# A retrospective review of Granulomatous Interstitial Nephritis (GIN) amongst HIV-infected patients at Groote Schuur Hospital Cape Town.

**By**

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# DECLARATION

I, ***Debbie Nel***, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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# Abstract

**A retrospective review of Granulomatous Interstitial Nephritis (GIN) amongst HIV-infected patients at Groote Schuur Hospital Cape Town .**

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**Introduction:** High numbers of granulomata have been identified on kidney biopsy at Groote Schuur Hospital in HIV positive patients. In the literature granulomatous interstitial nephritis (GIN) is most commonly attributed to sarcoid and drug reactions and occurs in 0.5-1.37% of kidney biopsies. Current data is only from developed countries and rarely in HIV positive individuals. As our yield of GIN appeared high we retrospectively reviewed the established HIV database of kidney biopsies to establish the likely causes of this histological finding in our HIV positive population. An extensive literature review was also performed with the intention of developing a diagnostic, and therapeutic, algorithm applicable to GIN in a South African setting.

**Subjects and Methods:** A database of 370 HIV positive kidney biopsies dating from January 2005 was retrospectively reviewed. All patients with GIN on kidney biopsy were analysed. Medication history, creatinine, urine protein/creatinine ratio, CD4 count and serological evidence of vasculitis were recorded. A radiological evaluation and search for positive TB cultures was performed. Patients were divided according to the likely aetiology of GIN, ranging from least to most likely TB-GIN, together with the likelihood of a drug-induced or ascending infection-related aetiology. Mortality data was obtained from reviewing the Clinicom system and patient records. Ethics was granted from the UCT ethics committee.

**Results:** 45 patients (12.2%) had evidence of (GIN). 26 (57.8%) were female. Median age was 33 years (IQR 29-37). TB-GIN was likely in 62.2% of patients .Median CD4 was 126 cells/mm3 (IQR 54-237). There were 6 cases of possible paradoxical TB IRIS identified. [median CD4 count of 74 cells/mm3 (IQR 36-170)]. 49% of patients were on a drug implicated in GIN, with 11% on >1 drug [The most common drug being cotrimoxazole]. 6 patients had evidence of ascending infection. No patients had vasculitis.14/45 (31%) patients died on follow up with a median time to death of 119 days (IQR 30-444 days).

**Interpretation:** GIN is common in our HIV population. TB is the most likely cause however other aetiologies require consideration, especially drugs. TB IRIS should be considered if cART has been recently initiated and the CD4 count is low. A proposed diagnostic algorithm was developed as part of this study, together with treatment guidelines. Further research is needed to evaluate the utility of these in a clinical setting.

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# Introduction

The introduction of combined antiretroviral therapy (ART) has changed the face of Human Immunodeficiency Virus (HIV) infection (Wyatt et al., 2006: 561), particularly in the developed world where antiretroviral therapy has been available since the mid-1990's. In contrast to developed countries, the rollout of antiretroviral therapy in South Africa only started in 2004 and although the roll out has been very effective, there are still significant numbers of patients who are unable to access this therapy.

According to statistics in 2011, 1.6 million of the 5.5 million HIV-infected people in South Africa were receiving ART (South Africa. Department of Health, 2012:51) (25% of all those who qualified for ART were not receiving this treatment). (South Africa. Department of Health, 2012:64). In developed countries, improved survival and slower rates of progression to Acquired Immune Deficiency Syndrome (AIDS) has increased the number of people living with HIV (Jones et al.,2007:991), and the incidence of opportunistic infections has declined. (Kalim, Szczech & Wyatt, 2008:556) As a result, there has been heightened interest in the non-infectious comorbidities in HIV-infected patients for example diabetes, hypertension, chronic obstructive pulmonary disease (COPD) and others. (Post &Holt, 2009:43) In South Africa, opportunistic infections still contribute significantly to the morbidity and mortality amongst HIV-infected patients while, at the same time, the prevalence of the above chronic conditions is increasing as ART becomes more widely available.

HIV infected patients are at an increased risk of developing kidney disease compared with the general population (Dellow, Unwin & Miller, 2000:71), and evidence of kidney dysfunction at the time of antiretroviral initiation has been found to be an independent predictor of mortality in this population. (Szczech et al., 2004:1203)

The spectrum of kidney disorders recognised in HIV-infected individuals is broad. While HIV-associated nephropathy (HIVAN) is the classic kidney pathology seen in HIV-infected individuals, (Vali et al., 2012:98) (Wearne et al., 2012) drug toxicities, acute tubular necrosis (ATN), lymphoma, granulomatous inflammation, glomerulonephritides unrelated to HIV infection, and others are increasingly being recognised. (Wearne et al., 2012) (Fine & Atta, 2007) Kidney biopsy is often necessary to determine the underlying diagnosis and guide treatment. (Wearne et al., 2012) (Gerntholtz, Goetsch & Katz, 2006) (Fine & Atta, 2007:814)

Regardless of the aetiology, early identification, accurate diagnosis and subsequent appropriate management result in improved outcomes. (Arendse, Okpechi & Swanepoel 2011: 44) (Fine &Atta, 2007: 814) (Wearne et al., 2012)

***Biopsy series amongst HIV-infected individuals performed at Groote Schuur Hospital (Wearne et al., 2012)***

Three kidney biopsy studies have been performed in South Africa amongst HIV-infected patients (see literature review). The biopsy series performed by Wearne et al. at Groote Schuur Hospital (GSH) in Cape Town is the largest study performed to date (221 biopsies) and serves as the basis for the current study. (Wearne et al., 2012) A database has been set up with new patients being enrolled as kidney biopsies are performed. There are currently 370 patients on the database. During data analysis, it was noted that 45 (12.2%) patients were found to have evidence of interstitial granulomata on biopsy (granulomatous interstitial nephritis, GIN). This is a **much higher** frequency than has been previously reported. (0.5 (Bijol et al., 2006:58) to 1.37%) (Javaud et al., 2007:171)

GIN is a variant of acute interstitial nephritis (AIN) in which granulomata (one or more distinct aggregates of epithelioid cells, with or without multinucleate giant cells) (Kim & Hee Sung, 2010) (Joss et al., 2007) (Chapagain et al., 2011) are seen in the kidney interstitium, often surrounded by inflammatory infiltrate. (Bijol et al., 2006) This high rate of GIN in HIV-infected patients has not been previously noted in the literature, and no studies have been performed in this group of patients related to possible aetiology and/or outcome of GIN.

There are many causes of GIN (see table 3 literature review), with most of the information in the form of case reports. In the larger reviews (all performed in developed countries) drug reactions and sarcoidosis accounted for the majority of cases, with numerous cases being documented as 'idiopathic' (i.e. no cause found after extensive investigation). (Bijol et al., 2006) (Javaud et al., 2007) (Mignon et al., 1984) (Viero & Cavallo, 1995) (Joss et al., 2007) Infections accounted for only 8% of the cases of GIN in these reviews, (Javaud et al., 2007) (Mignon et al., 1984) (Viero & Cavallo, 1995) with mycobacterial infections accounting for only 6% (Mignon et al., 1984) (Javaud et al., 2007) (TB in 4%). One might expect, particularly with the high tuberculosis (TB) and HIV burden in South Africa that infectious causes might account for a higher proportion of cases in this population when compared to developed countries. This has been noted amongst kidney transplant recipients, where mycobacterial and fungal infections are a major causative factor of GIN. (Chung et al., 2009:456) Only 2

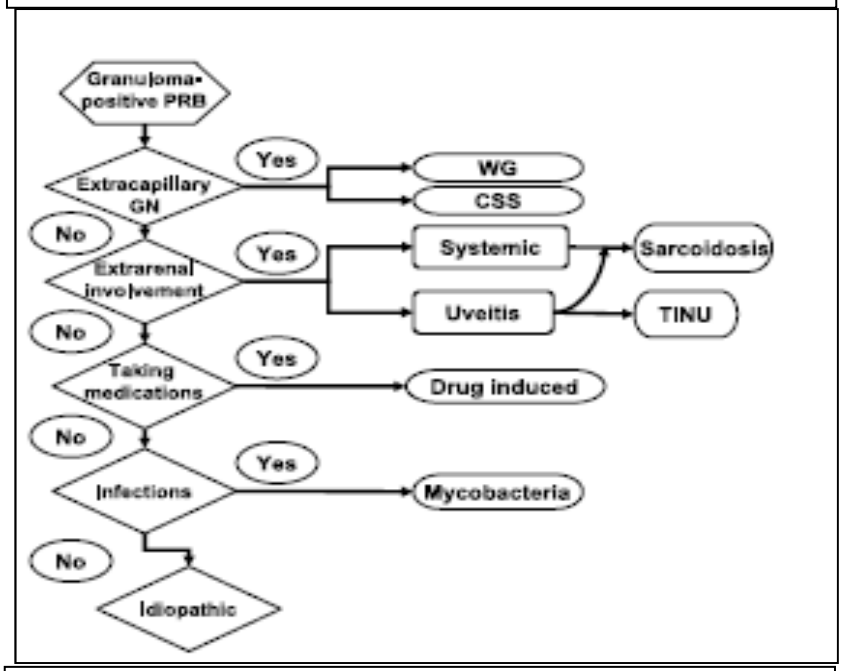
patients in the aforementioned studies were noted to be HIV-infected. (Javaud et al., 2007) The aetiology of the GIN in both of these patients was found to be infective. (Javaud et al., 2007)

The high prevalence of GIN in HIV-infected patients has proved a diagnostic dilemma for nephrologists at GSH. There is a broad differential diagnosis and currently there is no diagnostic algorithm to assist in the approach to this disease. This is compounded by the fact that many patients with GIN have evidence of TB elsewhere and the cause of GIN is hence attributed to TB with no further tests done to prove (or dispute) the diagnosis of TB-related GIN (TB-GIN). It is possible, therefore, that other (potentially treatable) aetiologies are being overlooked (e.g. drug reactions and other infections). Immune reconstitution inflammatory syndrome (IRIS) is another possibility in those patients recently started on ART. This has been shown, in case reports, to have a favourable response to corticosteroid therapy. Another complicating factor is that kidney biopsy specimens are not routinely sent for bacterial, mycobacterial or fungal culture. Therefore the definitive exclusion of infectious causes of GIN remains difficult.

