COVID-19** | 2 (0.6%) | 0 (0.0%) | 0.5 |
| **Neurocysticercosis** | 0 (0.0%) | 2 (1.0%) | 0.14 |
| **Total CD4 count** | 32.5 (13.3, 61.8) | 34.0 (16.0, 61.5) | 0.8 |
| **White cell count x109** | 5.2 (3.4, 7.6) | 5.8 (4.0, 8.9) | 0.076 |
| **Lymphocyte count x109** | 0.9 (0.4, 3.9) | 0.7 (0.4, 1.3) | 0.2 |
| **Neutrophils** | 3.7 (1.5, 8.3) | 3.3 (1.7, 7.4) | 0.8 |
| **Kidney medication** | 30 (8.8%) | 22 (10.6%) | 0.5 |

1Median (IQR); n (%); 2Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

**Comparison of patients with advanced HIV on admission by tertiles.**

When comparing patient characteristics of CD4 counts by tertiles, patients with the lowest CD4 count, were younger at enrolment *p*=0.013 As expected, the participants with lowest CD4 count had the highest viral load; *p*=0.001. In concert with the lowest CD4 count, the white cell, neutrophil and lymphocytes were also lower than the remaining tertiles. The only significant opportunistic infections by tertiles were candida which was more dominant in the youngest age group and Kaposi sarcoma which was more pronounced in the mid-tertile group

