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# 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 and disseminated opportunistic infections, which have been associated with both primary (PAI) and secondary adrenal insufficiency (SAI). We hypothesized that adrenal insufficiency (AI) may partially account for the high mortality in 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, topical or inhaled steroids in the previous 3 months. A tetracosactide test was performed in patients with a morning random cortisol concentrations of less than 500 nmol/L.

### Results:

A total of 559 patients were recruited, of these, 549 complete records were evaluated. The median interquartile range (IQR) age of patients at enrolment was 36.0 years (IQR: 31.0-43.0). The majority were Black Africans 75.7% and mixed race (22.9%), whites and Asians in (1.2%) and (0.3%), respectively. Of the 549 patients 33 (6.01%) had AI, of whom 24 (72.72%) had (SAI) whereas 9 (27.27%) had (PAI). Overall, 151 tetracosactide tests were performed identifying 27 AI patients, 6 more patients were presumed AI by random cortisol less than 200 nmol/L. Extra Pulmonary tuberculosis (EPTB) and *Cryptococcus neoformans* predominate in the AI group 33.3% vs 19.0%, *p*=0.045, 30.3% vs 3.8%, *p*<0.001, whereas pulmonary tuberculosis (PTB) predominates in the group without AI 42.4% vs 64.7%, *p*=0.01. The diastolic blood pressure was higher in AI versus Non-AI group 73.0 (66.4,80.8) versus 70.0 (60.0, 79.0); *p*=0.012. Fluconazole (33% versus 12.1%) and opiate use (36.4% versus 21.9%) were higher in the AI versus Non-AI group; *p*=0.002 and *p*=0.054, respectively. Duration of illness was longer in the SAI versus the PAI group: 14 days (IQR:14.0, 27.8) versus 10 days (IQR: 7.0, 14.0); *p*=0.006. The overall one-year mortality for the whole cohort was 27.5%. Although mortality was not different in the AI vs non-AI groups, it was higher in patients with AI patients and *Cryptococcus neoformans* 45.5% versus 4.3%; *p*<0.001. Regression analysis revealed that a 50 nmol/L cortisol increase, and 100 pg/L increase in the plasma ACTH was associated with a 11% and 97% increase in the odds of mortality (OR = 1.11, (95%CI: 1.05, 1.18), *p*<0.001) and (OR = 1.97, (95%CI:1.15, 3.59), *p<*0.019), respectively.

### Conclusion:

### AI does not account for the high mortality in advanced HIV even though its mortality rate is marginally higher than that in patients without AI. EPTB and Cryptococcus were predominant OI’s in AI, whereas PTB was the predominant OI in the non-AI group. Increased cortisol and ACTH Concentrations were associated with increased mortality risk.

PTB – pulmonary tuberculosis, EPTB – Extrapulmonary tuberculosis

### Introduction:

Adrenal insufficiency (AI) resulting from deficiency of glucocorticoids and mineralocorticoids is an invariably fatal medical condition without replacement. Prior to the availability of glucocorticoids, most patients with primary adrenal insufficiency (PAI) died within 2 years of diagnosis (1).

Autoimmune adrenal insufficiency predominates in European populations (2), and also in South Africa (3), despite the high background prevalence of tuberculosis (TB) (4). Our recent survey suggests that TB (34%) and Acquired Immune Deficiency Syndrome (AIDS) (29.8%) predispose to the development of PAI (5).

The background prevalence of Human Immunodeficiency Virus (HIV) in sub-Saharan Africa is 9% (6)((7). The mortality in HIV positive patients is higher in resource limited settings for example, sub-Saharan Africa, (8) due to late presentation and inadequate health resources. Patients with HIV may develop PAI due to *inter alia* TB, *Mycobacterium avium Intracellulare* (MAI), cytomegalovirus (CMV), toxoplasmosis, *Pneumocystis carinii*, histoplasmosis and malignancies for example, non-Hodgkin’s lymphoma and Kaposi sarcoma (KS). Additionally, fungal infections including cryptococcus, blastomycosis, and histoplasmosis and medications for example, ketoconazole and mitotane may precipitate PAI (9). Secondary adrenal insufficiency (SAI) may also be caused by *inter alia* TB, toxoplasmosis and CMV in HIV infection (10-12).

There are varied results describing the incidence of hypoadrenalism in patients with HIV. In a study in Pakistan of 64 HIV infected, predominantly male patients (84.9%), Afreen *et al*, reported the AI incidence of 9 (14.0%), using the 250 microgram intravenous tetracosactide test and a 60 minute cortisol concentration of less than 18 microgram/dL (500 nmol/L) (13), whereas in a Nigerian study of 43 newly diagnosed HIV positive patients who were antiretroviral treatment naïve, the AI incidence was 34.8%, using a 1 microgram tetracosactide test (14) and a 30 minute cortisol of less than 500 nmol/L. In India, Sharma et al reported the AI incidence of 24.23 % on HIV positive patients using the cortisol cutoff of 500 nmol/L following a 250 μg tetracosactide test.

Despite optimal replacement therapy in AI in general, patients demonstrate poorer survival due in part to cardiovascular, malignant and infectious diseases, compared to background populations (10, 15). Both overtreatment and insufficient replacement with glucocorticoids during infections and stress-related events confer an increased mortality (16).

We hypothesized that coexistent AI among patients with advanced HIV may accelerate mortality. Our objectives were to determine the incidence of AI among ill, hospitalized HIV-infected patients. In addition, we wished to explore the positive predictive clinical and biochemical characteristics for AI, and the predictors for survival.

### Materials and methods:

Approval 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 i declaration of Helsinki from 2013. We undertook a prospective case-finding study of HIV positive patients, presenting with advanced disease and an opportunistic infection (OI) to an acute tertiary care medical ward. All participants provided written informed consent. If participants were affected by delirium our research and ethics committee endorsed retrospective informed consent, with a view to limiting bias and providing an equal opportunity for life-saving treatment.

***Inclusion and exclusion criteria***

Inclusion criteria were age 18 years and older, and a CD4 count of 100 cells per mm3 or fewer and an opportunistic infection. The use of oral, topical or inhaled steroids in the previous 3 months represented an exclusion criterion.

***Data extraction***

Patients who met the inclusion criteria had blood samples taken for random serum cortisol and plasma ACTH between 08:00 and 09:00 on the day of enrolment analyzed at a private accredited laboratory. 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. 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***

Due to the unavailability of the gold standard LC/MS cortisol diagnostic test (17) we utilised the Roche COBAS ECLIA tests to measure both cortisol and ACTH concentrations. For the random plasma ACTH, the specimen was collected in EDTA vials on ice and for serum cortisol concentration, clotted blood was submitted.

***Methodology for serum Cortisol and ACTH***

Serum cortisol concentrations were assayed on the Roche Cobas 6000 e601 platform, using the Elecsys Cortisol II antibody. Serum cortisol concentrations were determined using Electro luminescence assay (ECLIA). This method has been standardized against the institute for reference materials and measurements in brackets (IRMM)/IFCC-45 panel. The measuring range is 1.5 nmol/L to 1750 nmol/L with a limit of detection (LOD) of 1.5 nmol/L (18).

***The tetracosactide test***

The samples of plasma ACTH collected in Ethylenediaminetetraacetic acid (EDTA) tubes on ice and serum cortisol obtained during the test were submitted to the National Health Laboratory Services (NHLS). Venous blood was taken at 0 minutes for serum cortisol, followed by 250 µg of intravenously administered tetracosactide and a 30-minute serum cortisol was taken at the conclusion of the test. AI was diagnosed if a 30-minute serum cortisol was less than 500 nmol/L. In patients with confirmed cortisol deficiency, a concomitant plasma ACTH above the upper limit of the reference range (67.7 pg/mL) was consistent with PAI (17), while a low or normal plasma ACTH was diagnostic of SAI (18). Serum cortisol analyses performed to evaluate the diagnostic sensitivity and specificity of stimulated cortisol concentration using cut-offs at 400 nmol/L, and 340 nmol/ L, respectively.

***Determination of survival***

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

### Statistical analysis:

Statistical analyses were performed using R-programing software *(*ref: R Core Team (2023*). \_R: A Language and Environment for Statistical Computing\_. R Foundation for Statistical Computing, Vienna, Austria. https://*[*www.R-project.org/)*.](http://www.R-project.org/).) 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. We ranked CD4 counts into tertiles of 0-33; 34-66 and 67-100, respectively to determine if AI was associated. We conducted a bivariate analysis using logistic regression to examine the basic relationship between each independent variable and mortality. To tackle the issue of missing data, we utilized the mice package in R for multiple imputations, allowing us to create a complete dataset for our analysis. Before diving into the model, we checked for collinearity among the independent variables to ensure that our regression estimates would be stable and reliable.

We then applied various feature selection methods to pinpoint the most relevant variables that should be included in our multivariable logistic regression model. The final model helped us identify the factors independently associated with mortality. We considered results statistically significant if the P-value was less than 0.05. Our findings were presented as Adjusted Odds Ratios (aORs), along with their 95% confidence intervals (95% CI) and corresponding P-values.

Recruited 589



Enrolled 563

AI 35

SAI : 26

PAI : 9

Excluded 26

No consent

Incomplete data

Topical steroid use

Non-AI 528

We conducted a bivariate analysis using logistic regression to examine the basic relationship between each independent variable and mortality. To tackle the issue of missing data, we utilized the mice package in R for multiple imputations, allowing us to create a complete dataset for our analysis. Before diving into the model, we checked for collinearity among the independent variables to ensure that our regression estimates would be stable and reliable.

We then applied various feature selection methods to pinpoint the most relevant variables that should be included in our multivariable logistic regression model. The final model helped us identify the factors independently associated with mortality. We considered results statistically significant if the P-value was less than 0.05. Our findings were presented as Adjusted Odds Ratios (aORs), along with their 95% confidence intervals (95% CI) and corresponding P-values.

### Results:

A total of 589 patients were recruited, of whom 10 withdrew from participation and 563 participants were included in the final analysis. Most of the patients were female 282 (50.6%) and the predominant opportunistic infection was tuberculosis in 459 (84.4%), followed by pneumonia 62 (11.4%), candida 35 (6.4%), and cryptococcus infection in 29 (5.3%). The median, interquartile range (IQR) age at enrollment was 36 years (31.0-43.0) years and when subdivided by CD4 count, patients were significantly younger in the lowest CD4 tertile, 35 years (30.0-42.0); ***p*=0.003**, compared to the remaining tertiles Table 1.

The distribution of gender and ethnicity and duration of illness did not differ by CD4 distribution. As expected, the participants with the lowest CD4 count, had the highest viral load**; *p*=0.001**. The white cell, lymphocyte and neutrophil counts were lower in the lowest CD4 tertile, ***p*<0.001, *p*=0.004** and ***p*=0.003,** respectively, compared with the remaining tertiles.

The incidence of opportunistic infections (OI’s) for example, tuberculosis, pneumonia, and cryptococcus did not differ across the CD4 tertiles, apart from candida which was highest in the lowest CD4 count, ***p*=0.049**.

### HAART vs HAART naïve

When comparing the subgroup of patients receiving HAART, compared with those who were antiretroviral treatment naïve, the patients did not differ apart from the incidence of cryptococcus infection which was greater in the treatment naïve group, compared with those patients on HAART 24 (7.0%) versus 6 (2.6%); ***p*=0.039**. Importantly the CD4 count, and viral load did not differ between these two groups.

**Table 1: Patient presentation by CD4 count in tertiles with the p-values comparing patient clinical characteristics across the tertiles**