There is evidence that GIN heals with fibrosis (Kaul et al., 2011:385) and that the cautious use of corticosteroids may decrease the extent of the inflammation in the acute phase and subsequent transformation to fibrosis. (Kaul et al., 2011:385) However, steroids should only be considered if an infectious aetiology (other than TB) has been excluded. The diagnosis of TB in the setting of GIN is difficult as the yield of urine cultures is suboptimal, and can take 6-8 weeks for a positive result. (Hemal et al., 2000:571) (Sun et al., 2010:341) (Colbert, Richey & Schwartz, 2012:238) There are newer, more rapid diagnostic tests for TB, which have not yet, been studied in the setting of GIN. These have been reviewed further in the following literature review, and may be included in a possible diagnostic algorithm in the future.

Javaud et al. devised a diagnostic approach based on a study of renal granulomatoses performed in Paris (Figure 1) (Javaud et al., 2007:178). However, this is based on patients in a developed country, with low tuberculosis incidence, and only 2 of the 43 patients (4.6%) of patients were HIV-infected. Therefore, this diagnostic algorithm has limited applicability in the South African context.

**Figure 1:** Schematised approach to diagnosing renal granulomatoses and their aetiologies (Javaud et al., 2007:178)




**CSS: Churg Strauss Syndrome; WG: Wegener's granulomatosis; PRB: puncture renal biopsy; TINU: tubulointerstitial nephritis with uveitis.**

This descriptive study was undertaken in an attempt to:

- Review all kidney biopsy cases in HIV-infected patients that showed evidence of GIN and correlate histological evidence with clinical, microbiological or radiological evidence of TB or other conditions/drugs known to cause granulomatous inflammation.
- Provide information on outcomes of GIN in this population group.
- Perform an extensive literature review which would assist in the possible formation of a diagnostic algorithm to follow in those patients with GIN on kidney histology in a South African setting, with possible therapeutic recommendations.
- Look at newer techniques for diagnosing TB that have been developed, with possible recommendations for studying these techniques in this setting in the future.

# Literature review.

## HIV and kidney disease

### Chronic kidney disease (CKD) in HIV infected patients

In the era of ART, the prevalence of CKD (according to estimated glomerular filtration rate, eGFR) amongst HIV-infected individuals in developed countries has been found to be 5.5% (range 4.7-8.7%). (Post & Holt, 2009:44)In contrast to studies performed in developed countries, where 84-93.5% of patients recruited are taking ART, (Wyatt et al., 2007:2102) (Cheung et al., 2007:3187) the face of CKD amongst HIV-infected individuals in sub-Saharan Africa (which is at the epicentre of the HIV pandemic) is strikingly different due to reduced access to ART and renal replacement therapy. (Fabian & Naicker, 2009:592)

The prevalence of CKD in HIV-infected patients in Africa ranges from 11.5% to 48.5%, depending on the criteria used. (Wools-Kaloustian et al., 2007:2210) (Emem et al., 2008:742) (Andia, Pepper & Matthieson, 2004)

The contribution of GIN to CKD is in Sub Saharan African is currently unknown. However with the relatively high frequency of this finding amongst our cohort of HIV-infected patients, it is possible that the contribution of GIN to CKD in this population is higher than anticipated.

### HIV and acute kidney injury (AKI)

Early detection of AKI is crucial, as many of the causes are reversible, provided the insult is removed timeously. (Izzedine, Baumelou & Deray, 2007:2757) HIV-infected patients have numerous risk factors for AKI including infections, nephrotoxic drug regimens and co-morbidities such as diabetes, hypertension and liver disease. (Kalim, Szczech and Wyatt, 2008:556)

HIV has been associated with an increased risk of AKI in one study (OR 4.62 in 1995(pre-ART) and OR 2.82 in 2003 (post-ART),) (Wyatt et al., 2006:563) and a nearly six-fold increase in in-hospital mortality. (Wyatt et al., 2006:564) AKI was significantly associated with traditional

risk factors for kidney disease: older age, black race, diabetes, pre-existing chronic kidney disease. (Wyatt et al., 2006:564)

In a retrospective study performed at GSH, higher CD4 count, lower creatinine at dialysis initiation, longer hospitalisation and diagnosis of acute tubular necrosis (ATN) significantly correlated with improved hospital survival and independence of dialysis at the end of a 6-week period. In this study, in-hospital mortality was 41%, which compares with mortality rates in HIV-negative intensive care cohorts, suggesting that patients may have a good short-term survival if treated adequately. (Arendse, Okpechi & Swanepoel, 2011)

GIN can also present as AKI (see later). It is possible that in HIV-infected patients who present acutely unwell with kidney dysfunction and opportunistic infections (particularly TB and fungal infections), that GIN contributes to the AKI. However, unless a biopsy is performed, many of these patients may be labelled as ATN +/- HIVAN.

## Causes of kidney disease amongst HIV infected individuals

It is useful to divide the causes of kidney disease into HIV-specific pathologies (primary) and secondary causes which are also seen in the general population, but which may be related to, or worsened by, the HIV-infection. (Fabian & Naicker, 2008:12) (Dellow, Unwin & Miller, 2000:72) (Fine & Atta, 2007:814). A summary of causes of kidney disease in HIV are outlined in Table 1.

Secondary causes can be divided into prerenal, intrarenal (including GIN) and postrenal (obstructive) causes. Infection remains an important cause of kidney disease in this population, even in the post-ART era (52% of cases of AKI were associated with infections in one study, 76% of which would be classified as opportunistic). (Dellow ,Unwin & Miller, 2000:73) (Franceschini et al., 2005:1528) Iatrogenic kidney injury (32% in one study) may result from the numerous drugs used to treat HIV infection as well as the many opportunistic infections associated with immunosuppression. (Franceschini et al., 2005:1529) There are also numerous electrolyte and acid-base disorders which have been documented in HIV infection. These may arise from HIV infection itself, from opportunistic infections or malignancies, or from the many drugs used in the treatment of these patients. (Fabian & Naicker, 2008:12)

## Table 1: Causes of kidney disease in HIV-Infected Patients
**(Fabian & Naicker, 2008) (Fauci et al., 2008:1753)**

| | | | | |
|---|---|---|---|---|
| **HIV associated nephropathies (primary)** | HIVAN[*]<br>HIV-ICGN[ɣ]<br>HIV-TTP/HUS[¥]<br>DILS[Ŧ] | | | |
| | **Prerenal** | **Intrarenal** | | **Postrenal** |
| **Non-HIV related (secondary)** | **True volume depletion**<br>• Nausea/vomiting<br>• Diarrhoea<br>• Renal losses (e.g diuretics)<br><br>**Effective volume depletion**<br>• Sepsis<br>• Low cardiac output states (e.g cardiomyopathy; tamponade etc)<br>• Cirrhosis<br>• Pancreatitis<br>• Nephrotic syndrome<br>**Impairment of autoregulatory mechanisms** (e.g. NSAIDs/ACE-I) | **Renovascular obstruction**<br>• Arterial or venous<br>**Acute tubular necrosis**<br>• Sepsis<br>• Nephrotoxins<br>  ○ Drugs (e.g. Tenofovir, Rifampicin, Isoniazid)<br>  ○ Contrast<br>  ○ Traditional medication<br>• Ischaemia<br>• Pigment induced<br>  ○ Rhabdomyolysis (Drug/infection)<br>  ○ Haemolysis<br>**Vascular causes**<br>• HUS/TTP<br>• Malignant hypertension<br>• Hypertensive nephrosclerosis<br>• Vasculitides<br>• Infection<br>• DIC | **Interstitial nephritis**<br>• Drugs<br>• Infection<br>• Malignant infiltration<br>• Inflammatory (sarcoidosis/TINU)<br>• **Granulomatous interstitial nephritis**<br>**Glomerular diseases**<br>• Other glomerulopathies (e.g. IgA, PIGN, SLE, RPGN)<br>• Amyloidosis (can also cause vascular disease)<br>• Diabetic glomerulosclerosis<br>**Intratubular obstruction**<br>• Endogenous<br>  ○ Myeloma<br>  ○ Uric acid<br>  ○ Oxalosis<br>• Exogenous<br>  ○ Acyclovir<br>  ○ Indinavir<br>  ○ Cotrimoxazole<br>**Infiltration (Dellow, Unwin & Miller, 2000:12) (Wearne et al., 2012)**<br>• Lymphoma<br>• Kaposi's sarcoma<br>• Amyloidosis | Renal calculi<br>Abnormalities of the urinary tract<br>Extrinsic compression<br>• Malignancies<br>• Lymph nodes<br>• Retroperitoneal disease |

**[*]HIVAN: collapsing focal segmental glomerulosclerosis with epithelial cell hypertrophy and hyperplasia with interstitial involvement that includes microcysts and a plasma cell predominant infiltrate.**

**[ɣ] HIV-ICGN: Immune complex glomerulonephritis defined by Gerntholtz as glomerular changes with varying numbers of immune deposits visible on light microscopy not fitting into any well-described pattern of classic immune-complex mediated glomerulonephritis; with overlapping features of post-infectious and membranous GN (Gerntholtz, Goetsch & Katz, 2006). ball-in-cup appearance with very large subepithelial deposit (Wearne et al., 2012), May be seen in isolation or together with HIVAN.**

**[¥]HIV-TTP/HUS: HIV associated thrombotic thrombocytopenic purpura/ haemolytic uraemic syndrome (thrombotic microangiopathies). These are characterised by thrombocytopenia, microangiopathic haemolytic anaemia, fever, renal abnormalities and neurological manifestations (Furlan et al., 1998:1578). Thought to be the result of widespread endothelial cell injury (Kimmel, 2000:118), and may be associated with cytomegalovirus infection (Peraldi et al., 1999:1579).**

**[Ŧ]DILS: Diffuse Infiltrative Lymphocytosis Syndrome.**

**NSAIDs: non-steroidal anti-inflammatory drugs; ACE-I: Angiotensin converting enzyme inhibitors; DIC: Disseminated Intravascular Coagulation. TINU: tubulointerstitial nephritis uveitis syndrome; IgA: IgA nephropathy; PIGN: post-infectious glomerulonephritis; SLE: systemic lupus erythematosis nephritis; RPGN: rapidly progressive glomerulonephritis**

## Kidney biopsy studies in South Africa

There is limited information regarding kidney biopsy histology in South African patients with HIV infection. Much of what has been published tends to concentrate on HIV associated nephropathy (HIVAN) and related HIV-specific kidney diseases. Primary HIV-related kidney disease is not the focus of this study. However, the results of three biopsy-based South African studies are included to demonstrate the variety of kidney diseases found in HIV-infected patients. Previous information regarding kidney disease in this population was limited due to the poor prognosis of HIV-related kidney disease without ART, which was previously denied to many South Africans. (Wearne et al, 2012:4109) There has also been a tendency to assume that kidney pathology in the setting of HIV infection is always related to HIVAN. (Wearne et al, 2012:4109) The findings of three biopsy series performed in South Africa are summarised in the following table. (Gerntholtz, Goetsch & Katz, 2006) (Han et al., 2006) (Wearne et al, 2012)

| Table 2: Kidney Biopsy series amongst HIV-infected patients in South Africa | | | | |
|---|---|---|---|---|
| | **Han et al 2006** | **Gerntholtz et al 2006** | **Wearne et al 2012** | |
| Number (n) | 30 | 99 | 192 | |
| HIVAN alone | 21 (70%) | 27 (27%) | 110 (57.2%) | |
| HIV-ICK* | - | 21 (21%) | 9 (4.6%) [#] | |
| Immune complex glomerulonephritides (ICGN) | | | | |
| | | | ICGN with HIVAN | ICGN alone |
| | 6 (20.3%) | 40 (40%) | 33 (17%) | 16 (8.3%) |
| Membranous GN | 4 (13.3%)[#] | 13 (13%) | 7 (3.6%) | 5 (2.6%) |
| Post-infectious GN | - | 8 (8%) | 3 (1.6%) | 3 (1.6%) |
| Mesangial hyperplasia | - | 6 (6%) | 8 (4%) | 1 (0.5%) |
| IgA nephropathy | - | 5 (5%) | 2 (1%) | 1 (0.5%) |
| IgM nephropathy | - | - | 2 (1%) | 0 |
| Non-HIVAN FSGS | - | 3 (3%) | 0 | 2 (1%) |
| Minimal change | - | 2 (2%) | 0 | 0 |
| Membranoproliferative GN | 2 (7%) | 2 (2%) | 11 (5.7%)[$] | 2 (1%) |
| Chronic GN[Ŧ]) | - | 1 (1%) | 0 | 1 (0.5%) |
| Crescentic GN | - | - | 0 | 1 (0.5%) |
| Diseases other than HIVAN/ICGN | | | | |
| Malignant hypertension | - | 3 (3%) | 1 (0.5%) | |
| Acute tubular necrosis | - | 2 (2%) | 5 (2.6%) | |
| Interstitial nephritis | 3 (10%) | 3 (3%) | 9 (4.7%) | |
| Diabetic nephropathy | - | 2 (2%) | 4 (2%) | |
| Lymphoma | - | - | 3 (1.6%) | |
| Granulomas | - | - | 2 (1%) | |
| Myeloma | - | - | 1 (0.5%) | |
| Amyloidosis | - | - | 1 (0.5%) | |
| HUS-TTP | - | - | 1 (0.5%) | |

* #: all with HIVAN; $ one with associated 'ball-in-cup'; Ŧ: unable to specify further due to global glomerulosclerosis

While HIVAN still accounts for a significant proportion of kidney pathology amongst HIV-infected individuals the differential diagnosis is diverse and often multifactorial. In the series by Wearne et al, 34.8% of patients with features of HIVAN had an additional pathology. (Wearne et al., 2012) Another study (from London) found that in 95% of HIV-infected patients with AKI, more than one aetiology was present. (Roe et al., 2008:245)These findings serve to emphasise the importance of kidney biopsy in this group. (Wearne et al., 2012:4118) Kidney biopsy may potentially diagnose a reversible pathology.

## Granulomatous interstitial nephritis (GIN).

GIN is usually seen as a component of acute interstitial nephritis, or in association with specific granulomatous diseases, such as sarcoidosis. (Bijol et al., 2006:57) The aetiology of granulomatous inflammation in the kidney may differ from those causing this type of inflammation in the skin, upper respiratory tract and lungs, where mycobacterial and fungal infections, together with sarcoidosis, predominate. (Bijol et al., 2006:57) Granulomata in the kidney may be isolated or extensive; necrotising or non-necrotising. In one study, granulomata were found, to be associated with other kidney disease in 30.4% of cases (IgA nephropathy, ATN and others). (Bijol et al., 2006:58)

Several reviews have been done to document the various causes, and prevalence, of GIN. Amongst these reviews the frequency was found to be low (0.5 (Bijol et al., 2006:58) to 1.37% (Javaud et al., 2007:171)). There are many causes of GIN (see Table 3), with most of the information in the form of case reports.

**Table 3: Possible causes of Granulomatous Interstitial Nephritis (GIN)**

| | |
|---|---|
| Drugs | BCG, intravesical (Bijol et al., 2006) (Kennedy, Shrinkanth & Charlesworth, 2006)<br>Antibiotics<br>• Methicillin; (Rossert, 2001)Ampicillin (Javaud et al., 2007) (Rossert, 2001); penicillin; (Javaud et al., 2007) (Viero & Cavallo, 1995) (Rossert, 2001) Vancomycin; (Hong et al., 2007) Cefuroxime; (Tong, Howell & Foreman, 2007) Fluoroquinolones; (Ramalakshmi, Bastacky & Johnson, 2003) (Lien, Hansen & Kern, 1993) Clarithromycin; (Audimoolam & Bhandari, 2006) Nitrofurantoin; (Javaud et al., 2007) (Korzets et al., 1994) (Rossert, 2001) Cotrimoxazole; (Vanhille et al., 1983) (Lapasia et al., 2010) (Javaud et al., 2007) (Bijol et al., 2006) (Rossert, 2001) Gentamycin (Viero & Cavallo, 1995)<br>TNF-α blockers (associated with sarcoidosis/sarcoid-like reaction) (Korsten et al., 2010)<br>NSAIDs (Singh & Colvin, 2003) (Javaud et al., 2007)<br>Paracetamol (Vanhille et al., 1983)<br>Aspirin (Viero & Cavallo, 1995)<br>Diuretics (Enriquez et al., 1995) (Rossert, 2001)<br>Captopril (Pena de la Vega et al., 2005) (Rossert, 2001)<br>Allopurinol (Magner, Sweet & Bear, 1986)<br>Anticonvulsants (lamotrigine; carbamazepine, phenytoin) (Fervenza et al., 2000) (Hegarty et al., 2002) (Ram et al., 2009)<br>Bisphosphonates (alendronate) (Pena de la Vega et al., 2005)<br>All-*trans* retinoic acid (Tomita et al., 2001)<br>Heroin (Do Sameiro Faria et al., 2003)<br>Omeprazole (Montseney & Meyrier, 1998) (Lapasia et al., 2010) |

| Table 3: Possible causes of Granulomatous Interstitial Nephritis (GIN) (Continued) | |
|---|---|
| Infection | Bacteria<br>• *E. Coli* (Viero & Cavallo, 1995) (Meehan,Josephson & Haas, 2000)<br>• *Rhodococcus equi* (Tse et al., 2004)<br>Mycobacteria<br>• Tuberculosis (Meehan, Josephson & Haas, 2000) (Mignon et al., 1984) (Gibson, Puckett & Shelly, 2004) (Colbert, Richey & Schwartz, 2012) (Sampathkumar et al., 2009) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) (Chapagain et al., 2011) Nontuberculous mycobacteria; (Javaud et al., 2007) Leprosy (Javaud et al., 2007)<br>Viral<br>• Adenovirus; (Singh & Nickeleit, 2004) (Alsaad et al., 2007) infectious mononucleosis (Mignon et al., 1984)<br>Fungi<br>• Histoplasma; (Adams & Cook, 2007) (Qian et al., 2011) (Nasr et al., 2003) (Lapasia et al., 2010) Candida; (Meehan, Josephson & Haas, 2000) (Ogura et al., 2012) Mucormycosis; (Mitwalli et al, 1994) Trichosporon (Ogura et al., 2012)Cryptococcus (Chung et al., 2009) (David et al., 2009)<br>Protozoa (toxoplasmosis) (Mignon et al., 1984) |
| IRIS | Related to opportunistic infections (Salliott et al., 2008) (Jehle et al., 2004) (Izzedine, Baumelou & Deray, 2007) (Croucher et al., 2010) (Martin-Blondel et al., 2011) (Daugus et al., 2006) |
| Inflammatory conditions | Sarcoidosis (Bijol et al., 2006) (Javaud et al, 2007) (Mignon et al, 1984) (Viero & Cavallo, 1995) (Joss et al., 2007) (Robson et al., 2003)<br>Wegener's granulomatosis (Bijol et al., 2006) (Javaud et al., 2007) (Mignon et al., 1984) (Viero & Cavallo, 1995)<br>TINU (Joss et al., 2007)<br>Crohn's disease (Unal et al., 2008) (Archimandritis & Weetch, 1993) (Javaud et al., 2007) |
| Malignancies | Chronic lymphocytic leukaemia (Inoue et al., 2010)Multiple myeloma (Bijol et al., 2006) |
| Foreign body giant cell reactions | Oxalate crystals (as a consequence of jejunoileal bypass) (Bijol et al., 2006) (Viero & Cavallo, 1995) Nephrocalcinosis (associated with chronic pyelonephritis) (Bouzourene, Bouzourene & Francke, 1998) |
| Idiopathic | (Bijol et al., 2006) (Javaud et al., 2007) (Mignon et al., 1984) (Viero & Cavallo, 1995) (Joss et al., 2007) |

**BCG**: *Bacille Calmette-Guérin* used to treat bladder carcinoma; TNF-α: **Tumour necrosis factor-α;** NSAIDs**: Non-steroidal anti-inflammatory drugs;** IRIS**: Immune reconstitution inflammatory syndrome;** TINU**: Tubulointerstitial nephritis and uveitis syndrome**

Drug reactions and sarcoidosis were the leading causes of GIN in many of the reviews, with many (8.3% (Viero & Cavallo, 1995) to 50% (Joss et al., 2007) being listed as idiopathic (i.e. no cause found after extensive investigation). Patients with isolated kidney disease labelled as idiopathic GIN may later develop other manifestations of the disease (extrarenal complications of sarcoidosis, or uveitis in the case of tubulointerstitial nephritis and uveitis syndrome (TINU)), and the diagnosis may need to be reviewed. Table 4 summarises the findings of the five largest studies of GIN.

**Table 4: Breakdown of the aetiology of GIN found in 5 retrospective reviews**

| | **Bijol et al** (2006) | **(%)** | **Javaud et al** (2007) | **(%)** | **Mignon et al** (1984) | **(%)** | **Viero et al** (1995) | **(%)** | **Joss et al** (2007) | **(%)** |
|---|---|---|---|---|---|---|---|---|---|---|
| Total number | 46 | | 43 | | 32 | | 12 | | 18 | |
| Prevalence in kidney biopsies | 0.5% | | 1.37% | | 0.9% | | 5.9%* | | <1% | |
| Sarcoidosis | 11 | 28.9 | 20 | 50 | 3 | 9.4 | 3 | 25.0 | 5 | 27.8 |
| Drug reaction | 17 | 44.7 | 7 | 17.5 | 10 | 31.3 | 3 | 25.0 | 2 | 11.1 |
| Mycobacterium | 0 | 0.0 | 5 | 12.5 | 3 | 9.4 | 0 | 0.0 | 0 | 0.0 |
| Miscellaneous infections | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 3 | 25.0 | 0 | 0.0 |
| BCG | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Wegener's | 2 | 5.3 | 2 | 5 | 8 | 25.0 | 1 | 8.3 | 0 | 0.0 |
| FBGCR | 2 | 5.3 | 0 | 0 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 |
| XPN | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| TINU | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 2 | 11.1 |
| Crohn's | 0 | 0 | 1 | 2.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Idiopathic | 4 | 10.5 | 5 | 12.5 | 8 | 25.0 | 1 | 8.3 | 9 | 50.0 |

BCG**: intravesical *Bacille Calmette-Guerin* used to treat bladder carcinoma;** FBGCR**: Foreign body giant cell granuloma;** XPN**: xanthogranulomatous pyelonephritis;** TINU**: tubulointerstitial nephritis and uveitis syndrome.**

* **This figure represents the percentage of GIN among patients with acute interstitial nephritis (not total kidney biopsies performed)**

# Non-infectious GIN

## Sarcoidosis

GIN is seen in 15-40% of patients with sarcoidosis undergoing kidney biopsy, (Javaud et al., 2007:177) (Robson et al., 2003) and may occur in the absence of extrarenal manifestations of the disease. (Robson et al., 2003) This figure may be an underestimate, as many patients (99% in one series) (Javaud et al., 2007:177)) had normal kidney function and hence were unlikely to undergo kidney biopsy, or the histological features of sarcoid may be missed on kidney biopsy due to sampling error. (Javaud et al., 2007:177)

### *Sarcoidosis and HIV*

The coexistence of sarcoidosis and HIV in the same patient is unusual. (Lenner et al., 2001:979) This is thought to be due to the deficiency of CD4+ T cells (the hallmark of HIV-infection), the main components of granulomatous inflammation seen in sarcoidosis. (Mirmirani et al., 1999:285) (Lenner et al., 2001:979) (Foulon et al., 2004:423) Although rare, the coexistence of HIV and sarcoidosis may be seen in patients with CD4 counts exceeding 200cells/$mm^3$. (Gomez et al., 2000:1278) The clinical spectrum resembles sarcoidosis seen in HIV-seronegative patients. (Lenner et al., 2001:980) There have been case reports of sarcoidosis developing in HIV-infected patients as part of the immune reconstitution inflammatory syndrome (IRIS) (Mirmirani et al., 1999) (Gomez et al., 2000) (Foulon et al., 2004) 29+/- 16 months after initiation of ART. (Foulon et al., 2004:419) . This is significantly longer than the delay between ART and development of TB-IRIS (see below). Interestingly, no patients with kidney disease were reported.

## Drugs

The true frequency of drug-induced acute interstitial nephritis (AIN) is likely underestimated as kidney biopsies are not always performed in this setting. (Javaud et al., 2007:177) Many drugs have been implicated in GIN, but the most common are antibiotics, anticonvulsants, non-steroidal anti-inflammatory drugs and diuretics. (Larsen et al., 2008:527) (Nasr et al., 2003) . Although the exact mechanism is unclear, it is thought that granuloma formation is secondary to a delayed type hypersensitivity reaction via the cell-mediated immune response (Th1 cells) to antigenic stimuli. (Hong et al., 2007:298) Of particular importance in South Africa is whether rifampicin is associated with GIN. On extensive literature review,

only three obscure references were found in relation to rifampicin and GIN. (Chung et al., 2009:455) (Javaud et al, 2007:172) (Rossert, 2001) For the purposes of this study we assumed no link.

Proving that a drug is causally responsible for GIN is difficult. The evidence is often circumstantial in the form of a temporal relationship between drug initiation and kidney dysfunction or improvement of kidney function upon cessation of the offending drug. (Kim & Hee Sung, 2010:141) (Robson et al., 2003) Patients may also be on numerous potential drugs. (Bijol et al., 2006:59) Drug-induced GIN may be accompanied by systemic symptoms of fever, rash, eosinophilia and arthralgia (up to 33% of cases) (Javaud et al., 2007:177) (Viero & Cavallo, 1995:1348) (Singh & Colvin, 2003)). These symptoms appear to be less frequent in those patients with GIN compared to those with 'classic' AIN, (Vanhille et al., 1983:648) (Javaud et al., 2007:177) (Viero & Cavallo, 1995:1348) such as that reported in methicillin-induced AIN. (Rossert, 2001:807) On histology, the granulomatous inflammation is typically non-necrotising, tends to have a diffuse distribution with poorly formed granulomata, and eosinophils and neutrophils may be a feature. (Bijol et al., 2006:61) Of note, 57% of the patients with drug-induced GIN in one series had evidence of cholestasis on liver profile testing. (Javaud et al., 2007:172)

## Wegener's Granulomatosis (WG)

WG was listed as a leading cause of GIN in one study, (Mignon et al., 1984) . In the setting of HIV, antineutrophil cytoplasmic antibody (ANCA) positivity has been reported (prevalence ranging from 20-83%), (Cornely et al., 1999) (Jansen et al., 2005) and is most likely related to polyclonal activation of B-lymphocytes. (Jansen et al., 2005:272) However, the occurrence of ANCA-associated vasculitis appears to be very rare, with only 2 cases reported in the literature. (Cornely et al., 1999:95).

## Treatment of non-infectious GIN

In the setting of sarcoidosis (including sarcoid IRIS), steroid therapy may need to be high-dose and prolonged. (Joss et al., 2007:227) (Robson et al., 2003) Idiopathic GIN has also been shown to respond to steroid therapy. (Joss et al., 2007) (Kim & Hee Sung, 2010)

In drug-induced GIN, withdrawal of the drug is mandatory. In addition, some authors recommend the use of corticosteroids, although kidney dysfunction may persist. (Javaud et al., 2007) (Hong et al., 2007) (Tong, Howell & Foreman, 2007) (Ramalakshmi, Bastacky & Johnson, 2003) (Lien, Hansen & Kern, 1993) (Audimoolam & Bhandari, 2006) (Enriquez et al., 1995) (Ram et al., 2009) (Pena de la Vega et al., 2005) (Lapasia et al., 2010) (Montseney & Meyrier, 1998) (Kennedy, Shrinkanth & Charlesworth, 2006) (Joss et al., 2007) (Viero & Cavallo, 1995) The use of steroids for the treatment of GIN has been extrapolated from the use in drug-induced AIN. (Gonzalez et al., 2008:941) A delay in initiation of steroid therapy significantly increased the risk of incomplete recovery from AIN. (Gonzalez et al., 2008:942) Further studies are needed to evaluate the use, dose and duration of steroid treatment together with alternative treatment options in GIN. (Javaud et al., 2007:179) (Joss et al., 2007:229)

## Infections and GIN

South Africa has a high rate of HIV and TB co-infection(estimated to be as high as 60%). (South Africa. Department of Health. 2012). Although *Mycobacterium tuberculosis* (*M. tuberculosis*) may be implicated in GIN, other infectious causes are possible including bacterial, fungal and viruses as well as other mycobacterium species (see table). (Javaud et al., 2007:178) There have been several case reports of TB-GIN treated with steroids (see below). However, treatment with steroids in the face of infection other than TB may lead to overwhelming sepsis, and indeed be fatal. (Chung et al., 2009:457) (Nasr et al., 2003)

Fungal infections, e.g. *Histoplasma*, *Cryptococcus*, *Mucormycosis*, *Candida*, have been described as the cause of GIN in both immunocompromised (e.g. transplant and HIV-infected) (Ogura et al., 2012) (Ahuja et al., 1998) (Mitwalli et al., 1994) (Lapasia et al., 2010) (Meehan, Josephson & Haas, 2000) (David et al., 2009) and immunocompetent patients. (Chung et al., 2009) (Nasr et al., 2003) (Adams & Cook, 2007) (Qian et al., 2011) Special stains may be required to identify fungal infections on kidney biopsy (Chung et al., 2009:454) (Nasr et al., 2003) The diagnosis may be difficult as cultures need to be specifically requested. (Adams & Cook, 2007:684) (Qian et al., 2011:1020) Fungal serology may also be misleading, especially early in the disease when antibodies have not yet formed, and in immunocompromised patients. (Chung et al., 2009:455) (Adams & Cook, 2007:684) (Qian et al., 2011:1020)

## Tuberculosis (TB) and the kidney (genitourinary TB; GUTB)

South Africa ranks third highest in the world in terms of TB burden (after India and China) (Dias et al., 2012), with an incidence that has increased by 400% in the past 15 years. (South Africa. Department of Health, 2012). GUTB may be the second most common form of extrapulmonary TB (after lymphadenitis) in the United States, Canada and the United Kingdom (Javaud et al., 2007:177) (Larsen et al., 2008:526) (Eastwood, Corbishley & Grange, 2001:1307) with prevalence figures ranging from 5.7% (Fain et al., 2000:145) to 54%. (Dolberg et al., 1991:177) The frequency of renal TB in South Africa is unknown as is the frequency of renal TB in HIV-infected patients. One autopsy study in India showed 17 cases of TB in 35 kidneys studied from patients with AIDS. (Lanjewar et al., 1999) Another autopsy study performed in Mexico identified *M. tuberculosis* in 23% of 87 HIV-infected patients with kidney disease. (Soriano-Rosas et al., 1998) TB has also been demonstrated to be a cause of end-stage kidney disease. (Chun & Hale, 2004:119) In 1991, 0.65% of new patients entered on the European Dialysis and Transplant Association registry had chronic kidney disease attributable to TB. (Chun & Hale, 2004:119)

Renal TB may be isolated (4% of patients (Chapagain et al., 2011:671)), or part of a generalised tuberculous infection. (Chaudhari, Ranganath & Pavan, 2011:208) (Larsen et al., 2008:526) In patients with previous pulmonary TB there may be a latent period of 5-40 years before kidney disease becomes evident. (Larsen et al., 2008:526) It may also present as part of disseminated primary infection. (Larsen et al., 2008:527) In the context of HIV infection, the presentation of TB is more often disseminated or extrapulmonary, and may be atypical. In the study by Nzerue et al. (2000), 66.6% of the patients with GUTB were HIV-infected, 43.8% of whom were diagnosed with concurrent pulmonary TB. (Nzerue et al., 2000) All patients with evidence of abdominal TB (splenic granulomata/abscesses and retroperitoneal adenopathy) were found to be HIV-infected. (Nzerue C, 2000)

## Pathophysiology

Mycobacteria undergo haematogenous spread from the primary site of infection (usually the lungs) to the kidney. (Nzerue et al., 2000) Granulomatous inflammation initially occurs around cortical glomeruli (Chaudhari, Ranganath & Pavan, 2011:208)where increased oxygen tension favours mycobacterial proliferation. (Gibson, Puckett & Shelly, 2004:254) (Larsen et al., 2008:526) Granulomata develop as a result of cell-mediated immunity, with

clonal expansion of CD4+ cells that drives activation of macrophages through secretion of Interferon γ. (Lawn, Bekker & Miller, 2005:363) An inflammatory milieu is established with chemokines and cytokines facilitating further mononuclear cell recruitment and activation. (Lawn, Bekker & Miller, 2005:363) The granulomata characteristically consists of macrophages that have undergone epithelioid differentiation and developed into multinucleate Langhans' giant cells, with CD4+ cells at the periphery that orchestrate the cell mediated immune response. (Lawn, Bekker & Miller, 2005:363) (Eastwood, Corbishley & Grange, 2001:1310) Caseation may or may not be present. In immunocompromised patients, granulomata may be poorly formed, and caseous necrosis is seen less frequently. (Eastwood, Corbishley & Grange, 2001:1310)

Granulomata may heal, particularly in patients with intact cellular immunity, and mycobacteria in the foci may remain dormant for many years. (Gibson, Puckett & Shelly, 2004:254) (Larsen et al., 2008) In some patients, either initially or later when immunity is impaired, granulomata may progress (Gibson, Puckett & Shelly, 2004:254) (Larsen et al., 2008) and rupture into the tubules or interstitium. (Chaudhari, Ranganath & Pavan, 2011:208) (Gibson, Puckett & Shelly, 2004:254) Rupture is random and is not often associated with symptoms. (Chaudhari, Ranganath & Pavan, 2011:208) An intense granulomatous inflammation ensues, (Colbert, Richey & Schwartz, 2012:237) with caseous necrosis and often cavitation, the result of which is often progressive disease with destruction, loss of function, fibrosis and calcification. (Chaudhari, Ranganath & Pavan, 2011:208)

There appears to be two distinct forms of renal TB: (Eastwood, Corbishley & Grange, 2001)

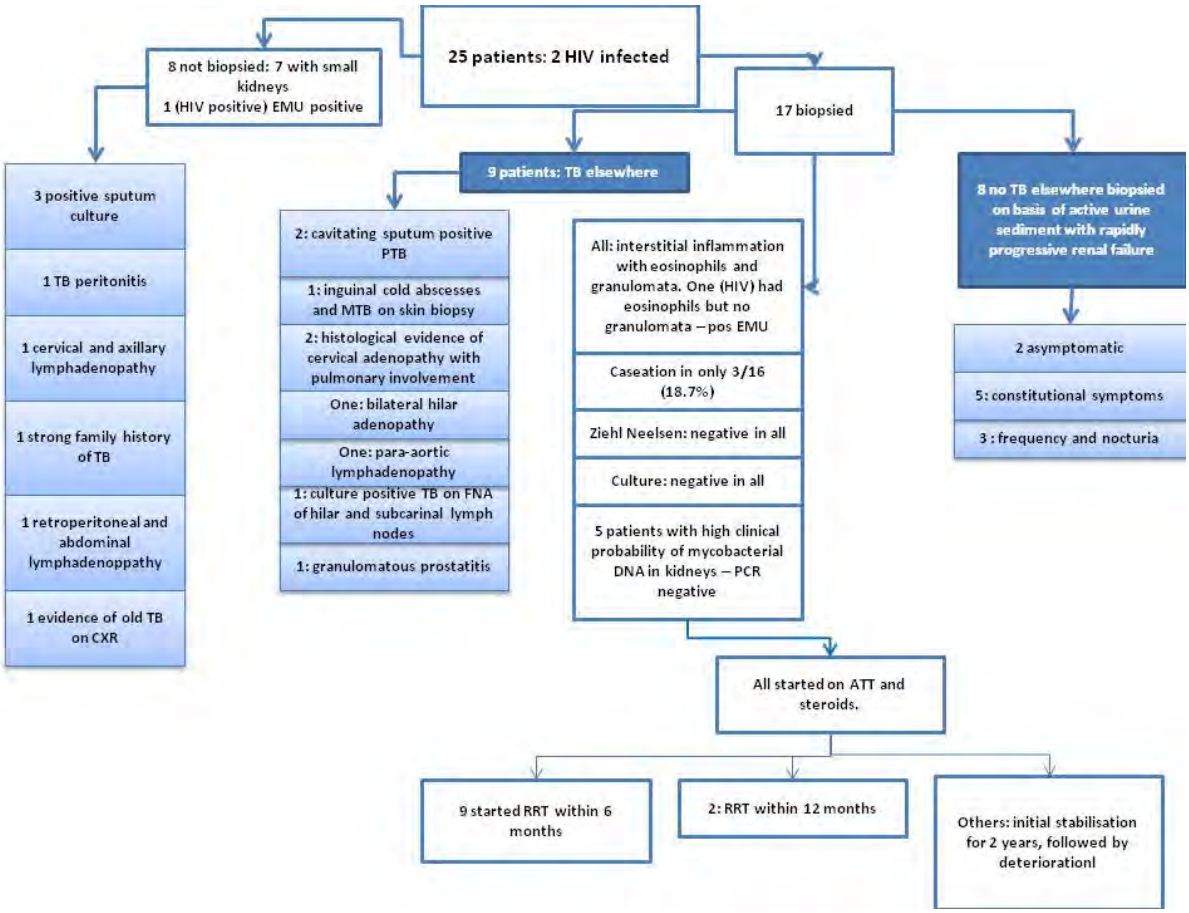
**1."Classical" renal TB** where there is contiguous spread of the pathogen from the interstitium into the renal pelvis, ureters and bladder and reproductive organs (Larsen et al., 2008) leading to fibrosis, calcification and stricture formation. (Gibson, Puckett & Shelly, 2004:255) This form of renal TB will not be discussed in further detail.

**2.TB-associated granulomatous interstitial nephritis (TB-GIN).** TB can also affect the kidney insidiously, causing kidney dysfunction but without the gross anatomical abnormalities noted in "classic" renal TB. (Eastwood, Corbishley & Grange, 2001:1308) The first description of GIN as the only manifestation of renal TB was made in 1981 (Kaul et al., 2011:383) It is usually due to the result of reactivation of tuberculosis, but can occur as part of the disseminated primary tuberculous infection. (Chapagain et al., 2011) It has been

described in the context of both CKD and rapidly progressive kidney impairment. (Chapagain et al., 2011:673) (Kaul et al., 2011:383) (Sampathkumar et al., 2009:844) It may even mimic glomerular disease with proteinuria and active urine sediment (Kaul et al., 2011:385) (Sampathkumar et al., 2009:844) (Khilji et al., 2012) Most patients have co-existent extrarenal TB. (Kaul et al., 2011:385) (Khilji et al., 2012:1) (Chapagain et al., 2011:673) Patients may have constitutional symptoms or symptoms related to extrarenal sites. (Chapagain et al., 2011:673) (Nzerue et al., 2000:300) Urinary tract symptoms are uncommon although many have pyuria. (Eastwood, Corbishley & Grange, 2001:1308) Patients may be completely asymptomatic. (Chapagain et al., 2011:673) (Colbert, Richey & Schwartz, 2012:237) (Eastwood, Corbishley & Grange, 2001:1307) (Chun & Hale, 2004:119). Complement or immune complex deposition is not a typical feature. (Chapagain et al., 2011:676)

Prior to 2011, the literature on TB-GIN was largely limited to case reports, but in 2011 Chapagain et al reported on twenty-five adult patients with tubulointerstitial nephritis secondary to TB, seventeen of whom had biopsy proven GIN. (Chapagain et al., 2011:671) Figure 2 summarises the findings of this study, and a table of the case reports of TB-GIN can be viewed in Appendix 4.

**Figure 2: Summary of 25 patients with tubulointerstitial nephritis due to TB**




## Immune reconstitution inflammatory syndrome (IRIS)

IRIS is a phenomenon occurring in HIV infected patients during the early period following ART initiation, when immune reconstitution can be complicated by the **"unmasking"** of previously subclinical infections (**unmasking IRIS**) or the deterioration of pre-existing partially treated opportunistic infections (**paradoxical IRIS**). (Lawn, Bekker & Miller, 2005:361) It is thought to result from immunopathological responses, previously suppressed by HIV infection, becoming activated with restoration of the host's immune system. (Lawn, Bekker & Miller, 2005:361)

IRIS has been reported in association with a wide variety of opportunistic infections (including cytomegalovirus, toxoplasmosis and *Cryptococcus neoformans)* . (Lawn, Bekker & Miller, 2005:361) However, IRIS in association with mycobacterial infection is the most frequently reported. Clinically apparent paradoxical reactions have been observed in 29-36% of HIV-infected patients with TB (on treatment) newly started on ART, with radiological

deterioration in 46% of patients. (Lawn, Bekker & Miller, 2005:368) IRIS can be triggered by viable or dead organisms, or shed antigen (which, in the case of TB, can persist in host tissues for many weeks after initiation of antimycobacterial therapy). (Lawn, Bekker & Miller, 2005:363) Mycobacterial culture performed as part of investigation may, therefore, be negative.

HIV disrupts the cell mediated immune response to *M. tuberculosis* by progressive CD4 lymphocytopenia (Lawn, Bekker & Miller, 2005:363) and impaired CD4 cell function with changes in cytokine secretion driving humoral immune response (type 2) as opposed to cell-mediated immune response (type1). (Lawn, Bekker & Miller, 2005:363) This in turn affects recruitment and function of macrophages, and results in failure of granuloma response, increased mycobacterial burden and risk of disseminated infection. (Lawn, Bekker & Miller, 2005:363) ART restores the host's ability to form granulomata. Paradoxical reactions are thought to be related to intensification of cell-mediated immunity, temporally related to a rise in serum TNF-α which may result from macrophage activation in response to certain mycobacterial antigens. (Lawn, Bekker & Miller, 2005:362,364) This may account for much of the subsequent tissue destruction and complications seen as part of IRIS.

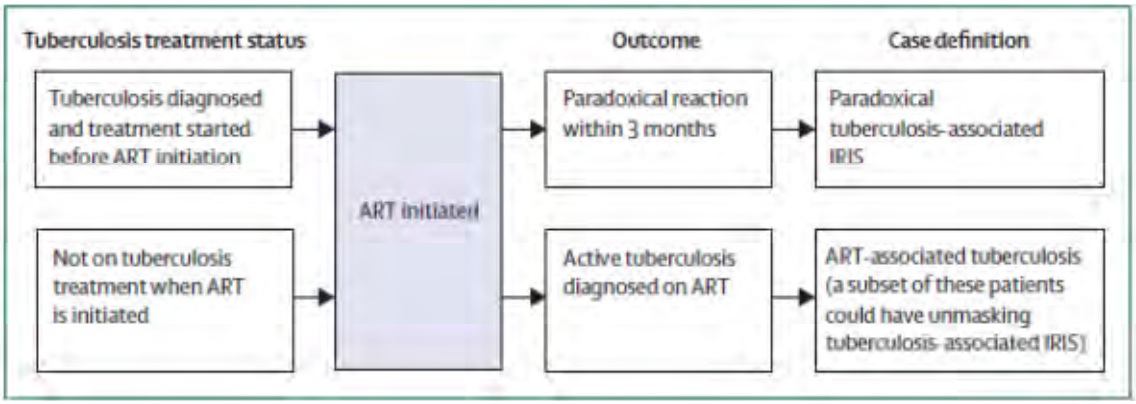
Risk factors for the development of IRIS include low nadir CD4 (less than 100 cells/µl), a high viral load and rapid response to ART. (Lawn, Bekker & Miller, 2005:370) The major risk factor for TB-IRIS is the initiation of ART within the first 2 months of antituberculous therapy, when the mycobacterial antigen load is still high. (Lawn, Bekker & Miller, 2005:370)

The diagnosis of IRIS should be considered when a patient presents or deteriorates with an opportunistic infection shortly after initiation of ART. (Lawn, Bekker & Miller, 2005:362) The interval between starting ART and IRIS onset varies from less than one week, to several months, with most cases occurring within 2-3 months. (Izzedine et al., 2007:534) (Meintjies et al., 2008:518) However other causes of deterioration need to be considered including other opportunistic infections, inadequate treatment (including drug resistance), drug reactions, and the possibility of a paradoxical reaction unrelated to ART initiation. (Lawn, Bekker & Miller, 2005:362) (Meintjies et al., 2008:520) There have been numerous proposed 'markers' to support a diagnosis of TB-IRIS, many of which are used only as research tools. (Lawn, Bekker & Miller, 2005:362) A rise in CD4 lymphocyte count and a reduction in viral load may support a diagnosis of IRIS. (Lawn, Bekker & Miller, 2005:362) (Meintjies et al., 2008:518) However, a rise in CD4 count does not necessarily indicate that the CD4 cells are functional, nor does failure of the CD4 lymphocyte count to rise indicate

that there has been no restoration of functional T-cell response. (Lawn, Bekker & Miller, 2005:362) (Meintjies et al., 2008:518)

Consensus case definitions for TB-associated IRIS for use in resource-limited settings have been developed. (Meintjies et al., 2008:516) – see Figure 3 below. Paradoxical TB IRIS occurs after commencement of ART in patients receiving antituberculous therapy and requires a preceding diagnosis of TB together with an initial clinical response to antituberculous therapy. ART-associated TB is defined as a new diagnosis of TB after initiation of ART. (Meintjies et al., 2008:518,520) Unmasking TB IRIS is reserved for those patients with "ART-associated TB" who have evidence of a marked or exaggerated inflammatory component associated with their presentation, or whose clinical course is complicated by paradoxical deterioration once they are established on TB treatment. (Meintjies et al., 2008:521)

**Figure 3: Schematic representation showing the different forms of tuberculosis-associated IRIS and ART-associated tuberculosis.** (Meintjies et al., 2008)




## IRIS and kidney disease.

To date there have only been 6 case reports of biopsy-proven IRIS-related kidney injury (see table) after initiation of antiretroviral therapy. (Martin-Blondel et al., 2011:2406) Five of these cases have been related to *M. tuberculosis*, and one case attributed to *Mycobacterium avium*.

**Table 5. Case reports of AKI thought to be due to IRIS related tubulointerstitial nephritis.**

(See appendix 7 for enlarged table.)

| Case report | CD4 count[a] | HIVVL[b] (copies/ml) | Mycobacterial diagnosis | cART initiated | Onset of renal disease | Other manifestations | CD4 count[a] | HIVVL[b] (Copies/ml) | Renal biopsy | Drug changes | Treatment | Renal function improvement | Follow up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jehle 2004 | 69 cells/µl | 1247786 | There are no sources in the current document. Miliary TB and urinary TB. Culture +, drug sensitive | 2 weeks after ATT | 6 weeks after cART | New pulmonary infiltrates | 82 cells/µl | 104 (↓4 $log_{10}$) | Severe GIN with interstitial infiltrates | Cotrimoxazole stopped[c] | Prednisone 1mg/kg | Within 10 days | Healthy at 1 year |
| Daugus 2006 | 26 cells/µl | 56732 | Pleural effusion (Culture + MTB) | 1 month after ATT | 4 weeks after cART (during steroid withdrawal) | Abdominal TB IRIS[a] | 127 cells/µl | Undetectable | Diffuse interstitial inflammation. No granulomas. | TDF dose adjusted | Steroids reintroduced at 1mg/kg | Within 1 week | Normal renal function after 1 year |
| Izzedine et al 2007 | 37 cells/µl | ~750000 | Symptoms: bone and liver granulomas | 3 months BEFORE ATT | 8 weeks after ATT 5 months after cART | Erythematous skin lesions: lymphadenopathy / splenomegaly; cholestasis | 148 cells/µl | >200 | Acute GIN | | Prednisone 1 mg/kg. | Over 2 weeks. | Normal renal function at 10 months. |
| Salliot et al 2008 | 88/mm³ | 177504 | Pulmonary, hepatic and cutaneous TB | 45 days after ATT | 15 days after cART | None | 326 cells/mm³ | 494 | Intense GIN | EMB, PZA and cART stopped; cART restarted after 3.5 months | Prednisone 1mg/kg initially; ↑ to 1.5mg/kg | Slow (needed higher dose of prednisone). | Normal renal function at 8 months. |
| Croucher et al 2010a | 79 cell/µl | 61573 | Pulmonary TB | 2 days before ATT (previously defaulted cART) | 50 days after restarting cART (after withdrawal of steroids)[a] | Fever | 320 cells/µl | 333 | Lymphocytic AIN (CD4+ predominant); No granulomas | TDF changed to ABC | Prednisone restarted. | Within 24 hours: normalised after 2 weeks. | Normal renal function at 18 months. |
| Martin-Blondel et al 2011 | 25 cells/mm³ | 6.5 $log_{10}$ | MAC (diagnosed 34 days AFTER ART) | 34 days prior to MAC diagnosis and treatment | 103 days after cART (69 days after MAC treatment) | Fever; cognitive impairment; interstitial pulmonary infiltrate; progression of retroperitoneal lymphadenopathy | 88 cell/mm³ | 2.4 $log_{10}$ | Severe acute GIN. Macrophage predominant (60%), T cells 40% (CD8+: 80%) | ART regimen changed | Prednisone 1mg/kg. | Over 1 month | Normal renal function at 10 months. |

The highlighted cases fall into the category of unmasking IRIS i.e. mycobacterial infection was diagnosed after ART initiation, with subsequent development of kidney dysfunction.

The second case, (Daugus et al., 2006) describes abdominal TB IRIS occurring four days after initiation of ART in a patient who had been on antituberculous therapy for one month. Abdominal symptoms responded to an initial course of corticosteroids, but kidney function deteriorated upon reduction of steroid dose. Kidney function improved dramatically with corticosteroid reintroduction, this was tapered and stopped after 6 months, with no recurrence of kidney dysfunction. (Daugus et al., 2006:595) Kidney disease (due to IRIS) occurring after steroid withdrawal was also seen in a case reported by Croucher et al. (Croucher et al., 2010)

In all of the above cases there was a rapid improvement after corticosteroid therapy. The use of corticosteroids has been shown to be efficient in a controlled clinical trial of South

African patients with non-life-threatening paradoxical TB IRIS. (Meintjies et al., 2010) It is important to note, however, that this trial did not look at kidney function specifically. While more infections occurred in the prednisone arm, they were mostly mild in nature, and corticosteroids were generally well-tolerated. (Meintjies et al., 2010, p. 2387) Alternative explanations for clinical deterioration (as mentioned above) should be excluded whenever possible, (Meintjies et al., 2008:520) as the administration of steroids in the face of an incorrect diagnosis of TB-IRIS could be detrimental. (Meintjies et al., 2010:2387)

ART was stopped in only one case due to severe kidney failure and severe histological lesions. (Salliott et al., 2008) In this case there was an initial partial response to prednisone therapy at 1mg/kg, but this was later increased to 1.5mg/kg because of the pharmacokinetic interaction between steroids and rifampicin. The higher dose of prednisone produced further (albeit slow) improvement, allowing reintroduction of ART after 3.5 months. (Salliott et al., 2008:180) Most authors would not recommend stopping ART unless IRIS is life-threatening, or non-responsive to steroid therapy, as there is a risk of progression of AIDS with ART interruption. (Martin-Blondel et al., 2011:2406).

## Diagnosis of renal TB

The diagnosis of renal TB may be challenging. The gold-standard for diagnosis is culture of *M. tuberculosis* from the urine. (Colbert, Richey & Schwartz, 2012) The preferred specimen is the first morning urine, and multiple specimens are advised to demonstrate the presence of mycobacteria (Dunlap et al., 2000:1382) (Larsen et al., 2008:527) as shedding of the organisms into the urine may be sporadic. (Hemal et al., 2000:571) Urine smear for acid-fast bacilli is usually negative and may not be cost-effective to perform, (Dunlap et al., 2000:1382) and cultures may take 6-8 weeks for a positive result, resulting in significant diagnostic delays, and frequent loss to follow up. (Hemal et al., 2000:571) (Colbert, Richey & Schwartz, 2012:238) (Sun et al., 2010:341) In addition, broad-spectrum antibiotics may inhibit growth of mycobacteria from urine, and result in negative cultures. (Dunlap et al., 2000:1382) Specificity of urine culture is 100%, (Colbert, Richey & Schwartz, 2012:238) but sensitivity varies depending on the clinical context and co-morbidities of the patient. (Hemal et al., 2000:571) (Sun et al., 2010:341) The highest quoted sensitivity of three morning urine specimens for mycobacterial culture in the literature is 80-90% in cases of GUTB, (Chun & Hale, 2004:120) but other studies have shown much lower sensitivities (10.7% to 37.14%).

(Hemal et al., 2000:571) (Sun et al., 2010:343) (Colabawalla, 1990) There are, however, no studies specific to TB-GIN.

Intermittent shedding of *M. tuberculosis* in the urine may account for false negative results from urinary-based tests. (Sun et al., 2010:341) Kidney biopsy specimens to diagnose renal TB have been reported as having a low sensitivity and specificity for detection of microorganisms (see below). (Larsen et al., 2008:527) In histological specimens, the finding of "chronic granulomatous inflammation" is non-specific (see table of causes of GIN). (Goel & Budhwar, 2007:24) The finding of caseous necrosis on biopsy favours the diagnosis of TB. In the absence of caseation, special stains may be used. (Larsen et al., 2008:527) However, staining for acid fast bacilli (AFB) with Ziehl-Niellsen (ZN) stain on histological specimens has a low sensitivity (32-43%). (Larsen et al., 2008:527) (Goel & Budhwar, 2007:24) Auramine O has a better sensitivity, but a lower specificity, (Larsen et al., 2008:527)and should be used in conjunction with ZN staining, rather than as a replacement test. In a study of kidney transplant patients with mycobacterial infection causing GIN in the kidney allograft, organisms were visualised in only 3 out of 13 patients (23%) (Meehan, Josephson & Haas, 2000) Confirmation of tuberculosis by culture in granulomatous biopsies is <10% is some series (particularly in HIV-infected patients with advanced immune suppression), and is not consistently performed. (Diaz et al., 1996:360)

### *Polymerase chain reaction (PCR) techniques for diagnosis of renal TB*

Improved diagnostic tests for renal TB are clearly needed. PCR performed on urine specimens can be used to amplify small amounts of specific genomic sequences allowing the detection of the presence of even minute amounts of mycobacterial DNA within 24-48 hours. (Hemal et al., 2000:571) PCR does not obviate the need for culture; although it can detect resistance to INH/RIF, extended sensitivities may still be needed. (Larsen et al., 2008:528) Urinary PCR has been shown to have a sensitivity of 84- 95% when compared to culture, with a specificity of 85-98%. (Hemal et al., 2000:572) (Moussa et al., 2000:586) (Larsen et al., 2008:527) TB-PCR has also been used in formalin-fixed, paraffin embedded histological specimens as an adjunct to routine stains and mycobacterial culture (Park et al., 2003:326) (Diaz et al., 1996:359) (Li, Lo & Ng, 2000) (Sampathkumar et al., 2009:843) This would be particularly useful in the retrospective diagnosis of tuberculosis in specimens where culture is not possible due to prior formalin fixation of the tissue specimen. (Rish et al., 1996:1419) (Diaz et al., 1996:362) The reported sensitivity of this technique varies from 31.9% (Alvarado-Esquivel et al., 2009:3) to 78% (compared with a sensitivity of culture of

42%). (Li, Lo & Ng, 2000) (Park et al., 2003:328) and appears to decrease in older specimens. (Chapagain et al., 2011:674) This technique has not been studied specifically in the context of TB-GIN.

### *Immunohistochemical techniques*

Immunohistochemical studies have been performed to establish the aetiology of granulomata in tissue specimens. (Goel & Budhwar, 2007:24) Good results have been reported with monoclonal antibodies directed towards a 38kDa protein of *M. tuberculosis* (100% positivity in histological specimens of proven extrapulmonary tuberculosis with no false positives or false negatives). (Goel & Budhwar, 2007:29) However, there were no kidney biopsy specimens included in this study, (Goel & Budhwar, 2007:25) and these tests are, as yet, not available in South Africa.

## New TB diagnostics

### *Urinary lipoarabinomannan (LAM)*

There is significant interest in the use of urine to diagnose TB (other than GUTB). It is easily accessible and readily available, with low infection risk to healthcare workers. (Peter et al., 2010:263) Lipoarabinomannan (LAM) is a 17.5 kD glycolipid found in the outer cell wall of mycobacteria. (Peter et al., 2010:263) It is filtered by the kidneys and may become detectable in the urine. Enzyme linked immunosorbent assay (ELISA) tests for LAM antigen in the urine, as well as a dipstick test are now available. (Peter et al., 2010) The best clinical utility has been found in HIV-infected patients with advanced immunosuppression, (Peter et al., 2010:263) who may have a higher antigen burden (Lawn et al., 2009) and/or changes in glomerular filtration as a result of HIV-related podocyte dysfunction. (Peter et al., 2010:266) This is in contrast to most diagnostic modalities in which test performance often declines as immunosuppression advances. (Lawn et al., 2009) In the setting of patients with advanced immune suppression (CD4<100), urinary LAM had a sensitivity that far exceeded that of sputum smear microscopy (SSM), with a specificity of 100%, (Lawn et al., 2009) with incremental value over SSM in extrapulmonary TB. (Peter, Theron, van Zyl-Smit et al. 2012:1219) In HIV-infected hospitalised patients with CD4 <200, urine LAM was positive in 50% of all sputum scarce or smear negative patients, potentially allowing a diagnosis of TB to be made without the use of further invasive, and costly, special investigations. (Peter, Theron, van Zyl-Smit et al. 2012:1219) Urine LAM may be useful as a rule-in test in the diagnostic algorithm for tuberculosis in HIV-infected patients with advanced

immunosuppression who are sputum smear negative. (Peter et al., 2010:267) There are no studies on the use of urinary LAM in the diagnosis of genitourinary TB or GIN.

### *XPert MTB/RIF*

There has been significant interest in the development and roll-out of the point of care XPert MTB/RIF molecular diagnostic tool. (Lawn et al., 2011) This is a novel, automated TB diagnostic which is able to detect the presence of *M*. *tuberculosis*DNA, together with drug resistance to rifampicin in less than two hours using real-time PCR technology. (Peter, Theron, Muchinga et al., 2012) (Lawn & Nicol, 2011:1069) Compared with conventional PCR, this test is not prone to cross-contamination, does not require extensive biosafety facilities, and can be performed by personnel with minimal training, allowing for testing to be performed away from centralised laboratories in resource limited settings. (Lawn & Nicol, 2011) (Vadwai et al., 2011: 2540) South Africa has been the leading purchaser of these tests (37% of the world's purchased tests). (Dias et al., 2012:10)

This test has been shown to have a high accuracy in sputum samples in the diagnosis of pulmonary TB. (Lawn & Nicol, 2011) A study performed in hospitalised HIV infected patients showed that the use of urinary XPert MTB/RIF in the diagnosis of TB (other than GUTB) had an overall sensitivity of 48%, comparable to that of SSM and urinary LAM, with improved performance at lower CD4 counts. (Peter, Theron Muchinga et al., 2012) It was also shown that combination with urinary LAM improved the sensitivity of the test to 70%. This may be important in patients who are unable to produce sputum, with the added benefit of being able to detect rifampicin resistance. (Peter, Theron, Muchinga et al., 2012).

Data on the use of XPert MTB/RIF in the diagnosis of extrapulmonary TB have largely been opportunistic laboratory-based studies rather than systematic prospective studies, (Lawn & Nicol, 2011:1079) although one study showed moderate to good sensitivity for most extrapulmonary specimens, except cerebrospinal fluid. (Vadwai et al., 2011:2544) The use of XPert MTB/RIF testing of urine or kidney biopsy specimens has not yet been evaluated in the setting of renal or genitourinary tuberculosis or GIN. (Lawn & Nicol, 2011:1079)

## Treatment and outcome of TB- GIN

Antituberculous therapy should be given as per protocol. There are no studies on corticosteroid therapy in TB-GIN, and neither the British Thoracic Society/National Institute for Clinical Excellence in the United Kingdom nor the Centre for Disease Control/American Thoracic Society in the United States recommend steroid therapy for this condition. (Chapagain et al., 2011:672) However, these guidelines are based on the 'classical' deforming form of genitourinary TB rather that GIN specifically. (Chapagain et al., 2011:672)

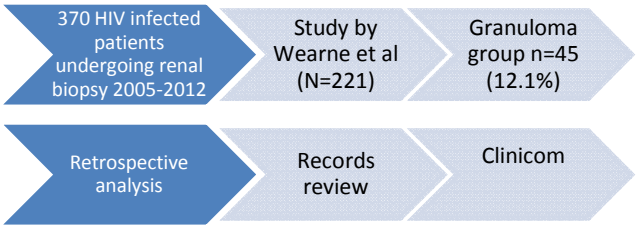
Concomitant steroid therapy at a dose of (0.5- 1mg/kg) has been noted to result in favourable kidney outcomes in patients with TB-GIN, (Sampathkumar et al., 2009:844) (Chapagain et al., 2011:672) The hypothesis is that GIN heals by fibrosis and steroids may decrease the extent of the inflammation in the acute phase and subsequent transformation to fibrosis. (Kaul et al., 2011:385) Chapagain et al. (2011) started lower dose steroids initially (prednisone 20mg/day) and tapered by 5mg/week with a view to stopping steroids at the end of the first month of treatment. (Chapagain et al., 2011:672) Although rare [<1% of cases of end-stage kidney disease] (Colbert, Richey & Schwartz, 2012:238), TB-GIN has been documented as a cause of end stage kidney disease (ESKD), (Kaul et al., 2011:385( (Khilji et al., 2012:2). Of the 25 patients in the study by Chapagain et al, 9 patients (36%) required renal replacement therapy within 6 months of presentation, (Chapagain et al., 2011:674) despite showing a good symptomatic response to antituberculous therapy. Another 2 patients progressed to ESKD within 12 months of presentation. (Chapagain et al., 2011:676) It was also found that kidney function stabilised in the subgroup of patients who presented with an eGFR of >15ml/min with less interstitial fibrosis and glomerular atrophy, lending credence to the hypothesis that earlier diagnosis (through kidney biopsy) and treatment may result in an improved kidney outcome. (Chapagain et al., 2011:676) However, despite initial stabilisation, a number of patients showed deterioration in kidney function 2 years after treatment began (likely due to tubular atrophy and glomerulosclerosis from the previous disease). (Chapagain et al., 2011:676) Only one of three patients with TB- GIN in the study by Javaud et al. (2007) received oral corticosteroids in addition to standard antituberculous therapy, although all of the patients had a favourable kidney outcome. (Javaud et al., 2007:174) .

# Methods

## Setting

This study was conducted amongst patients referred to the Division of Nephrology and Hypertension at Groote Schuur Hospital in Cape Town, a tertiary level hospital. The unit receives referrals from the Department of General Medicine at this hospital, from three secondary level hospitals as well as many community health centres within the Cape Town Metropole.

**Figure 4: General overview of the study**




## Participants

Participants were selected from HIV-infected patients (both hospitalised and outpatients) seen by the Division of Nephrology and Hypertension for investigation of kidney disease. A study by Wearne et al of all kidney biopsies performed in HIV-infected patients from January 2005 until December 2010 was performed among the same patient group. (Wearne et al., 2012) Data was obtained retrospectively in patients from 2005 until September 2008 (*n*=116) and subsequently prospectively. (Wearne et al., 2012)The study reported on 221 biopsies, and a database has subsequently been established with new patients being enrolled as kidney biopsies are performed. Kidney biopsy was only performed in those patients in whom it was clinically indicated (seeTable 6).

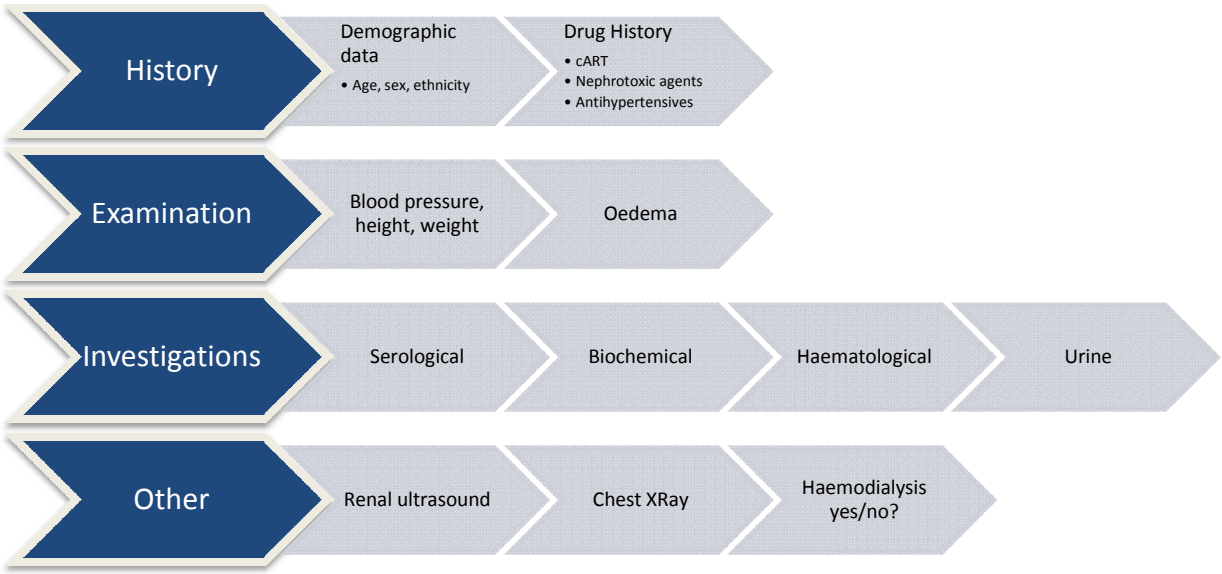
| Table 6: Inclusion and Exclusion Criteria | |
|---|---|
| Inclusion criteria: | • HIV positive.<br>• Older than 18 years.<br>• Meeting criteria for kidney biopsy, including,<br>  ○ Unexplained kidney dysfunction and/or<br>  ○ Unexplained proteinuria or haematuria.<br>  ▪ Kidney biopsy was only offered to patients if deemed clinically relevant and part of standard practice.<br>• Able and willing to provide informed consent for kidney biopsy and inclusion in the study. |
| Exclusion criteria: | • HIV negative.<br>• Younger than 18 years.<br>• Unwilling or unable to provide informed consent.<br>• Not fulfilling criteria for kidney biopsy.<br>• "Inadequate" specimen to assess for kidney pathology. |

This study utilised this pre-existing database to analyse kidney biopsies performed between January 2005 and October 2012 (*n*=370). Those patients with granulomata on kidney biopsy were incorporated into a new database ("Granuloma group", *n*=45) and analysed for this descriptive study.

## Procedures and measurements.

All participants (those in the original and "Granuloma group" database) underwent kidney biopsy as part of the investigation into the aetiology of their kidney disease. This biopsy was performed as part of best clinical practice and not for research purposes alone. Biopsy was performed under ultrasound guidance, and all specimens sent for histological analysis at the National Health Laboratory Service Division of Anatomical Pathology. No specimens were sent for culture.

**Figure 5: Data collected at the time of biopsy. For further information on investigations see Table 7 below.**




All patients had kidney ultrasound scans performed, with kidney size estimation recorded. On occasion, this was reported as "normal" which, in consultation with the renal unit (particularly Dr Wearne) was taken to be 10 centimetres for statistical purposes. Kidney size was described as small if <9 cm; normal if 9.1-13cm and large >13.1cm. Kidney size discrepancy existed when there was >1.5cm difference between the two kidney measurements. All of the above information was documented, together with the results of kidney biopsy (see Appendix 2: Clerking sheet).

## Informed consent

Informed consent was taken from all participants prior to kidney biopsy (as per standard clinical practice). The risks of the procedure were clearly communicated, and patients informed that they were under no obligation to proceed with the biopsy if they so chose.

Further informed consent for inclusion into the study was taken in the patient's own language. Consent forms (see Appendix 1) outlined the procedures involved as well as the inclusion of information regarding the biopsy and related investigations into the database.

| Table 7: Investigations done at the time of biopsy. | |
|---|---|
| **Blood test performed at biopsy** | **Abbreviation** |
| **Creatinine** | