4.0 DISCUSSION AND CONCLUSIONS

4.1 URINARY DIPSTICK SCREENING: LEUCOCYTURIA AND HAEMATURIA

This study found a high prevalence of leucocyturia and microscopic haematuria, in addition to proteinuria on urinary screening of ART-naive HIV infected outpatients. With regard to leucocyturia, the surprisingly high prevalence of 30.28% was second only to a Ugandan study which showed 44.1% (Andia et al., 2005). It appears that HIV infected patients in African countries have a much higher prevalence (16 - 44.1%) of leucocyturia than in developed countries (1-11%) but no studies have investigated why this occurs nor its potential significance (Andia et al., 2005, Janabi et al., 2002, Mortier et al., 2003, Smith et al., 1995, Wools-Kaloustian et al., 2007).

Based on current literature, this is the only African study that has investigated leucocyturia on dipstick by performing urine MCS. Specimens submitted for culture yielded an organism in 29.1% of cases, unlike the findings from HERS (HIV Epidemiology Research Study) which identified an organism in 60% of cultures (Smith et al., 1995). The corollary may be more significant in that 70.9% of urine cultures were sterile in this study compared with 40% in HERS, which offered no explanation for their findings.

There are no published studies to date that have investigated the causes of (sterile) leucocyturia in HIV infection, in particular, in African populations. Well known causes in the non-HIV infected population are listed below (Dieter, 2000):

- A recently (within last 2 weeks) treated UTI or inadequately treated UTI
- UTI with ‘fastidious’ organisms (chlamydia, mycoplasma)
- Renal tract tuberculosis
- Pregnancy
- Urethritis (STI)
- False negative culture due to contamination with antiseptic
- Contamination of sample with vaginal leucocytes (may be seen in STI)
• Interstitial nephritis: analgesic nephropathy, sarcoidosis
• Urinary tract stones
• Renal papillary necrosis: diabetes, sickle-cell disease, analgesic nephropathy
• Urinary tract neoplasm
• Polycystic kidneys
• Interstitial cystitis
• Radiation cystitis
• Prostatitis, epididymitis, orchitis
• Other reported associations include appendicitis, fever, systemic lupus erythematosus and Kawasaki disease

In HIV infected patients additional potential causes of sterile leucocyturia may be:

• Interstitial nephritis: directly due to HIV infection and / or due to drugs used in the management of HIV infection e.g. co-trimoxazole, atazanavir, tenofovir, indinavir, possibly efavirenz through a hypersensitivity reaction (Roling et al., 2006)
• In advanced disease, opportunistic genitourinary pathogens e.g. parvo-B19, herpes, cytomegalovirus, polyoma viruses, candida infection, aspergillosis of the renal tract (Heyns and Fisher, 2005)

After observing this pattern during screening, it became apparent that certain infections may be associated with sterile leucocyturia, namely TB (16.6%) and STI (11.1%). In those screened, only the group with sterile leucocyturia were asked about symptoms and signs of STI and TB. This introduced selection bias making the results difficult to interpret as there are no data on symptoms and prevalence of TB / STI in those screened without sterile leucocyturia. In addition, those with suspected STI were treated syndromically and no genital swabs or cultures were done to confirm the clinical diagnosis. It is interesting to note that in those with a diagnosis of TB and sterile leucocyturia, TB was diagnosed from organs other than the genitourinary tract. Haematuria, sterile leucocyturia and proteinuria have been described in association with genitourinary TB (GUTB) in
HIV (Golden and Vikram, 2005, Nzerue et al., 2000, Orakwe and Okafor, 2005). Because urine was not sent for TB culture we could not determine whether there was concomitant GUTB in these patients, possibly as part of disseminated disease.

It is obvious that many causes of sterile leucocyturia exist but whether these are different in the HIV infected population is unknown. There may be an association with concomitant infection (TB; STI), but these data can only serve as an hypothesis that needs to be explored in further research. This is potentially significant as sterile leucocyturia may act as a clinical indicator for screening this sub-group of patients for easily treatable conditions. The reasons for why leucocyturia is more common in ART-naïve HIV infected Africans, compared with studies from non-African countries is not clear. From published studies, a potential explanation may be that African patients tended to have more advanced HIV disease at screening. Similarly in this study, 84% of patients had AIDS making them more susceptible to opportunistic genitourinary pathogens, hence the leucocyturia (Andia et al., 2005, Borkan et al., 1992, Janabi et al., 2002, Mortier et al., 2003, Smith et al., 1995).

Microscopic haematuria occurred in 33.05% of those screened in this study, a relatively high prevalence when compared with other studies: 1.6% (Janabi et al., 2002); 15.5% (Borkan et al., 1992); 25% (Cespedes et al., 1995). This finding is considered acceptable in the presence of a urinary tract pathogen, which may be the explanation in most cases but further investigation was not performed. Having said this, isolated microscopic haematuria has been described in HIV infection and after investigation, no serious pathology was found in young asymptomatic patients with normal renal function (Cespedes et al., 1995). Cystoscopy in nine patients with dysuria, frequency and haematuria without demonstrable urinary tract infection (including CMV) showed congested bladder mucosae in all patients – the authors implicated HIV in the pathogenesis but bladder biopsy with testing for HIV protein was not performed (Elem and Patil, 1991). It may be plausible that uncontrolled HIV replication in uroepithelial tissue may be a source of isolated microscopic haematuria, but this has not been proven.
A positive urine culture prevalence of 7.1% of the total number screened, was found in this study. This is relatively low compared with published rates of 7-50% in those with AIDS (Heyns and Fisher, 2005; Kaplan et al., 1987). This low prevalence may be ascribed to the outpatient population setting for this study, compared with many published studies which were hospital-based. In the culture positive group, organisms identified in this study were: E.Coli (70%), Klebsiella species (12%), Staphylococcus aureus (5%) and equally small percentages of Enterobacter species, Enterococcus faecalis, Proteus mirabilis, Streptococcus agalactiae and viridans (2.5% each). A similar spectrum of organisms has been reported in other studies (Heyns and Fisher, 2005). In contrast however, to these studies, the following organisms were not seen in this population: Pseudomonas aeruginosa, Acinetobacter, Serratia and Salmonella (Heyns and Fisher, 2005). This may be a reflection of patterns of local pathogen exposure, inpatient versus outpatient populations or may be a manifestation of inadequate sample size. High levels of co-trimoxazole resistance occurred with E.coli and Klebsiella species (90 and 80% respectively). These findings concurred with published results, confirming that co-trimoxazole prophylaxis may not protect against UTI, most likely because of rapid emergence of uropathogen resistance, in spite of its efficacy against prevention of bacterial pneumonias and pneumocystis (Evans et al., 1995, Heyns and Fisher, 2005, Schonwald et al., 1999, Wiktor et al., 1999, Zachariah et al., 2002).

At present, urinary screening of new HIV infected patients in outpatient clinics is not considered standard practice. From the results of this study regarding leucocyturia, this means that 28.5% of patients had an abnormality that would not have been investigated or treated. Untreated UTI could potentially be a source for ascending infection in the urinary tract and septicaemia in the immunocompromised clinic attendee. In those with sterile leucocyturia, common and easily treatable conditions such as STI or TB could be detected and treated, with obvious benefit to patients.

Ongoing inflammation provoked by untreated genitourinary tract infections may promote access of HIV, either as free virus or in CD4-infected T-lymphocytes, through an inflamed uroepithelial barrier. In support of this hypothesis, it has long been known that HIV virions can cross epithelial cell line
barriers using transcytosis, the characteristic epithelial transcellular vesicular pathway. These transcytosed HIV particles can productively infect mononuclear cells located at the basolateral side of the epithelium (Bomsel, 1997). In-vitro studies with a human bladder cell line have shown that E.coli promotes inflammation through expression of cytotoxic necrotising factor type 1 (CNF1) which in turn upregulates cellular transcription of proinflammatory cytokines TNF-α, IFN-γ, IL-6 and IL-8 (Falzano et al., 2003). The expression of bladder epithelial cell line co-receptors, CXCR4 and CCR5, can be altered by the presence of proinflammatory cytokines. In-vitro studies have shown that increased expression of IFN-γ, favours expression of the macrophage-tropic CCR5 receptor, promoting epithelial infection with M-tropic strains of HIV (Dolei et al., 2000). This may have implications for patients who acquire super-infection with different strains of HIV and may play a role in promoting the systemic upregulation of proinflammatory cytokines.

It would have been useful to investigate the causes of all dipstick abnormalities more aggressively with better history taking, physical examination and ‘clean catch’ techniques for urine collection - especially in light of the unexpected levels of leucocyturia but this was not the primary aim of the study. Further studies comparing findings in this cohort with urine findings of those with less profound immunodeficiency and those who are HIV-negative may yield deeper understandings about the impact of immunodeficiency on the genitourinary tract. With the high prevalence of dipstick abnormalities found in this study, routine urine screening of all new patients attending ART clinics in South Africa should be considered standard practice with a potential algorithm (figure 4.2) for guidelines on management.

**4.2 URINARY DIPSTICK SCREENING: PROTEINURIA**

This study showed a high prevalence of dipstick positive proteinuria of 43.7% (253/578). Part of the high prevalence was due to the inclusion of dipstick testing for microalbuminuria, not routinely done due to their relative expense. The laboratory confirmed proteinuria in 76% of specimens submitted. Some specimens for microalbuminuria were excluded because the levels were too low for the definition of microalbuminuria applied in this study. The final analysis thus confirmed 27.3%
proteinuria on screening, of which 18.5% were microalbuminuric, 6.4% overtly proteinuric and 2.4% nephrotic.

There are few published studies on screening for detection of proteinuria (even fewer with microalbuminuria) in ART-naive HIV infected populations, particularly in Africa. A screening study was conducted in Kenya, applying similar exclusion criteria to those in this study. In total 23/373 (6.2%) outpatients tested ≥1+ for proteinuria on dipstick (equivalent to ≥ 300mg/l of proteinuria), which correlated with findings in this study of 8.8%. The mean CD4 count in the Kenyan study was 391 cells/mm$^3$ and 21.9% of patients had a CD4 count <200 cells/mm$^3$ (Wools-Kaloustian et al., 2007). In a study from Uganda, 60/299 (20%) of outpatients tested ≥1+ for proteinuria on dipstick and although CD4 counts were not done, 67.6% were classified as WHO clinical stage three disease. This study did not exclude those with co-morbid proteinuric disease such as uncontrolled hypertension and diabetes, possibly explaining the higher prevalence of proteinuria compared with this and the Kenyan study (Andia et al., 2005). In a Tanzanian study, albuminuria was present in 28.4% of HIV infected versus 16.8% of HIV non-infected patients and the mean CD4 count in HIV-infected patients was 289 cells/mm$^3$ (Janabi et al., 2002). It is interesting to note in these studies that the participants were at less severe stages of HIV infection than what was found in this study where 84% had AIDS and the mean CD4 count was 130 cells/mm$^3$. These findings may suggest that risk for HIV-associated renal disease is not necessarily related to disease progression.

Unfortunately the proteinuria was not quantified in any of these studies, proteinuric patients did not undergo renal biopsy to confirm HIV-associated renal disease, nor was screening done for microalbuminuria, which may have detected early renal disease. The surprising feature in the Tanzanian study was the prevalence of albuminuria of 16.8% in the non-HIV infected group, the causes of which were not mentioned.

Racial disparities within the spectrum of renal disease do exist. Studies in hypertensive black Americans have shown higher rates of microalbuminuria with a 6 – 18 fold increased risk of hypertensive renal failure than white participants who were matched for age, sex, blood pressure,
body mass index, diabetes, creatinine, duration of hypertension (Summerson et al., 1995). In the NHANES III (National Health and Nutrition Examination Survey) 20 050 participants were surveyed and it was shown that racial and ethnic minorities, both with and without diabetes have greater odds for albuminuria than whites, particularly if the estimated GFR was <60ml/min/1.73m². Independent risk factors associated with development of albuminuria were metabolic control of diabetes, systolic blood pressure, diuretic use, hypertensive treatment status and income (Bryson et al., 2006). A recent publication examined racial differences in microalbumin excretion in healthy adolescents. After a period of stress-induced pressure natriuresis, blacks had significantly higher albumin excretion rates (AER) than whites (Hanevold et al., 2008). It has often been debated whether these differences could be genetic or environmental. In the USA it has been proven that ethnic minorities are disadvantaged regarding access to and quality of health care, but in a study comparing AER in a diabetic population, racial / ethnic differences occurred in spite of comparable access to health care (Ramirez, 2005, Young et al., 2005). Most studies describe the findings in African Americans compared with Caucasian populations. Studies in sub-Saharan African blacks are scarce. Some studies among diabetic populations showed varying AER from 10.7 – 57% but do not compare prevalence to a control Caucasian population (Lutale et al., 2007). A study from Cape Town showed increased AER in non-diabetic blacks with features of metabolic syndrome, but no comparison was made with a Caucasian population (Okpechi et al., 2007). The paucity of local data highlights the need for epidemiological studies in sub-Saharan African black populations to elucidate the prevalence and significance of albuminuria in the general population and in those at risk for renal disease such as diabetes, hypertension and HIV.

Mention needs to be made regarding the definitions of microalbuminuria used, which have not been validated in black Africans nor in HIV infected populations. In this study, with specific regard to microalbuminuria, there appeared to be little difference between various definitions applied to the data (figure 3.4), particularly the use of albumin concentration (mg/L) versus the microalbumin: creatinine ratio (mg/mmol). The reason for including creatinine in the ratio is to correct for muscle bulk, thus correcting for differences between men and women and for example, conditions with
severe muscle wasting – as seen in advanced HIV disease (de Jong and Curhan, 2006). Analysis was performed on the microalbuminuric group to assess whether there was any correlation between weight (as a surrogate marker for muscle bulk) and albumin concentration or weight and the microalbumin: creatinine ratio and no correlations were found (figure 3.5). These data were not sufficiently powered to undergo analysis for statistical significance between various definitions used but they do suggest that further studies should be done to evaluate various definitions of microalbuminuria in black African HIV infected populations. Of most significance from this data is that urinary albumin concentration was equivalent to the albumin: creatinine ratio but the former is cheaper, easier to perform and may be sufficient for mass screening programmes, particularly in resource-limited settings.

Microalbuminuria was found in 107/578 (18.5%) of those screened, which is similar to results of other studies, although the numbers in this study are much larger. Two studies showed microalbuminuria in 14/72 (19.4%) and 9/48 (19%) patients (Luke et al., 1992, Monje et al., 1996). Microalbuminuria in the study by Luke et.al. correlated with lower CD4 and white cell counts, and higher levels of TNF-alpha and beta 2-microglobulin, suggesting an association between AIDS progression and microalbuminuria (Luke et al., 1992). The study by Monje et.al. corroborated Luke’s findings with a positive correlation between microalbuminuria, HIV p24 antigenemia and levels of beta 2-microglobulin (Monje et al., 1996). Unfortunately levels of TNF-alpha and beta 2-microglobulin were not measured in this study. Both of these studies did not investigate co-morbid pathology nor follow up patients for persistent microalbuminuria and renal biopsies were not done. Fortunately in this study, aside from larger numbers screened, the above investigations were performed.

This study is the first to show that co-morbid disease in HIV-infected patients with microalbuminuria (69.4%) is common and that by treating co-morbid disease, particularly infection, microalbuminuria may be reversible – seen in 17% of cases. The percentage of those with reversible microalbuminuria may have been underestimated because of lack of follow up: many were
excluded for various reasons on first screening and 26% of those screened were lost to follow up. It was previously thought that the onset of microalbuminuria heralded an inexorable decline in renal function in diabetic populations but it has been shown that regression may occur with effective interventions (Perkins et al., 2003). This has not previously been shown in the setting of HIV infection. This finding is important as the significance of microalbuminuria, whether persistent or transient, in the setting of HIV infection is still to be determined.

With regard to persistent microalbuminuria in this study, those with persistent microalbuminuria on follow up comprised 11/107 (10.3%) which correlated with the study by Han et.al. in which 6% had persistent proteinuria (Han et al., 2006). Of those who underwent renal biopsy (8/11), all had renal pathology on histology, which was also seen in the biopsy series from Durban (Han et al., 2006). These data suggest that a single screening microalbuminuria may have different implications from persistent microalbuminuria in HIV. A single positive microalbumin may correlate with advanced HIV disease and possibly co-morbid disease but whether this translates to an increased risk for morbidity and / or mortality is unclear. It would have been interesting to see whether there was any correlation between the rapid resolution of microalbuminuria and the suppression of HIV viral load with ART in this group. In contrast, persistent microalbuminuria correlated in both this and the study by Han et.al. with the presence of established HIV-associated renal disease on histology in 100% of cases (Han et al., 2006). The subjects were not followed for a sufficiently long period of time to determine whether the presence of established renal disease with microalbuminuria translated to reduced renal or patient survival. This highlights the need for long term prospective trials to address these issues.

Microalbuminuria has been associated with increased risk of cardiovascular disease, death and cardiac failure in both diabetic and non-diabetic populations (Gerstein et al., 2001). As an independent risk factor, the link between cardiovascular risk and albuminuria may be systemic endothelial dysfunction (ED) (Clausen et al., 2001). A recent study showed a five-fold increase in the prevalence of microalbuminuria in HIV infected individuals on ART compared with HIV negative
controls (Szczech et al., 2007). Those with HIV infection and microalbuminuria had more known cardiovascular risk factors: insulin resistance, systolic hypertension, and family history of hypertension, than the matched HIV controls. Studies assessing systemic ED (by assessing flow-mediated dilation of the brachial artery) in HIV infection have had contradictory outcomes. A study in HIV infected children showed that HIV infection, with and without treatment was associated with ED in the absence of traditional risk factors (Bonnet et al., 2004). Another study showed that HIV patients on ART had significant ED compared with HIV negative controls. The ART naïve HIV infected group also had ED but the effect was not statistically significant (Blanco et al., 2006). Other studies have not confirmed these findings and in fact have shown that the use of lopinavir/ritonavir may improve endothelial function (Gupta et al., 2007). The point must be made that potential concerns regarding increased cardiovascular and /or other risk with PI regimens in HIV infected patients have not been validated in long term studies, perhaps in part, because of the relatively short clinical history of these drugs. These concerns have driven strategies such as structured treatment interruptions to reduce drug exposure. The SMART study has however shown that uncontrolled HIV replication and its cardiovascular and renal effects (possibly through ED) are more detrimental than the effects of sustained treatment (Neaton, 2007). A potential hypothesis from this study data may be that persistent inflammation due to HIV infection, often in combination with TB and / or recurrent bacterial or opportunistic infections may promote systemic ED. There may be additional co-morbid diseases in HIV patients such as DM or hypertension which are independently associated with ED. As a result, glomerular ED may manifest clinically as microalbuminuria and less frequently, overt proteinuria. The final common pathway, independent of aetiology, is the upregulation of cytokines that link these different pathological processes (figure 4.1).

It has been shown that when HIV binds the CD4 receptor, peripheral blood monocytes are stimulated to release TNF-α and interleukin (IL)-1 (Merrill et al., 1989). HIV infection is also associated with increased serum levels of IL-18, which rise with increasing viral load. Interleukin -18 is a potent inducer of TNF-α, IL-1-β and IFN-γ, with TNF-α in turn stimulating further production of IL-1 (Orphanidou et al., 1996, Wiercinska-Drapalo et al., 2004). Similarly, TB is associated with
increased levels of TNF-α, IL-1 and IL-6 – with increased IL-6 levels due to enhanced transcription of the IL-6 gene by mycobacterial protein lipoarabinomannan (LAM)) (Zhang et al., 1994). Recurrent bacterial infection and opportunistic infection, common in advanced HIV, are also associated with increased levels of these cytokines as part of the acute phase response to bacterial cell wall lipopolysaccharide. All of these cytokines are potent stimulators of inflammation and TNF-α is a potent inducer of endothelial as well as macrophage production of IL-1 (Orphanidou et al., 1996). It is interesting to see that the link between ED, inflammation and increased circulating levels of IL-18, TNF-α and IL-6 has been well described in atherosclerosis, insulin resistance, metabolic syndrome and type 2 diabetes (Moriwaki et al., 2003). The end result of the various disease processes is upregulation of proinflammatory cytokines.

Figure 4.1 Potential mechanisms for endothelial dysfunction in HIV – a final common pathway
In support of this hypothesis, it is noteworthy that patient 12 had overt proteinuria which regressed within two weeks of corticosteroid and TB therapy without any ART (protein: creatinine ratio: 1.2g/mmol reduced to microalbumin: creatinine ratio: 9.7mg/mmol). Another potential mechanism may be due to the responsiveness of podocytes to corticosteroids. In-vitro work on a human podocyte cell line by Xing et al., showed that dexamethasone augmented podocyte survival, increased podocyte expression of nephrin and tubulin-α which are essential for the function of the slit diaphragm and suppressed expression of interleukin 6 by podocytes (Xing et al., 2006).

In the group with overt proteinuria, in comparison with the microalbuminuric group: infection, similarly, was the most common co-morbidity and hypertension, diabetes and malignancy comprised the remainder of co-morbid disease. The notable differences in this group were that CKD featured more prominently (23 vs.10.3%); resolution of proteinuria was less frequent (5.4 vs 17%) and those requiring renal biopsy almost doubled (18.9 vs 10.3%). The prevalence of those with reversible proteinuria in this group may have been underestimated for the same reasons as the microalbuminuric group. This finding suggests that proteinuria be confirmed in a second test before considering renal biopsy, especially in those with co-morbid disease. In the nephrotic group, there was little co-morbid disease, no detected infection and all had CKD. Based on these results, it would be prudent for clinicians to refer those with persistent, unexplained microalbuminuria, persistent overt proteinuria and all those with nephrotic proteinuria to a nephrologist for further investigation and possible renal biopsy as the prevalence of renal disease in these groups approaches 100%.

Urinary screening for the presence of kidney disease in ART clinics in South Africa is not considered standard of care. These data suggest that routine urine dipstick screening be performed on all new patients attending ART clinics, an easy and affordable intervention.
**Risk factor assessment for kidney disease:**
Race, family history of kidney disease, WHO criteria for AIDS, co-morbid disease: diabetes mellitus, hypertension, CD4 count\(^1\), HIV-viral load\(^1\), hepatitis B and C co-infection\(^1\), serum creatinine\(^1\), calculate estimated GFR\(^2\)

**Urine dipstick:** if leucocytes and/or nitrites send for MCS\(^3\)
If leucocytes present look for following and repeat dipstick at follow up:
- Symptoms of UTI\(^4\) → empiric treatment for UTI
- Symptoms of STI\(^5\) → syndromic management, syphilis serology
- Symptoms of TB\(^6\) → investigate, treat if indicated

**Urine dipstick:** if positive for proteinuria:
Excluding other causes: fever, infection (especially TB\(^5\)), pregnancy, uncontrolled diabetes, hypertension, cardiac failure. Start ART and repeat dipstick at next visit
- If dipstick still positive for protein and/or creatinine clearance <60 ml/min/1.73m\(^2\): kidney ultrasound\(^1\), spot urine for protein:creatinine ratio\(^1\) and refer for investigation/renal biopsy
- If unable to refer: ART\(^7\) (dose adjust for kidney failure) and monitor response
  - If still proteinuric after 3 months on ART: check K\(^+\) start ACE-I\(^8\)
  - Long term management as for CKD

Urine dipstick: if negative for proteinuria:
- Annual dipstick in those at risk for development of kidney disease

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\(^1\)If test available  
\(^2\)Calculate estimated glomerular filtration rate (GFR) using Cockcroft and Gault formula  
\(^3\)MCS: microscopy, culture, sensitivity  
\(^4\)UTI: urinary tract infection  
\(^5\)STI: sexually transmitted infection  
\(^6\)TB: tuberculosis (pulmonary and extrapulmonary)  
\(^7\)ART: antiretroviral therapy according to local protocol  
\(^8\)ACE-I: angiotensin-converting enzyme inhibitors

*Figure 4.2 Management algorithm for urinary screening of HIV infected ART-naïve patients*
The Infectious Diseases Society of America (IDSA) published guidelines to this effect in 2005 (Gupta et al., 2005). Their recommendation is that all individuals be assessed for kidney disease at the time of diagnosis of HIV infection with a screening urine analysis for proteinuria and a calculated estimate of renal function. This allows for early detection of renal disease and dose adjustment of ART and/or other commonly used drugs in HIV infection. The IDSA does not however include a management approach for additional abnormalities found on urine dipstick during screening, which were frequent in this study. Based on these findings, an algorithm (figure 4.2) has been proposed to include not only screening for renal disease, but also management of additional abnormalities found on urinary screening which seems more appropriate for our population.

4.3 PERSISTENT PROTEINURIA, RENAL BIOPSY AND FOLLOW UP

To date, there are no published studies, to the author’s knowledge, that have:

- Prospectively assessed the effect of ART with/out ACE-I on HIV-associated renal disease in ART-naïve patients
- Included in the prospective study, histological lesions other than HIVAN and documented the renal response to treatment with histology pre- and post initiation of ART

4.3.1 EFFECT OF ART WITH/OUT ACE-I ON HIV-ASSOCIATED RENAL DISEASE IN ART-NAÏVE PATIENTS

Statistical analysis showed that there was a significant response to the treatment intervention, both immunologically and with regard to renal function, which has not been previously documented. In the assessment of renal response to ART two parameters were used, resolution of proteinuria and improvement in eGFR. In the microalbumiuric group, the regression of microalbuminuria was rapid (figure 3.11) with most reduction occurring within the first three months of treatment. It is important to observe that none of these patients were on ACE-I within the first three months of treatment suggesting the resolution of microalbuminuria was directly related to ART. This response may be correlated with the effect of ART on viral replication, potentially supporting the findings in the SMART study. In the other groups, ACE-I therapy was either initiated at the time of ART or soon
after commencement of ART because of the severity of proteinuria. The decline of proteinuria in the nephrotic group was also rapid and occurred predominantly in the first three months but it is difficult to assess the independent effects of ART versus ACE-I (figure 3.17). For the assessment of estimated GFR (eGFR), using two different formulae, statistical significance was achieved with both the modified MDRD equation and the Cockroft and Gault formulae. This is encouraging, because the Cockroft and Gault formula is easier to use but thought to be less accurate for assessing eGFR in African Americans. The numbers used in the analysis are small and the findings need to be verified in larger studies before interpreting these results as significant.

4.3.2 HISTOLOGICAL LESIONS AND ASSESSMENT OF HISTOLOGICAL RESPONSE TO TREATMENT

In the microalbuminuric group, of eight biopsies done, none had HIVAN on histology. This is in contrast with the findings from Durban where 6/7 biopsies in the microalbuminuric group demonstrated HIVAN (86%) (Han et al., 2006). In this series looking at all ranges of proteinuria, HIV-associated immune complex disease (HIV-ICD) (8/20) was more frequent than HIVAN (1/20), with 1/20 as a combination of HIVAN and HIV-ICD. This again is in contrast with the study from Durban, where 19/23 of those with proteinuria had HIVAN (Han et al., 2006). These findings are more in keeping with those published by Gerntholtz et al., where HIVAN comprised 27% and HIV-associated immune complex disease (HIV-ICD) 21% of biopsies (Gerntholtz et al., 2006). Both these findings and those from Gerntholtz et al. contradict assumptions made by researchers in the developed world that HIV-ICD is more common in Caucasians (Nochy et al., 1993). What compounds the data in this study is that some of those with HIV-ICD had other infections that may have contributed to their renal disease. One patient with both hepatitis B and C declined ART and demised. Two other patients with hepatitis B received a lamivudine containing regimen and demonstrated an impressive response to treatment with respect to eGFR and regression of proteinuria. It is possible that both HIV and hepatitis B were treated concomitantly. Staining of the specimens for both hepatitis B and HIV DNA/RNA may clarify which infection, if not both, contributed to the renal disease. Two others had hepatitis C, one of which showed a remarkable
response to ART, from being dialysis dependant to having a normal eGFR and complete resolution of proteinuria over a period of 3 months. Clinically this may suggest the renal disease was more likely to be HIV-related, but on repeat biopsy, there was only partial regression of the immune complex disease - suggesting perhaps that part of the immune-complex deposition may be Hepatitis C related. The other patient required high dose ACE-I to induce remission of proteinuria and demonstrated progression of his renal lesions on histology, suggesting again that renal disease may be hepatitis C-related.

It is important to note that 9/20 biopsies had mesangial and interstitial changes that did not fulfil histological criteria for HIVAN but clearly showed a response to ART on follow up biopsy. On follow up histology, the mesangial disease appeared more responsive to ART when compared with interstitial disease, a finding not been previously described. This suggests the current histological classification may not be accurate nor sufficiently comprehensive. It may be with screening and detection of earlier disease, we are seeing a different spectrum of HIV-associated renal disease that if untreated, would evolve into typical histological patterns that have been well described.

The striking feature in this study, again, not previously described, has been the relatively rapid response of proteinuria to treatment across all three groups: microalbumin, overt and nephrotic proteinuria. Most patients showed regression to normal levels of urinary protein excretion within 3-6 months of treatment regardless of severity of proteinuria. The proteinuria may, in part, be mediated by pro-inflammatory cytokines and regression of proteinuria achieved by suppression of HIV viral replication and damping down the pro-inflammatory cytokine milieu. Studies have shown a correlation with microalbuminuria and levels of TNF-alpha and beta 2-microglobulin, thought to be markers of advanced HIV-1 disease (Luke et al., 1993, Monje et al., 1996). In this study most patients had AIDS with a high HIV-1 viral load, confirming advanced HIV disease. It would be interesting to track regression of proteinuria with suppression of HIV viral load and levels of other pro-inflammatory markers in both blood and urine to determine whether there is a causal association.
What is noteworthy is the lack of correlation between resolution of proteinuria and histological changes on follow up biopsy. The nephrological community assumes that certain clinical indicators, such as proteinuria and eGFR are accurate markers for resolution of renal disease. Perhaps these assumptions need to be questioned. This study demonstrated that the immunological (viral load and CD4 count) and renal (proteinuria and eGFR) response to treatment was in most cases relatively dramatic when compared to the histological response, which in some cases was unchanged or showed only partial regression. Only one patient 17 (SN) showed complete regression of glomerular and tubular lesions on follow up biopsy, but the interstitial component of the disease persisted. This contrast was most clearly captured in the serial biopsies from patient 3 (EM). He demonstrated complete resolution of microalbuminuria within 2 months of ART but slow, progressive and still incomplete resolution of immune complex disease at 25 months follow up on histology. In this study, proof of virological suppression was obtained by monitoring HIV viral load in peripheral blood, which was achieved in all patients who adhered to their treatment regimens. It may not be safe to assume that virological suppression in peripheral blood necessarily correlates with suppression of replication in renal tissue or that resolution of proteinuria correlates with histological regression of renal disease. One explanation may be that ART does not penetrate, or only partially penetrates the renal compartment or that HIV viral resistance develops independently within the renal compartment. At present, there is no non-invasive way to determine whether virological suppression has been achieved in the renal compartment. An alternative explanation is that changes at an ultrastructural level have occurred in response to ART that were not depicted on light microscopy.

It is planned that further immunohistochemical staining/processing of the current specimens will be performed as part of the doctoral component of this work to compare before and after treatment biopsies for the following:

1. HIV-RNA, to confirm whether ART prevents viral replication in renal tissue, in spite of achieving confirmed viral suppression in blood
2. Pro-inflammatory cytokines known to be upregulated in HIV-associated renal disease: TGF-beta, TNF-alpha, interleukins 6, 8 are known to stimulate mesangial matrix deposition – this may be related to the mesangial changes seen in the biopsies; FGF-2 has been implicated in interstitial disease

4.4 CONCLUSION

In ART-naïve HIV infected patients screened at the HIV clinic, urinary abnormalities, specifically proteinuria (including microalbuminuria), leucocyturia (+nitrites) and microscopic haematuria were common on dipstick testing, at a prevalence of 44%; 30.28% and 33.05% respectively. In those who underwent renal biopsy, ART with/out ACE-I showed a statistically significant immunological and renal response to treatment. The response of proteinuria to treatment was relatively rapid and sustained but the histological response on follow up biopsies did not correlate with clinical parameters used to assess renal function. HIV-associated immune-complex disease was more prominent than HIVAN and a number of biopsies showed mesangial lesions that could not be classified according to current histological criteria, but did show response to treatment.

This study has confirmed, unequivocally, that HIV-associated renal disease is treatable especially if detected early. In addition to answering some research questions, it has also proposed more unanswered questions which need to be investigated. The ideal forum for further work in the field of HIV-associated kidney disease would be a multi-centre study involving collaboration between sub-Saharan African countries – for both clinical and laboratory research. It is hoped, with the results from this research report and others in sub-Saharan Africa, that a working group for the compilation of local guidelines for early detection and treatment of kidney disease in HIV can be established that would be appropriate for our population.