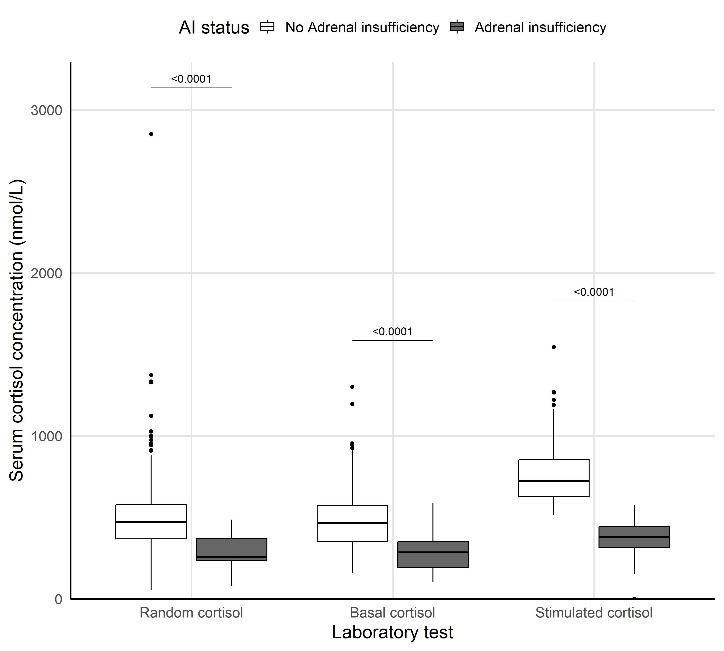
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variable** | **Overall**, N = 558*1* | **0 - 33**, N = 278*1* | **34 - 66**, N = 156*1* | **67 - 100**, N = 124*1* | **p-value***2* |
| **Age at enrolment, median (IQR) (years)** | 36.0 (31.0, 43.0) | 35.0 (30.0, 42.0) | 37.0 (32.0, 44.0) | 38.0 (33.0, 46.0) | **0.003** |
| **Gender, n(%)** |  |  |  |  | 0.2 |
| Female | 282 (50.6%) | 139 (50.0%) | 87 (55.8%) | 56 (45.5%) |  |
| **Duration of current illness, median (IQR) (days)** | 14.0 (12.0, 21.0) | 14.0 (14.0, 21.0) | 14.0 (11.5, 21.0) | 14.0 (7.0, 21.0) | 0.067 |
| **Weight loss** | 465 (86.4%) | 236 (87.1%) | 133 (87.5%) | 96 (83.5%) | 0.6 |
| **log10 viral load** | 10.7 (7.3, 12.4) | 11.6 (9.3, 12.8) | 8.9 (6.3, 12.3) | 7.6 (3.9, 10.3) | **<0.001** |
| **Opportunistic infections** |  |  |  |  |  |
| Tuberculosis | 459 (84.4%) | 229 (84.5%) | 131 (86.2%) | 99 (81.8%) | 0.6 |
| Pneumonia | 62 (11.4%) | 30 (11.1%) | 19 (12.5%) | 13 (10.7%) | 0.9 |
| Candida albicans | 35 (6.4%) | 22 (8.1%) | 11 (7.2%) | 2 (1.7%) | **0.049** |
| Cryptococcus neoformans | 29 (5.3%) | 15 (5.5%) | 5 (3.3%) | 9 (7.4%) | 0.3 |
| Gastroenteritis | 23 (4.2%) | 12 (4.4%) | 7 (4.6%) | 4 (3.3%) | 0.8 |
| Hepatitis B | 18 (3.3%) | 9 (3.3%) | 8 (5.3%) | 1 (0.8%) | 0.12 |
| Syphilis | 17 (3.1%) | 8 (3.0%) | 8 (5.3%) | 1 (0.8%) | 0.11 |
| Kaposis sarcoma | 6 (1.1%) | 6 (2.2%) | 0 (0.0%) | 0 (0.0%) | 0.056 |
| Urinary tract infection | 7 (1.3%) | 4 (1.5%) | 0 (0.0%) | 3 (2.5%) | 0.2 |
| Pneumocystis Jiroveci Pneumonia | 5 (0.9%) | 4 (1.5%) | 1 (0.7%) | 0 (0.0%) | 0.5 |
| Herpes simplex virus | 8 (1.5%) | 3 (1.1%) | 4 (2.6%) | 1 (0.8%) | 0.4 |
| Bacterial meningitis | 6 (1.1%) | 3 (1.1%) | 1 (0.7%) | 2 (1.7%) | 0.8 |
| **Haematological parameters** |  |  |  |  |  |
| White cell count x109 | 5.4 (3.7, 8.2) | 5.1 (3.0, 7.4) | 5.7 (4.2, 8.6) | 6.9 (4.5, 9.9) | **<0.001** |
| Lymphocyte count x109 | 0.8 (0.4, 1.8) | 0.6 (0.3, 1.3) | 0.9 (0.5, 4.9) | 1.3 (0.8, 2.9) | **0.004** |
| Neutrophils x109 | 3.4 (1.5, 8.5) | 2.4 (1.1, 6.2) | 6.6 (1.9, 15.2) | 5.2 (3.1, 11.3) | **0.003** |
| **Medical history** |  |  |  |  |  |
| HAART exposure | 204 (36.6%) | 100 (36.0%) | 61 (39.1%) | 43 (34.7%) | 0.7 |
| Anti-tuberculous therapy | 13 (2.3%) | 5 (1.8%) | 3 (1.9%) | 5 (4.0%) | 0.4 |
| Antifungal therapy | 6 (1.1%) | 2 (0.7%) | 1 (0.6%) | 3 (2.4%) | 0.3 |

*1* Median (IQR); 2Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test IQR-interquartile range, TB-tuberculosis, HAART Highly active antiretroviral therapy

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
| incremental\_cortisol\_percent\_increase | 55.6 (31.4, 101.0) | 51.3 (29.1, 95.7) | 47.0 (29.4, 88.3) | 106.3 (49.1, 136.4) | 0.003 |

### Diagnosis of adrenal insufficiency

Following random ACTH and cortisol concentrations done upon recruitment, 151 tetracosactide tests were done on patients with random cortisol less than 500 nmol/L. Of the 35 AI patients, 26 were identified as SAI and 9 PAI. The proportions of patients with a stimulated cortisol of 340 nmol/L, 400 nmol/L and 500 nmol/L, thus 12 patients would be diagnosed if the stimulated cut-off was 340 nmol/L, 18 patients would be diagnosed if a cut off of 400 nmol/L and 33 if 500 nmol/L was utilized.



**Fig 2:** Cortisol concentrations in patients with adrenal insufficiency compared with those without adrenal insufficiency.

### Diagnosis of adrenal insufficiency

The differences between the patients with SAI versus those with PAI were duration of illness, which was longer in the SAI group,14 days (IQR:14.0-23.3) versus 10 days (IQR:7.0-14.0), *p*=0.024 and vomiting which occurred more often in the PAI group, compared with SAI 66.7% versus 23.1%, ***p*=0.038**, respectively.

**Table 2: Comparison of the demographics, history, clinical findings and biochemical findings between patients with SAI and those with PAI**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **SAI, *N* = 261** | **PAI, *N* = 91** | **p-value2** |
| **Age at enrolment, median (IQR) (years)** | 37.0 (33.5, 47.0) | 39.0 (31.0, 43.0) | >0.9 |
| **Female-gender, n(%)** | 13 (50.0%) | 4 (44.4%) | >0.9 |
| **Ethnicity, n(%)** |  |  | >0.9 |
| Black African | 22 (84.6%) | 8 (88.9%) |  |
| Other | 4 (15.4%) | 1 (11.1%) |  |
| **Duration of current illness, median (IQR) (days)** | 14.0 (14.0, 23.3) | 10.0 (7.0, 14.0) | **0.024** |
| **HISTORY** |  |  |  |
| Weight loss | 21 (80.8%) | 9 (100.0%) | 0.3 |
| Weakness | 22 (84.6%) | 6 (66.7%) | 0.3 |
| Tiredness | 23 (88.5%) | 7 (77.8%) | 0.6 |
| Poor appetite | 22 (84.6%) | 7 (77.8%) | 0.6 |
| Increased skin pigmentation | 10 (40.0%) | 4 (57.1%) | 0.7 |
| Nausea | 15 (57.7%) | 7 (77.8%) | 0.4 |
| Vomiting | 6 (23.1%) | 6 (66.7%) | **0.038** |
| Liking for salt | 15 (57.7%) | 5 (55.6%) | >0.9 |
| Diarrhoea | 11 (42.3%) | 2 (22.2%) | 0.4 |
| Dizziness | 14 (53.8%) | 5 (62.5%) | >0.9 |
| Anorexia | 9 (34.6%) | 6 (66.7%) | 0.13 |
| Loss of axillary and pubic hair, if female | 3 (11.5%) | 2 (22.2%) | 0.8 |
| HAART exposure | 8 (30.8%) | 2 (22.2%) | >0.9 |
| **CLINICAL FEATURES** |  |  |  |
| BP (systolic) mmHg | 120.0 (100.0, 130.0) | 120.0 (120.0, 127.0) | 0.5 |
| BP (diastolic) mmHg | 73.0 (60.0, 80.0) | 79.0 (70.0, 85.0) | 0.5 |
| Any postural drop in blood pressure | 3 (11.5%) | 0 (0.0%) | 0.6 |
| Heart rate beats per minute | 84.0 (76.0, 96.0) | 97.0 (84.0, 105.0) | 0.058 |
| Hypotension | 2 (7.7%) | 0 (0.0%) | >0.9 |
| Presence of anaemia | 15 (60.0%) | 6 (66.7%) | >0.9 |
| **INVESTIGATIONS** |  |  |  |
| Viral load (log10 Copies/mL) | 12.3 (11.4, 12.3) | 11.6 (11.6, 11.6) | >0.9 |
| Random cortisol | 255.5 (235.5, 358.3) | 281.0 (209.0, 375.0) | 0.8 |
| Basal cortisol | 284.0 (188.5, 359.0) | 286.0 (194.5, 326.5) | 0.8 |
| Stimulated cortisol | 360.0 (306.5, 440.5) | 415.5 (371.0, 449.8) | 0.4 |
| Delta cortisol | 116.0 (41.5, 186.5) | 119.0 (28.3, 183.8) | >0.9 |
| Tuberculosis | 21 (80.8%) | 6 (66.7%) | 0.4 |
| PTB | 12 (46.2%) | 4 (44.4%) | >0.9 |
| EPTB | 9 (34.6%) | 2 (22.2%) | >0.7 |
| *Cryptococcus neoformans* | 7 (26.9%) | 3 (33.3%) | 0.7 |
| Pneumonia | 3 (11.5%) | 1 (11.1%) | >0.9 |
| Kaposis sarcoma | 1 (3.8%) | 0 (0.0%) | >0.9 |
| Herpes simplex virus (HSV) | 1 (3.8%) | 0 (0.0%) | >0.9 |
| Hepatitis B virus | 3 (11.5%) | 0 (0.0%) | 0.6 |
| *Candida albicans* | 2 (7.7%) | 0 (0.0%) | >0.9 |
| Total CD4 count | 39.0 (13.3, 57.5) | 46.0 (23.0, 76.0) | 0.3 |
| White cell count X109 | 4.7 (3.1, 10.2) | 5.2 (3.3, 6.5) | 0.7 |
| Lymphocyte count X109 | 0.9 (0.5, 1.1) | 1.6 (1.4, 1.7) | 0.2 |
| Neutrophils | 1.6 (1.1, 2.5) | 3.2 (2.0, 4.3) | >0.9 |
| Sodium mmol/L | 135.0 (132.0, 137.0) | 133.0 (131.0, 135.0) | 0.3 |
| Potassium mmol/L | 4.0 (3.5, 4.6) | 3.7 (3.3, 5.4) | >0.9 |
| Haemoglobin g/dL | 8.8 (7.7, 11.0) | 10.2 (8.1, 10.4) | 0.7 |
| Mortality | 7(26.9%) | 4 (44.4%) | ??? |

1 Median (IQR); n (%); 2 Wilcoxon rank sum test; Fisher’s exact test; Wilcoxon rank sum exact test; HAART: Highly Active Antiretroviral Therapy

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **SAI, *N* = 261** | **PAI, *N* = 91** | **p-value2** |
| incremental\_cortisol\_percent\_increase | 34.9 (15.2, 100.0) | 39.9 (10.0, 115.3) | 0.9 |

### Biochemistry: Comparison of cortisol concentrations in patients with AI and those without

The random cortisol was lower in the AI group, 332 nmol/L (252.0-382.0), compared with 478 nmol/L (372.5-578.0) in those patients without AI; **(*p*<0.001).** The basal cortisol was 300 nmol/L (188.5-359) in the AI group, compared with 466 nmol/L (352-573); **(*p*<0.001)** without AI. The stimulated cortisol was

379 nmol/L (314.5-440) in the AI group, compared with 722 nmol/L (630-858); **(*p*<0.001)** without AI (Figure 1).

**Comparison of clinical characteristics in patients with adrenal insufficiency with those without**

Extrapulmonary tuberculosis and cryptococcal infection occurred more often in the AI, compared with the remaining groups, whereas pulmonary tuberculosis occurred less often among patients diagnosed with AI. The diastolic blood pressure was higher in the AI, compared with the those without AI groups; ***p*=0.010**. There was greater use of fluconazole and opiates in the AI, compared with group without AI, 11 (31.4%) vs 63 (11.5.1%); ***p*<0.002** and 12 (34.3%) vs 114 (20.7%); *p*=0.058, respectively. (Table 2)

**Table 3: Comparison of clinical characteristics in patients with adrenal insufficiency with those without**

|  |  |  |  |
| --- | --- | --- | --- |
| ***Variable*** | ***Non-AI, N = 5281*** | ***AI, N = 351*** | ***p- value2*** |
| Age at enrolment, median (IQR) (years) | 36.0 (31.0, 43.0) | 39.0 (33.0, 45.5) | 0.2 |
| Female gender, N (%) | 267 (50.6%) | 17 (48.6%) | 0.8 |
| Duration of current illness, median (IQR) (days) | 14.0 (11.8, 21.0) | 14.0 (11.0, 21.0) | >0.9 |
| **HISTORY** |  |  |  |
| Weight loss | 435 (86.1%) | 30 (85.7%) | >0.9 |
| Weakness | 430 (85.5%) | 28 (80.0%) | 0.4 |
| Tiredness | 431 (85.5%) | 30 (85.7%) | >0.9 |
| Poor appetite | 381 (76.2%) | 29 (82.9%) | 0.4 |
| Increased pigmentation of the skin | 250 (50.7%) | 14 (43.8%) | 0.4 |
| Nausea | 267 (53.1%) | 22 (62.9%) | 0.3 |
| Vomiting | 139 (27.7%) | 12 (34.3%) | 0.4 |
| Liking for salt | 264 (52.7%) | 20 (57.1%) | 0.6 |
| Hypoglycaemia | 11 (2.2%) | 0 (0.0%) | >0.9 |
| Loss of consciousness | 7 (1.4%) | 0 (0.0%) | >0.9 |
| Diarrhoea | 224 (44.9%) | 13 (37.1%) | 0.4 |
| Dizziness | 241 (48.1%) | 19 (55.9%) | 0.4 |
| Shock | 5 (1.0%) | 0 (0.0%) | >0.9 |
| Anorexia | 238 (47.4%) | 15 (42.9%) | 0.6 |
| Loss of axillary and pubic hair, if female | 97 (19.0%) | 5 (14.3%) | 0.5 |
| **CLINICAL FEATURES** |  |  |  |
| BP (diastolic) mmHg | 70.0 (60.0, 78.5) | 73.0 (64.8, 82.3) | **0.01** |
| Heart rate beats per minute this | 91.0 (79.0, 109.0) | 86.5 (77.0, 101.5) | 0.4 |
| Hypotension | 47 (9.4%) | 2 (5.7%) | 0.8 |
| Any postural drop in blood pressure | 20 (4.0%) | 3 (8.6%) | 0.2 |
| Presence of anaemia | 299 (59.8%) | 21 (61.8%) | 0.8 |
| **INVESTIGATIONS** |  |  |  |
| Viral load (log10 copies/mL) | 10.7 (7.1, 12.4) | 11.9 (11.3, 12.3) | 0.4 |
| Pulmonary tuberculosis | 332 (64.5%) | 16 (45.7%) | **0.026** |
| Extra pulmonary tuberculosis | 99 (19.2%) | 11 (31.4%) | 0.081 |
| Cryptococcus neoformans | 19 (3.7%) | 10 (28.6%) | **<0.001** |
| Pneumonia | 58 (11.3%) | 4 (11.4%) | >0.9 |
| Hepatitis B | 15 (2.9%) | 3 (8.6%) | 0.1 |
| Candida albicans | 33 (6.4%) | 2 (5.7%) | >0.9 |
| Kaposis sarcoma | 5 (1.0%) | 1 (2.9%) | 0.3 |
| Herpes simplex virus | 7 (1.4%) | 1 (2.9%) | 0.4 |
| Gastroenteritis | 23 (4.5%) | 0 (0.0%) | 0.4 |
| Total CD4 count | 33.0 (14.0, 63.0) | 42.0 (16.0, 58.0) | 0.8 |
| White cell count X109/L | 5.6 (3.8, 8.2) | 5.2 (3.1, 8.0) | 0.5 |
| Lymphocyte count X109/L | 0.8 (0.4, 2.0) | 1.1 (0.6, 1.3) | 0.4 |
| Neutrophils x109/L | 3.7 (1.6, 9.0) | 1.6 (1.0, 3.4) | 0.077 |
| Sodium mmol/L | 133.0 (130.0, 137.0) | 135.0 (131.3, 136.8) | 0.2 |
| Potassium mmol/L | 4.1 (3.6, 4.6) | 4.0 (3.3, 4.7) | 0.4 |
| Haemoglobin g/dL | 8.7 (7.4, 10.3) | 9.5 (7.8, 10.8) | 0.2 |
| **MANAGEMENT & OUTCOMES** |  |  |  |
| Mortality | 156 (67.8%) | 11 (68.8%) | >0.9 |
| Fluconazole | 63 (11.5%) | 11 (31.4%) | **0.002** |
| Opiates | 114 (20.7%) | 12 (34.3%) | 0.058 |
| HAART exposure | 194 (35.3%) | 10 (28.6%) | 0.4 |

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

HAART: Highly Active Antiretroviral Therapy

|  |  |  |  |
| --- | --- | --- | --- |
| ***Variable*** | ***Non-AI, N = 5281*** | ***AI, N = 351*** | ***p- value2*** |
| incremental\_cortisol\_percent\_increase | 60.4 (34.7, 102.6) | 35.6 (14.2, 99.4) | 0.042 |

|  |  |
| --- | --- |
| Time point | P-value |
| 3 | 0.8515620 |
| 6 | 0.9807383 |
| 12 | 0.5435858 |

The overall mortality at one-year follow-up was 11/35 (31.4%) among the AI group compared to the group without 156/528 (29.5%); (*p*=0.272). There was no significant difference in mortality at 3, 6 and 12 months for the two groups, respectively. There were, however, differences in the random cortisol, basal cortisol, stimulated cortisol, and incremental cortisol, with 519, (418, 640) versus 281, (244, 341); ***p*=0.001**, 484, (411, 625) versus 284, (201, 365); ***p*<0.001**, 791, (627, 916) versus 375, (321, 426); ***p*<0.001** and 263,(179, 360) versus 32, (17, 107); ***p*<0.001**, respectively, between the subgroups of deceased patients with AI versus patients without. As expected, higher cortisol concentrations were found in patients without AI. The mortality associated with *Cryptococcal neoformans* was however greater in the AI than in the non-AI groups, 45.5% vs 4.6%; ***p*<0.001**.

**Table 4 : Comparisons of the characteristics of patients who died without adrenal insufficiency versus those with AI**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | ***Non-AI, N = 1561*** | ***AI, N = 111*** | **p-value2** |
| Age at enrolment, median (IQR) (years) | 37 (30, 44) | 41 (37, 48) | 0.13 |
| Female gender, n(%) | 83 (53.2%) | 6 (54.5%) | >0.9 |
| Duration of current illness, median (IQR) (days) | 14 (14, 21) | 14 (10, 14) | 0.2 |
| **HISTORY** |  |  |  |
| Weight loss | 129 (89.0%) | 9 (81.8%) | 0.6 |
| Weakness | 127 (87.6%) | 9 (81.8%) | 0.6 |
| Tiredness | 128 (88.9%) | 11 (100.0%) | 0.6 |
| Poor appetite | 119 (82.1%) | 11 (100.0%) | 0.2 |
| Increased pigmentation of the skin | 83 (58.5%) | 4 (44.4%) | 0.5 |
| Nausea | 84 (57.9%) | 9 (81.8%) | 0.2 |
| Loss of consciousness | 4 (2.7%) | 0 (0.0%) | >0.9 |
| Diarrhoea | 71 (49.0%) | 3 (27.3%) | 0.2 |
| Dizziness | 73 (50.7%) | 5 (45.5%) | 0.7 |
| Shock | 2 (1.4%) | 0 (0.0%) | >0.9 |
| Anorexia | 73 (50.7%) | 4 (36.4%) | 0.4 |
| Loss of axillary and pubic hair, if female | 35 (23.6%) | 2 (18.2%) | 0.8 |
| **CLINICAL FEATURES** |  |  |  |
| BP (systolic) | 110 (100, 124) | 127 (114, 129) | 0.11 |
| BP (diastolic) | 70 (60, 80) | 79 (67, 83) | 0.2 |
| Heart rate | 92 (79, 108) | 82 (70, 103) | 0.3 |
| Hypotension | 16 (11.0%) | 0 (0.0%) | 0.6 |
| Any postural drop in blood pressure | 7 (4.9%) | 0 (0.0%) | >0.9 |
| Presence of anaemia | 93 (64.6%) | 6 (54.5%) | 0.5 |
| **INVESTIGATIONS** |  |  |  |
| Sodium mmol/L | 133 (129, 137) | 134 (132, 139) | 0.11 |
| Potassium mmol/L | 4.00 (3.50, 4.60) | 3.90 (3.65, 4.65) | >0.9 |
| Haemoglobin g/dL | 8.40 (7.10, 9.60) | 8.70 (7.45, 10.45) | 0.5 |
| Syphilis | 4 (2.6%) | 0 (0.0%) | >0.9 |
| Candida | 7 (4.6%) | 1 (9.1%) | 0.4 |
| Hepatitis B | 5 (3.3%) | 1 (9.1%) | 0.3 |
| Herpes simplex virus HSV | 2 (1.3%) | 0 (0.0%) | >0.9 |
| Pneumonia | 14 (9.2%) | 0 (0.0%) | 0.6 |
| Pneumocystis Jiroveci Pneumonia | 2 (1.3%) | 0 (0.0%) | >0.9 |
| Pulmonary tuberculosis | 101 (66.4%) | 5 (45.5%) | 0.2 |
| Extrapulmonary tuberculosis | 28 (18.4%) | 4 (36.4%) | 0.2 |
| Cryptococcus neoformans | 7 (4.6%) | 5 (45.5%) | **<0.001** |
| Total CD4 count | 28 (12, 57) | 43 (17, 67) | 0.3 |
| Viral load (log10 Copies/mL) | 11.6 (7.6, 13.0) | 12.3 (12.3, 12.3) | 0.8 |
| Random cortisol | 519 (418, 640) | 281 (244, 341) | **<0.001** |
| Basal cortisol | 484 (411, 625) | 284 (201, 365) | **<0.001** |
| Stimulated cortisol | 791 (627, 916) | 375 (321, 426) | **<0.001** |
| Incremental Cortisol | 263 (179, 360) | 32 (17, 107) | **<0.001** |

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | ***Non-AI, N = 1561*** | ***AI, N = 111*** | **p-value2** |
| incremental\_cortisol\_percent\_increase | 51 (37, 73) | 12 (10, 42) | 0.033 |

### Predictors of mortality:

The increased odds of mortality were associated with skin pigmentation (51%) and poor appetite (77%). With respect to the lab tests random cortisol, ACTH were associated with increased mortality risk, whereas opiate use was protective against mortality.

The logistic regression analysis is seen in In Table 5. At, a 50 nmol/L increase in the basal cortisol was associated with a 14% increase in the odds of mortality (OR = 1.14, (95%CI:1.08, 1.21), ***p*<0.001**). Opiates were associated with a 45% reduction in the odds of mortality (OR = 0.55, (95%CI: 0.0.33, 0.87), ***p*=0.013**. Increased A 100 pg/L increase in the ACTH was associated with a 17% increase in the odds of mortality (OR = 2.17, (95%CI:1.35, 3.72); ***p<*0.003**). Patients increased skin pigmentation was associated with 51% increase in the odds of mortality (OR = 1.51 (95%CI: 1.03, 2.22) *p* = 0.033) while having a poor appetite was associated with a 77% increase of mortality (OR=1.77 (95%CI: 1.11, 2.90) *p* = 0.02)

**The multivariate analysis demonstrating factors independently predictive of mortality are shown in Table 5**.

After adjusting for several factors, as shown in Table 5, the only features which were associated with patient mortality were systolic and diastolic blood pressure, random cortisol and use of Opiates. Every 10 unit increase in Diastolic blood pressure was associated with a 30% increase in the odds of mortality (aOR = 1.30 (95%CI: 1.07, 1.59), p=0.01) after adjusting for other factors, as shown in Table 5. Every 10 unit increase Systolic blood pressure was associated with an 18% reduction in the odds of mortality (aOR = 0.82 (95%CI: 0.71, 0.95) *p*=0.010) after adjusting for other factors in the model as shown in Table 5. Every 50 nmol/L increase in random cortisol was associated with 14.7% higher odds of mortality (aOR = 1.147 (95%CI:1.082, 1.217) ***p*<0.001**) after adjusting for other variables in the table. While the use of opiates was associated with a 44.5% reduction in the odds of mortality (aOR = 0.555 (95%CI:0.33, 0.933), ***p*=0.026**) after adjusting for other factors, as shown in Table 5.

### Table 5: Bivariate and Multivariate analysis of factors associated with time to mortality of patients in the entire cohort.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristic** | **N** | **Bivariate** | | | **Multivariable** | |
| **OR***1* | **95% CI***1* | **p-value** | **Adj. OR3 (95% CI)1** | **p-value** |
| **History** |  |  |  |  |  |  |
| Increased\_pigmentation\_skin | **525** | **1.51** | **1.03, 2.22** | **0.033** | 1.26 (0.76, 2.08) | 0.379 |
| Poor\_appetite | **535** | **1.77** | **1.11, 2.90** | **0.02** | 1.25 (0.72, 2.19) | 0.425 |
| Liking\_for\_salt | 536 | 1.33 | 0.92, 1.95 | 0.13 | 1.27 (0.85, 1.90) | 0.244 |
| Nausea | **538** | **1.4** | **0.96, 2.05** | 0.08 | 1.08 (0.66, 1.78) | 0.754 |
| BP\_diastolic | 553 | 1.13 | 0.98, 1.30 | 0.088 | **1.30 (1.07, 1.59)** | **0.010** |
| BP\_systolic | 553 | 0.93 | 0.84, 1.03 | 0.2 | **0.82 (0.71, 0.95)** | **0.010** |
| **Investigations** |  |  |  |  |  |  |
| Haemoglobin | **558** | **0.92** | **0.85, 1.00** | 0.06 | 0.93 (0.85, 1.02) | 0.138 |
| GE\_c\_diff | 549 | 0.49 | 0.14, 1.32 | 0.2 | 0.61 (0.19, 1.91) | 0.394 |
| Random\_cortisol | **548** | **1.14** | **1.08, 1.21** | **<0.001** | **1.15 (1.08, 1.22)** | **<0.001** |
| Basal\_cortisol | 167 | 1.06 | 0.98, 1.15 | 0.13 |  |  |
| ACTH | **548** | **2.17** | **1.35, 3.72** | **0.003** | 1.25 (0.95, 1.65) | 0.115 |
| Cryptococcus\_neoformans | 549 | 1.72 | 0.79, 3.67 | 0.2 | 1.77 (0.71, 4.40) | 0.217 |
| Pneumonia | 549 | 0.66 | 0.34, 1.21 | 0.2 | 0.68 (0.35, 1.34) | 0.269 |
| cd4 | 557 | 0.77 | 0.56, 1.06 | 0.11 | 1.02 (0.70, 1.47) | 0.935 |
| vl | 126 | 1.08 | 0.98, 1.21 | 0.14 | 1.02 (0.95, 1.10) | 0.554 |
| **Medications** |  |  |  |  |  |  |
| Opiates | **563** | **0.55** | **0.33, 0.87** | **0.013** | **0.56 (0.33, 0.93)** | **0.026** |
| Fluconazole | 563 | 1.64 | 0.98, 2.71 | 0.056 | 1.61 (0.90, 2.87) | 0.108 |

1 Odds Ratio; 2 95% Confidence interval; 3 Adjusted Odds Ratio

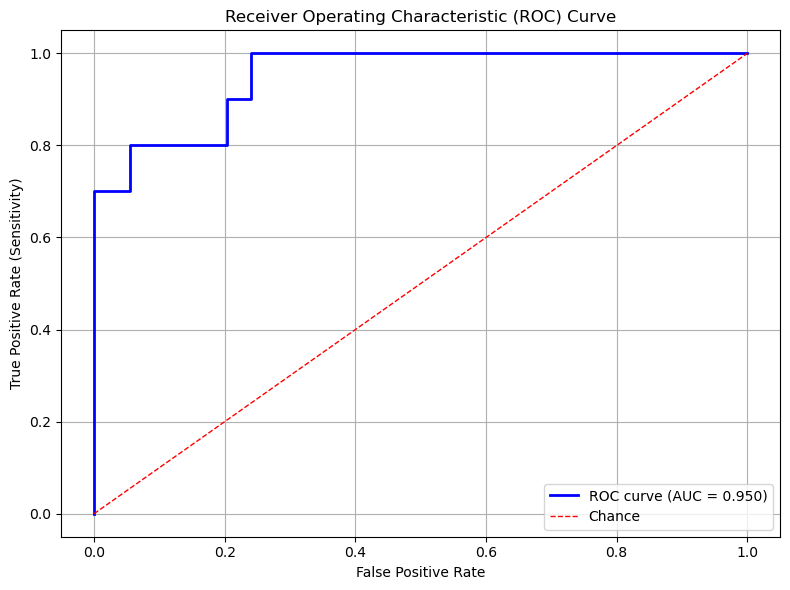
**A.I.-Based Prediction of AI**

In this study, we explored the utility of machine learning to predict adrenal insufficiency among 550 patients, of whom 43 were identified as having probable adrenal insufficiency. The dataset was divided into training and testing subsets, with 33 adrenal-insufficient patients and 453 non-adrenal-insufficient patients in the training set, and 10 adrenal-insufficient patients and 54 non-adrenal-insufficient patients in the testing set. After initial attempts with logistic regression proved unsatisfactory, a small fully connected neural network with two hidden layers (16 and 8 units, respectively) outperformed other approaches. As shown in **Table 1** (Performance Metrics of the Best Neural Network), this model achieved a training sensitivity of 1.00 and specificity of 0.95, and a testing sensitivity of 0.80 and specificity of 0.94.

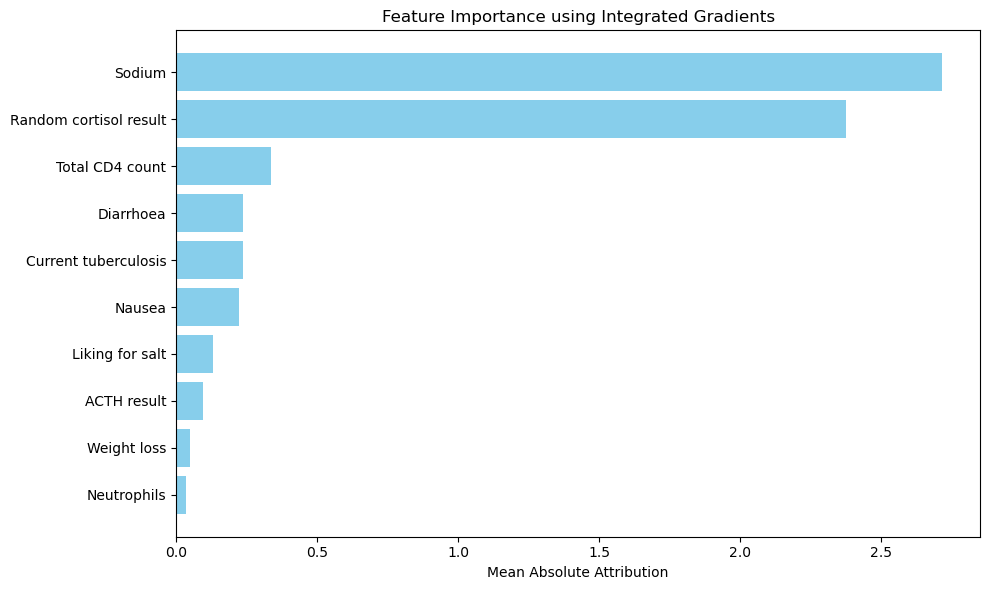
**Figure 1** (ROC Curve for the Testing Data; AUC = 0.950) illustrates the network’s strong discriminative capability. **Figure 2** (Feature Importances According to the Best Neural Network Model) highlights the relative influence of each variable, underscoring the high predictive value of Sodium and Random Cortisol Results. Though the limited size of this dataset precludes definitive conclusions, these proof-of-concept results underscore the promise of targeted machine learning strategies in anticipating adrenal insufficiency.

|  |  |  |
| --- | --- | --- |
| **Performance Metric** | **Train Dataset** | **Test Dataset** |
| **Accuracy** | 0.953 | 0.922 |
| **Sensitivity** | 1.000 | 0.800 |
| **Specificity** | 0.949 | 0.944 |
| **Precision** | 0.589 | 0.727 |
| **Recall** | 1.000 | 0.800 |
| **F1 Score** | 0.742 | 0.762 |
| **ROC AUC Score** | 0.994 | 0.950 |

**Table 1: Performance Metrics of the Best Neural Network**

****

**Figure 1: ROC Curve for the Testing Data (AUC = 0.950)**

****

**Figure 2: Feature Importances According to the Best Neural Network Model**

**Link to the code:**<https://anonymous.4open.science/r/adrenal-insufficiency-prediction-with-AI-F59C/>

## Discussion:

To our knowledge this is the largest study looking at the incidence of adrenal insufficiency and its mortality in patients with advanced HIV in Africa. Inspired by the knowledge that undiagnosed AI can be fatal in the event of complications, we sought to establish its presence and contribution to mortality in patients HIV and AIDS, with intention to mitigate this risk through early diagnosis and intervention. A 250 µg tetracosactide test was performed on 151 patients with random cortisol less than 500 nmol/L, using the Roche COBAS II ECLIA test. Using the 30 minute diagnostic cortisol concentrations cutoff’ of less than 500nmol/L, 35 patients were confirmed AI. Further breakdown of this total showed majority SAI (26) and PAI (9). In view of possible overdiagnosis of AI using the 500 nmol/L cortisol cutoff, lower cortisol cutoffs of 400 nmol/L and 340 nmol/L yielded, 18 and 12 AI patients, respectively.

Patients in the lower CD4 tertile were younger. Paradoxically, Sodium levels and Diastolic BP were higher in the AI versus the non-AI group. Overall, AI incidence in this cohort was 6.21% with a predominant SAI group. While there was significant vomiting in the PAI group, duration of illness was longer in the SAI versus PAI group, in contrast with Sharma et al who found that duration of illness was predictive of AI in HIV patients, with longer duration associated with PAI group. Overall, the most common opportunistic infection was tuberculosis at (84%), followed by pneumonia (11.35%) and candida (6.2%). The most common OI in the Non-AI group was PTB whereas Extra pulmonary tuberculosis and cryptococcus predominate in the AI group.

As expected, the WCC, lymphoctes, and neutrophils were lowest in the lowest CD4 tertile. The neutrophil counts were lower in the AI versus the non-AI group, though not significantly so. AI associated Natural killer cell cytotoxicity (NKCC) dysfunction has been associated with increased infections leading to increased mortality (19). The observed significant cryptococcal fungal infection in the adrenal insufficiency group may be explained by both numeric and functional decline in lymphocytes. There was significantly higher fluconazole, and opiate use in the AI group, both of which can reduce enzyme activity, leading to lower adrenal cortisol production.

Mortality was higher in the AI versus Non-AI group, though not significant. The causes of death in 64% the AI group were pneumonia, cryptococcal meningitis, liver disease, chronic kidney disease, seizures and sepsis. The rate of death began to increase after three months, and tuberculosis was the predominant cause of death in the non-AI group. The overall mortality was 27.50%, with no significant difference in the AI versus non-AI groups, at 33.3% and 26.9%, respectively. The increased odds of mortality was associated with skin pigmentation, nausea, poor appetite and liking of the salt., random cortisol and ACTH, whereas opiates use and CD4 increase were protective against mortality. Patients with adrenal insufficiency were associated with higher risk of extrapulmonary TB and cryptococcus neoformans, both of which are AIDS- defining illnesses and potential causes of AI. This begs the question, should the simultaneous presence of the two AIDS defining illnesses EPTB and cryptococcus infection become AI-defining?

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At multivariate analysis after adjusting for systolic BP, loss of consciousness, the only features which were associated with patient mortality were opiate use and elevated random cortisol. This finding is consistent with a study by Christ-Crain et al (20) in which elevated cortisol levels were associated with poor outcomes in AI patients with community acquired pneumonia (CAP) in ICU.

Despite the limited size of the dataset, the application of a small fully connected neural network provided encouraging evidence that key features—most notably Sodium and Random Cortisol Results—may be particularly influential in predicting adrenal insufficiency. The model demonstrated high sensitivity and specificity, and its receiver operating characteristic curve showed a strong area under the curve (AUC = 0.950), underscoring its capacity to discriminate between individuals at heightened risk. These findings suggest that a targeted machine learning approach could complement standard clinical evaluations and laboratory tests for the early identification of adrenal insufficiency. Nonetheless, the modest cohort size and variations in feature documentation limit the broader generalizability of these preliminary outcomes, making further research with larger, more comprehensive datasets essential to confirming and refining the practical utility of such models in clinical settings.

Strengths and Weaknesses:

**This is the biggest study on patients with advanced HIV Africa confirming AI, its associated aetiologies and mortatlity.**

The weaknesses

**A small AI sample size.**

**It is a single center study and may thus its findings may not be generalizable.**

**Unavailability of the LC/MS which is the new GOLD Standard for steroid analysis.**

**Conclusion:**

The largest African study on hypoadrenalism in advanced HIV revealed the AI incidence of 6.21% and overall mortality of 26.9%. We feel that screening for AI is warranted in advanced HIV, to mitigate the risk of death. The majority of the opportunistic infections were tuberculosis, pneumonia and cryptococcal infection. Tuberculosis and cryptococcal infections invariably correlated with the aetiology of mortality and may be AI-defining illnesses in advanced HIV, while elevated cortisol was associated with increased odds of death. Early AI detection, appropriate treatment, sick-day treatment adjustments education and shorter initial follow up intervals of HIV positive patients with AI is advised to prevent post discharge death

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