**Table 3: Should compare Total AI with Non-AI patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | Non-AI vs AI | | | | | PAI vs SAI patients | | |
| no-AI, N = 5221 | | AI, N = 271 | | p-value2 | SAI, N = 201 | PAI, N = 71 | p-value2 |
| Age at enrolment, median (IQR) (years) | 36.0 (31.8,43.0) | | 36.0 (32.0,47.5) | | 0.4 | 36.0 (32.0, 48.5) | 40.0 (35.0, 45.0) | 0.6 |
| Gender, n(%) | | |  | | >0.9 |  |  | 0.7 |
| Female | 266 (51.1%) | | 14 (51.9%) | | | 11 (55.0%) | 3 (42.9%) |  |
| Male | 255 (48.9%) | | 13 (48.1%) | | | 9 (45.0%) | 4 (57.1%) |  |
| Ethnicity, n(%) | | |  | | 0.4 |  |  | >0.9 |
| Black African | 407 (78.0%) | | 23 (85.2%) | | | 17 (85.0%) | 6 (85.7%) |  |
| Other | 115 (22.0%) | | 4 (14.8%) | |  | 3 (15.0%) | 1 (14.3%) |  |
| Duration of current illness, median (IQR) (days) | 14.0 (11.8, 21.0) | | 14.0 (14.0, 27.8) | | 0.4 | 21.0 (14.0, 30.0) | 10.0 (7.0, 12.5) | 0.019 |
| Weight loss | 430 (86.3%) | | 22 (81.5%) | | 0.4 | 15 (75.0%) | 7 (100.0%) | 0.3 |
| Viral load (log10 Copies/mL) | 10.8 (7.1, 12.3) | | 11.0 (10.7, 11.3) | | 0.9 | 10.5 (10.5, 10.5) | 11.6 (11.6, 11.6) | >0.9 |
| Tuberculosis | 440 (84.3%) | | 21 (77.8%) | | 0.4 | 16 (80.0%) | 5 (71.4%) | 0.6 |
| Cryptococcus neoformans | 20 (3.8%) | | 10 (37.0%) | | <0.001 | 7 (35.0%) | 3 (42.9%) | >0.9 |
| Pneumonia | 59 (11.3%) | | 3 (11.1%) | | >0.9 | 3 (15.0%) | 0 (0.0%) | 0.5 |
| Staph aureus | 0 (0.0%) | | 1 (3.7%) | | 0.049 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| Kaposis sarcoma | 5 (1.0%) | | 1 (3.7%) | | 0.3 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| Cytomegalovirus | 0 (0.0%) | | 1 (3.7%) | | 0.049 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| HSV | 7 (1.3%) | | 1 (3.7%) | | 0.3 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| HepB | 16 (3.1%) | | 2 (7.4%) | | 0.2 | 2 (10.0%) | 0 (0.0%) | >0.9 |
| Candida | 33 (6.3%) | | 1 (3.7%) | | >0.9 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| GE/c diff | 23 (4.4%) | | 0 (0.0%) | | 0.6 | 0 (0.0%) | 0 (0.0%) |  |
| Parvo B19 | 0 (0.0%) | | 1 (3.7%) | | 0.049 | 0 (0.0%) | 1 (14.3%) | 0.3 |
| Total CD4 count | 34.0 (15.0, 63.0) | | 28.0 (13.5, 49.5) | | 0.4 | 19.0 (10.0, 48.8) | 46.0 (25.5, 61.0) | 0.2 |
| White cell count X109 | 5.6 (3.8, 8.1) | | 5.2 (2.8, 8.9) | | 0.5 | 5.3 (2.8, 11.9) | 3.5 (3.0, 5.5) | 0.4 |
| Lymphocyte count X109 | 0.8 (0.4, 2.0) | | 0.9 (0.5, 1.2) | | 0.7 | 0.7 (0.5, 1.0) | 1.3 (1.3, 1.3) | 0.6 |
| Neutrophils | 3.8 (1.6, 8.9) | | 1.3 (0.9, 1.6) | | 0.037 | 1.5 (1.0, 1.7) | 0.9 (0.9, 0.9) | 0.7 |
| Sodium mmol/L | 133.0 (130.0, 137.0) | | 135.0 (133.0, 137.5) | | 0.033 | 135.5 (134.0, 137.3) | 133.0 (131.5, 136.5) | 0.2 |
| Potassium mmol/L | 4.1 (3.6, 4.6) | | 3.9 (3.3, 4.3) | | 0.069 | 4.0 (3.3, 4.5) | 3.6 (3.3, 3.9) | 0.4 |
| Haemoglobin g/dL | 8.7 (7.4, 10.3) | | 8.7 (7.6, 10.4) | | 0.6 | 8.7 (7.6, 10.7) | 10.2 (7.9, 10.3) | 0.7 |
| BP (systolic) | 111.0 (102.0, 125.0) | | 120.0 (102.5, 128.5) | | 0.4 | 118.0 (99.5, 129.3) | 120.0 (120.0, 123.5) | 0.7 |
| BP (diastolic) | 70.0 (60.0, 79.0) | | 71.0 (66.5, 80.0) | | 0.080 | 72.0 (60.0, 80.0) | 70.0 (70.0, 82.0) | 0.8 |
| Heart rate | 91.0 (79.0, 108.8) | | 87.0 (78.5, 107.0) | | 0.7 | 85.0 (76.8, 102.3) | 97.0 (88.0, 111.0) | 0.10 |
| Hypotension | 46 (9.3%) | | 1 (3.7%) | | 0.5 | 1 (5.0%) | 0 (0.0%) | >0.9 |
| Weakness | 422 (85.1%) | | 21 (77.8%) | | 0.3 | 17 (85.0%) | 4 (57.1%) | 0.3 |
| Tiredness | 423 (85.1%) | | 23 (85.2%) | | >0.9 | 18 (90.0%) | 5 (71.4%) | 0.3 |
| Poor appetite | 374 (75.9%) | | 22 (81.5%) | | 0.5 | 16 (80.0%) | 6 (85.7%) | >0.9 |
| Increased pigmentation of the skin | 247 (50.8%) | | 9 (37.5%) | | 0.2 | 6 (31.6%) | 3 (60.0%) | 0.3 |
| Nausea | 263 (53.0%) | | 16 (59.3%) | | 0.5 | 11 (55.0%) | 5 (71.4%) | 0.7 |
| Vomiting | 137 (27.7%) | | 8 (29.6%) | | 0.8 | 4 (20.0%) | 4 (57.1%) | 0.14 |
| Liking for salt | 262 (53.0%) | | 19 (70.4%) | | 0.078 | 14 (70.0%) | 5 (71.4%) | >0.9 |
| Hypoglycaemia | 11 (2.2%) | | 0 (0.0%) | | >0.9 | 0 (0.0%) | 0 (0.0%) |  |
| Loss of consciousness | 7 (1.4%) | | 0 (0.0%) | | >0.9 | 0 (0.0%) | 0 (0.0%) |  |
| Diarrhoea | 219 (44.5%) | | 8 (29.6%) | | 0.13 | 7 (35.0%) | 1 (14.3%) | 0.6 |
| Dizziness | 236 (47.8%) | | 13 (50.0%) | | 0.8 | 9 (45.0%) | 4 (66.7%) | 0.6 |
| Shock | 5 (1.0%) | | 0 (0.0%) | | >0.9 | 0 (0.0%) | 0 (0.0%) |  |
| Anorexia | 234 (47.3%) | | 8 (29.6%) | | 0.073 | 4 (20.0%) | 4 (57.1%) | 0.14 |
| Loss of axillary and pubic hair, if female | | 95 (18.9%) | | 3 (11.1%) | 0.5 | 2 (10.0%) | 1 (14.3%) | 0.8 |
| Any postural drop in blood pressure | 21 (4.3%) | | 2 (7.4%) | | 0.3 | 2 (10.0%) | 0 (0.0%) | >0.9 |
| Presence of anaemia | 290 (58.8%) | | 13 (50.0%) | | 0.4 | 9 (47.4%) | 4 (57.1%) | >0.9 |
| mortality | 60 (43.5%) | | 6 (50.0%) | | 0.7 | 4 (44.4%) | 2 (66.7%) | >0.9 |
| ART exposure | 201 (38.5%) | | 6 (22.2%) | | 0.089 | 4 (20.0%) | 2 (28.6%) | 0.6 |
| Kidney medication | 47 (9.0%) | | 5 (18.5%) | | 0.2 | 3 (15.0%) | 2 (28.6%) | 0.6 |

1Median (IQR); n (%); 2Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

A graph of a number of different colored squares

Description automatically generated with medium confidence

**Comparison of patients with adrenal insufficiency and those without**

There were no significant differences between the adrenal insufficiency (AI) and the non-AI groups in respect of age, gender, ethnicity, duration of presenting illness and clinical symptoms. As expected, there were differences in the random basal and stimulated cortisol between the AI and non-AI groups, with the former group demonstrating lower concentrations of all serum cortisol parameters; *p*<0.001. Patients with AI demonstrated lower potassium concentrations albeit that unexpectedly there was no difference between potassium of patients with primary versus secondary hypoadrenalism, and lower neutrophil counts that the non-AI group.

**Mortality analysis among the AI and Non-AI patients**

Mortality at three months follow up was 5% versus 11.5% for the non-AI vectors to AI. At six months of follow-up patients with AI demonstrated a higher mortality of 19.2% compared with 6.8% in the non-AI group; *p*=0.045. At 12 months of follow-up the mortality was 19.2% for the AI versus 8.5% for non-AI groups; *p*-value versus the overall 19.2% for the AI group. The majority of the mortality was accounted for by tuberculosis in both groups of 83% and 80% for the AI and non-AI groups, respectively. Cryptococcal infection appeared to contribute to a higher mortality in the AI compared with the non-AI groups 50% versus 5.2% Non-AI, respectively; *p*=0.009.

**Table 3a: Comparison of Mortality among Non-AI vs AI patients**

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | **1**, N = 601 | **2**, N = 61 | **p-value**2 |
| **Age at enrolment, median (IQR) (years)** | 38 (33, 44) | 41 (33, 46) | 0.6 |
| **Female gender, n(%)** | 32 (53.3%) | 3 (50.0%) | >0.9 |
| **Black African ethnicity, n (%)** | 52 (86.7%) | 4 (66.7%) | 0.2 |
| **Duration of current illness, median (IQR) (days)** | 14 (14, 21) | 14 (11, 14) | **0.079** |
| **Random cortisol** | 477 (368, 615) | 307 (262, 336) | 0.004 |
| **Basal cortisol** | 500 (433, 636) | 308 (246, 365) | <0.001 |
| **Stimulated cortisol** | 795 (662, 890) | 375 (338, 424) | <0.001 |
| **Weight loss** | 51 (87.9%) | 4 (66.7%) | 0.2 |
| **Viral load (log10 Copies/mL)** | 11.74 (9.55, 13.08) | - |  |
| **Tuberculosis** | 49 (81.7%) | 5 (83.3%) | >0.9 |
| **Cryptococcus neoformans** | 3 (5.0%) | 3 (50.0%) | **0.008** |
| **Pneumonia** | 7 (11.7%) | 0 (0.0%) | >0.9 |
| **HSV** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **HepB** | 0 (0.0%) | 1 (16.7%) | **0.091** |
| **Candida** | 4 (6.7%) | 0 (0.0%) | >0.9 |
| **Syphilis** | 3 (5.0%) | 0 (0.0%) | >0.9 |
| **B menigitis** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **UTI / Leptospirosis** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **PCP** | 2 (3.3%) | 0 (0.0%) | >0.9 |
| **COVID-19** | 0 (0.0%) | 0 (0.0%) |  |
| **Neurocysticercosis** | 0 (0.0%) | 0 (0.0%) |  |
| **Total CD4 count** | 28 (14, 55) | 30 (16, 45) | 0.7 |
| **White cell count x109** | 5.2 (3.4, 7.4) | 8.3 (4.3, 27.5) | 0.2 |
| **Sodium mmol/L** | 133.0 (128.0, 136.0) | 136.5 (133.3, 139.0) | 0.15 |
| **Potassium mmol/L** | 3.95 (3.60, 4.57) | 3.70 (3.37, 3.88) | **0.077** |
| **Haemoglobin g/dL** | 8.20 (7.00, 9.63) | 9.60 (8.25, 10.95) | 0.3 |
| **BP (systolic)** | 110 (102, 120) | 128 (122, 130) | **0.059** |
| **BP (diastolic)** | 71 (67, 80) | 74 (65, 78) | 0.8 |
| **Heart rate** | 91 (82, 108) | 87 (72, 98) | 0.3 |
| **Hypotension** | 7 (12.1%) | 0 (0.0%) | >0.9 |
| **Weakness** | 54 (93.1%) | 4 (66.7%) | **0.093** |
| **Tiredness** | 53 (93.0%) | 6 (100.0%) | >0.9 |
| **Poor appetite** | 51 (87.9%) | 6 (100.0%) | >0.9 |
| **Increased pigmentation of the skin** | 36 (63.2%) | 2 (40.0%) | 0.4 |
| **Nausea** | 37 (63.8%) | 5 (83.3%) | 0.7 |
| **Vomiting** | 15 (25.9%) | 2 (33.3%) | 0.7 |
| **Liking for salt** | 38 (65.5%) | 4 (66.7%) | >0.9 |
| **Hypoglycaemia** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Loss of consciousness** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Diarrhoea** | 33 (56.9%) | 1 (16.7%) | **0.090** |
| **Dizziness** | 34 (59.6%) | 3 (50.0%) | 0.7 |
| **Shock** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Anorexia** | 35 (60.3%) | 2 (33.3%) | 0.2 |
| **Loss of axillary and pubic hair, if female** | 19 (33.3%) | 1 (16.7%) | 0.8 |
| **Any postural drop in blood pressure** | 4 (7.0%) | 0 (0.0%) | >0.9 |
| **Presence of anaemia** | 38 (65.5%) | 3 (50.0%) | 0.7 |

1Median (IQR); n (%); 2Wilcoxon rank sum test; Fisher's exact test.

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 4: Comparison of mortality among the Non-AI vs AI patients.** | | |  |
|  |  |  |  |
| Variable | **Died without AI**, N = 58 | **Died with AI**, N = 6 | ***p*-value** |
| **Age at enrolment median (IQR) (years)** | 39 (33, 45) | 41 (33, 46) | 0.7 |
| **Gender, n(%)** |  |  | >0.9 |
| Female | 32 (55.2%) | 3 (50.0%) |  |
| Male | 26 (44.8%) | 3 (50.0%) |  |
| Unknown | 0 (0.0%) | 0 (0.0%) |  |
| **Ethnicity, n(%)** |  |  | 0.2 |
| Black African | 50 (86.2%) | 4 (66.7%) |  |
| Other | 8 (13.8%) | 2 (33.3%) |  |
| **Duration of current illness, median (IQR) (days)** | NA (NA, NA) | NA (NA, NA) |  |
| **Random cortisol** | 481 (369, 616) | 307 (262, 336) | **0.003** |
| **Basal cortisol** | 500 (433, 636) | 308 (246, 365) | **<0.001** |
| **Stimulated cortisol** | 795 (662, 890) | 375 (338, 424) | **<0.001** |
| **ACTH** | 40 (25, 59) | 42 (26, 78) | 0.8 |
| **BP (systolic)** | 110 (102, 120) | 128 (122, 130) | **0.051** |
| **BP (diastolic)** | 70 (66, 80) | 74 (65, 78) | >0.9 |
| **Heart rate** | 93 (82, 109) | 87 (72, 98) | 0.2 |
| **Hypotension** | 7 (12.1%) | 0 (0.0%) | >0.9 |
| **Weakness** | 52 (89.7%) | 4 (66.7%) | 0.2 |
| **Tiredness** | 50 (86.2%) | 6 (100.0%) | >0.9 |
| **Poor appetite** | 49 (84.5%) | 6 (100.0%) | 0.6 |
| **Weight loss** | 50 (86.2%) | 4 (66.7%) | 0.2 |
| **Increased pigmentation of the skin** | 35 (60.3%) | 2 (33.3%) | 0.2 |
| **Nausea** | 36 (62.1%) | 5 (83.3%) | 0.4 |
| **Vomiting** | 15 (25.9%) | 2 (33.3%) | 0.7 |
| **Liking for salt** | 38 (65.5%) | 4 (66.7%) | >0.9 |
| **Hypoglycaemia** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Loss of consciousness** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Diarrhoea** | 32 (55.2%) | 1 (16.7%) | 0.10 |
| **Dizziness** | 33 (56.9%) | 3 (50.0%) | >0.9 |
| **Shock** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **Anorexia** | 34 (58.6%) | 2 (33.3%) | 0.4 |
| **Loss of axillary and pubic hair, if female** |  |  | 0.8 |
| No | 16 (27.6%) | 2 (33.3%) |  |
| Not applicable | 23 (39.7%) | 3 (50.0%) |  |
| Yes | 19 (32.8%) | 1 (16.7%) |  |
| **Any postural drop in blood pressure** | 4 (6.9%) | 0 (0.0%) | >0.9 |
| **Presence of anaemia** | 36 (62.1%) | 3 (50.0%) | 0.7 |
| **Tuberculosis** | 47 (81.0%) | 5 (83.3%) | >0.9 |
| **Cryptococcus neoformans** | 3 (5.2%) | 3 (50.0%) | **0.009** |
| **Pneumonia** | 7 (12.1%) | 0 (0.0%) | >0.9 |
| **Staph aureus** | 0 (0.0%) | 0 (0.0%) |  |
| **Kaposis sarcoma** | 0 (0.0%) | 0 (0.0%) |  |
| **Cytomegalovirus** | 0 (0.0%) | 0 (0.0%) |  |
| **HSV** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **HepB** | 0 (0.0%) | 1 (16.7%) | 0.094 |
| **Candida** | 4 (6.9%) | 0 (0.0%) | >0.9 |
| **GE/c diff** | 0 (0.0%) | 0 (0.0%) |  |
| **Parvo B19** | 0 (0.0%) | 0 (0.0%) |  |
| **Syphilis** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **B menigitis** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **UTI / Leptospirosis** | 1 (1.7%) | 0 (0.0%) | >0.9 |
| **PCP** | 2 (3.4%) | 0 (0.0%) | >0.9 |
| **COVID-19** | 0 (0.0%) | 0 (0.0%) |  |
| **Neurocysticercosis** | 0 (0.0%) | 0 (0.0%) |  |
| **Viral load (log10 Copies/mL)** | 12.00 (10.29, 13.14) | NA (NA, NA) |  |
| 10.4579184937828 |  |  |  |
| 11.60516730587 |  |  |  |
| **Total CD4 count (Copies/mm3)** | 26 (13, 54) | 30 (16, 45) | 0.7 |
| **Sodium mmol/L** | 133.0 (127.5, 136.0) | 136.5 (133.3, 139.0) | 0.2 |
| **Potassium mmol/L** | 3.90 (3.60, 4.60) | 3.70 (3.37, 3.88) | 0.092 |
| **Haemoglobin g/dL** | 8.35 (7.05, 9.67) | 9.60 (8.25, 10.95) | 0.4 |
| **White cell count X109** | 5.0 (3.4, 7.4) | 8.3 (4.3, 27.5) | 0.2 |
| **Lymphocyte count X109** | 0.80 (0.40, 0.99) | NA (NA, NA) |  |

a Only two people had viral load results

**Linear regression and multivariate analysis.**

When we performed linear regression analyses, only random cortisol, basal cortisol and stimulated cortisol and Other opportunistic infections, were associated with AI. A 10 nmol/L increase in random cortisol was associated with an 18% increase in the odds of being diagnosed with AI, 1.18 (95%CI:1.13, 1.25). A 10 nmol/L increase in basal cortisol was associated with a 10% increase in the odds of being diagnosed with AI, 1.10 (95%CI:1.06, 1.15), while a 10 nmol/L increase in stimulated cortisol was associated with a 34% increase in the odds of being diagnosed with AI, 1.34 (95%CI:1.20, 1.59).

At multivariate analysis, after adjusting for lymphocyte count, both random morning cortisol and ACTH were independently associated with AI. A 10 nmol/L increase in random morning cortisol was associated with a 19.6% increase in the odds of being diagnosed with AI, 1.196 (95%CI:1.131, 1.265), while a 10 pmol/L increase in ACTH was associated with a 12.6% reduction in the odds of being diagnosed with AI, 0.874 (95%CI:0.786, 0.971) after adjusting for lymphocyte count.

On their varied analysis ACTH was significant together with incremental cortisol poor appetite nausea liking of soul and lots of axillary hair PCP infection in predicting mortality You can see that at multivariate analysis only basal cortisol predicted mortality. Do you need increase in basal cortisol was associated with an increased hazard of mortality.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristic | Bivariate | | | multivariate - Cox PH | | |
| HR1 | 95% CI1 | p-value | HR1 | 95% CI1 | p-value |
| Age at enrolment | 1.02 | 0.99, 1.04 | 0.2 |  |  |  |
| Gender | 0.92 | 0.56, 1.49 | 0.7 |  |  |  |
| Black African Ethnicity | 1.59 | 0.8116, 3.117 | 0.176 |  |  |  |
| Random cortisol | 1.00 | 1.00, 1.00 | 0.6 |  |  |  |
| Basal cortisol | 1.00 | 1.00, 1.00 | 0.15 | 1.09 | 1.01, 1.17 | **0.027** |
| Stimulated cortisol | 1.00 | 1.00, 1.00 | >0.9 |  |  |  |
| ACTH | 1.01 | 1.00, 1.01 | **<0.001** |  |  |  |
| BP systolic | 0.99 | 0.98, 1.01 | 0.3 |  |  |  |
| BP diastolic | 1.02 | 1.00, 1.03 | 0.066 |  |  |  |
| incremental cortisol | 1.00 | 0.99, 1.02 | **0.032** |  |  |  |
| Heart rate | 1.00 | 0.99, 1.02 | 0.5 |  |  |  |
| Hypotension | 1.25 | 0.57, 2.75 | 0.6 |  |  |  |
| Weakness | 1.78 | 0.77, 4.12 | 0.2 |  |  |  |
| Tiredness | 2.67 | 0.97, 7.35 | 0.057 |  |  |  |
| Poor appetite | 2.66 | 1.21, 5.83 | **0.015** |  |  |  |
| Weight loss | 0.98 | 0.48, 1.98 | >0.9 |  |  |  |
| Increased pigmentation of the skin | 1.60 | 0.96, 2.66 | 0.072 |  |  |  |
| Nausea | 1.73 | 1.03, 2.89 | **0.038** |  |  |  |
| Vomiting | 0.95 | 0.55, 1.65 | 0.9 |  |  |  |
| Liking for salt | 1.69 | 1.01, 2.83 | **0.046** |  |  |  |
| Hypoglycaemia | 1.49 | 0.36, 6.07 | 0.6 |  |  |  |
| Loss of consciousness | 2.57 | 0.63, 10.5 | 0.2 |  |  |  |
| Diarrhoea | 1.48 | 0.91, 2.42 | 0.12 |  |  |  |
| Dizziness | 1.59 | 0.96, 2.63 | 0.069 |  |  |  |
| Shock | 3.85 | 0.94, 15.7 | 0.061 |  |  |  |
| Anorexia | 1.62 | 0.99, 2.66 | 0.057 |  |  |  |
| Loss of axillary and pubic hair | 2.62 | 1.32, 5.18 | **0.006** |  |  |  |
| Any postural drop in blood pressure | 1.50 | 0.54, 4.13 | 0.4 |  |  |  |
| Presence of anaemia | 1.28 | 0.77, 2.14 | 0.3 |  |  |  |
| Tuberculosis | 0.84 | 0.45, 1.58 | 0.6 |  |  |  |
| Cryptococcus neoformans | 1.82 | 0.78, 4.21 | 0.2 | 1.98 | 0.85, 4.63 | 0.11 |
| Pneumonia | 0.94 | 0.43, 2.06 | 0.9 |  |  |  |
| HSV | 1.00 | 0.14, 7.18 | >0.9 |  |  |  |
| HepB | 0.44 | 0.06, 3.15 | 0.4 |  |  |  |
| Candida | 0.99 | 0.36, 2.73 | >0.9 |  |  |  |
| Syphilis | 1.44 | 0.45, 4.58 | 0.5 |  |  |  |
| B menigitis | 1.41 | 0.20, 10.2 | 0.7 |  |  |  |
| UTI / Leptospirosis | 1.19 | 0.17, 8.61 | 0.9 |  |  |  |
| PCP | 4.24 | 1.04, 17.3 | **0.044** |  |  |  |
| Viral load | 1.19 | 1.01, 1.41 | **0.036** | 1.05 | 0.94, 1.17 | 0.4 |
| CD4 count | 0.99 | 0.98, 1.00 | 0.2 | 1.00 | 0.99, 1.01 | 0.7 |
| Sodium | 1.00 | 0.98, 1.02 | >0.9 |  |  |  |
| Potassium | 0.98 | 0.94, 1.03 | 0.5 |  |  |  |
| Haemoglobin | 0.97 | 0.87, 1.08 | 0.6 |  |  |  |
| White cell count | 1.00 | 1.00, 1.00 | >0.9 |  |  |  |
| Lymphocyte count | 0.92 | 0.81, 1.05 | 0.2 |  |  |  |
| ART exposure | 1.16 | 0.71, 1.89 | 0.6 |  |  |  |
| Kidney medication | 2.04 | 1.07, 3.91 | **0.030** |  |  |  |
| Addisons disease | 2.11 | 0.91, 4.89 | 0.081 |  |  |  |

There was accelerated mortality in the AI group as shown by statistically significant deaths between six and twelve months in the adrenal insufficiency group than the non-AI group with *p*=0.014.

Table: Bivariate analysis for Adrenaline insufficiency

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | OR1 | 95% CI1 | p-value |
| Age at enrolment | 1.02 | 0.98, 1.07 | 0.2 |
| Male gender | 0.97 | 0.44, 2.11 | >0.9 |
| Ethnicity | 0.62 | 0.18, 1.64 | 0.4 |
| Duration of current illness | 1.00 | 0.98, 1.00 | 0.7 |
| Weight loss | 0.70 | 0.27, 2.13 | 0.5 |
| log10 viral load | 1.11 | 0.75, 1.84 | 0.6 |
| Tuberculosis | 0.65 | 0.27, 1.82 | 0.4 |
| Cryptococcus neoformans | 14.8 | 5.88, 36.1 | <0.001 |
| Pneumonia | 0.98 | 0.23, 2.92 | >0.9 |
| Kaposis sarcoma | 3.98 | 0.20, 25.9 | 0.2 |
| HSV | 2.83 | 0.15, 16.8 | 0.3 |
| HepB | 2.53 | 0.39, 9.57 | 0.2 |
| Candida | 0.57 | 0.03, 2.82 | 0.6 |
| Total CD4 count | 0.99 | 0.98, 1.01 | 0.4 |
| White cell count X109 | 1.00 | 1.00, 1.00 | 0.4 |
| Neutrophils | 0.24 | 0.02, 0.82 | 0.2 |
| Sodium mmol/L | 1.23 | 0.97, 1.54 | 0.089 |
| Potassium mmol/L | 0.96 | 0.55, 1.13 | 0.8 |
| BP (systolic) | 1.00 | 0.98, 1.02 | 0.7 |
| BP (diastolic) | 1.02 | 0.99, 1.05 | 0.2 |
| Any postural drop in blood pressure | 1.80 | 0.28, 6.63 | 0.4 |
| Heart rate | 1.00 | 0.98, 1.01 | 0.7 |
| Hypotension | 0.38 | 0.02, 1.83 | 0.3 |
| Weakness | 0.61 | 0.25, 1.72 | 0.3 |
| Tiredness | 1.01 | 0.37, 3.50 | >0.9 |
| Poor appetite | 1.40 | 0.56, 4.25 | 0.5 |
| Increased pigmentation of the skin | 0.58 | 0.24, 1.33 | 0.2 |
| Nausea | 1.29 | 0.59, 2.91 | 0.5 |
| Vomiting | 1.10 | 0.44, 2.49 | 0.8 |
| Liking for salt | 2.10 | 0.93, 5.18 | 0.085 |
| Diarrhoea | 0.52 | 0.21, 1.18 | 0.13 |
| Dizziness | 1.09 | 0.49, 2.43 | 0.8 |
| Anorexia | 0.47 | 0.19, 1.06 | 0.079 |
| Presence of anaemia | 0.70 | 0.32, 1.56 | 0.4 |
| ART exposure | 0.46 | 0.17, 1.08 | 0.10 |
| Kidney medication | 2.30 | 0.74, 5.91 | 0.11 |
| mortality | 1.30 | 0.39, 4.35 | 0.7 |

1OR = Odds Ratio, CI = Confidence Interval

A graph with numbers and lines

Description automatically generated

Fig 1: The Kaplan-Meier survival curve over time

**Discussion**

HIV is highly prevalent, and it has a high mortality, especially if patients are severely immunocompromised. The majority of the patients were black African females who were significantly younger at the median age of 35 years. The overall median age at presentation was 36 years with the older age group associated with higher CD4 counts. The females were significantly younger at enrollment *p*=0.017, and they also had significantly lower haemoglobin p<0.001. The median duration of illness was 14 days. Most of the patient presented with weight loss and CD4 counts of around 33 cells /mm3. The opportunistic infections of note. In this cohort were tuberculosis, pneumonia, cryptococcus, candida, hepatitis B virus and syphilis. Most of the patients were found in the lowest tertile of 0 to 33 L and they had significantly higher viral loads together with low CD4 counts, white cell counts and neutrophils. Significant Kaposi sarcoma was exclusively found in the lowest CD4 tertile together candida which was more prevalent in the same tertile. There was significant KS in the youngest age group at p=0. 036. (Table 1.3) removed

The patients with hypoadrenalism 26/528, looked the same as the rest of the cohort in all respect except random, baseline, stimulated cortisol, potassium and neutrophils which were significantly lower in the hypoadrenal group. When comparing patients with hypoadrenalism, subdivided into primary 20/26 and secondary 6/26 hypoadrenalism, there were no significant differences respect of demographics, clinical history, clinical signs and investigations. When examining the etiology for primary and secondary hypoadrenalism we identified mainly tuberculosis at 83%, followed by cryptococcus (42%).

As expected, comparison of hypoadrenal patients with the rest of the cohort revealed statistically significant differences only in the random, baseline and stimulated cortisol levels. There were also differences in the median diastolic blood pressure and sodium levels which were higher in the hypoadrenal group, though not statistically significant. In contrast, the median neutrophil levels were higher in the rest of the cohort, though also not statistically significant. Neutrophil levels can be elevated by steroid-induced de-margination during inflammatory states, including HIV infection(17). We consider the lower neutrophil count in the AI group to be inappropriate and perhaps a marker of low cortisol state in advanced HIV which we consider to be a state of medical stress. Steroids can exert both pro and anti-inflammatory effect on the neutrophils depending on the inflammatory microenvironment (18). On the other hand, natural killer cell cytotoxicity (NKCC) lymphocyte dysfunction leading to increased infections has been associated with adrenal insufficiency(19), and increased mortality. In our study, the bivariate analysis a unit rise in lymphocytes was protective against AI, though not statistically significant.

Compared to the SAI group, the PAI group had a statistically significant shorter duration of illness at 14 days versus 30 days, and higher median ACTH levels at 42.4 pmol/L versus 9.5 pmol/L.The rest of the history, clinical signs and investigations of the PAI and the SAI groups were not significant. The surprising finding was the insignificant difference between the stimulated cortisol levels in both the PAI and SAI group.

The result of the Bivariate and multivariate analysis is counterintuitive, unless we assume that advanced HIV predicts higher cortisol output due to the metabolic and inflammatory stress, which may lead to adrenal cortisol depletion leading to AI. An attempt at generating an Artificial Intelligence (Ai) tool which can predict adrenal insufficiency yielded mixed results due an uneven distribution of the AI and non-AI populations. The Random Forest classifier was the most promising of several classifiers that we experimented with; its recall was good as evidenced by its ability to identify 92.4%. However, it was only able to accurately predict AI in 52.6% of the patients with advanced HIV. Refinement of this predictive tool is work in progress.

**Morbidity & Mortality:**

The Kaplan-Meier curve confirms the higher mortality rate in AI group over time. The mortality at six months follow-up was statistically significant (*p*=0.045) at 19.2% versus 6.8% for AI versus non-AI groups, respectively. Mortality occurred throughout the tertiles in non-AI groups. In contrast to the PAI mortality which occurred in the second and third tertiles, the SAI group experienced intermediate mortality of 1/6 (17.0%). The main causes of mortality in this cohort were tuberculosis 81% vs 83.3% and Cryptococcus 5.2% vs 50% in non-AI vs AI groups, respectively.

**Strengths and Weaknesses:**

This is the largest prospective study of patients with advanced HIV in a draining area with a high background of tuberculosis. One weakness is the population selection bias with the black Africans making most of the participants due to the state hospital draining area being predominantly Black African.

**AI predictive tool:** Our current ten variable machine learning (ML) model which is based on unbalanced data is biased towards the non-AI group due to its over-representation. One can artificially oversample AI or under sample non-AI thus making it harder to generalize the hypothesis of Adrenal insufficiency using the current model. It is our belief that in future, with increased AI population or improved classifier sensitivity, it may be possible to improve the precision in predicting AI in patients with advanced HIV.

**Conclusion:**

The largest study on hypoadrenalism in advanced HIV in Africa revealed overall mortality of 12.1%. The overall mortality in the AI group was 23.1% versus 11.6% for the non-AI groups, respectively. Mortality was bigger in the PAI group at 25% compared to the 16.7% in the SAI group. Higher mortality rate in the PAI group which is double the non-AI group suggests that hypoadrenalism carries a higher risk of mortality in patients with advanced HIV and thus justifies screening for it in these patients.

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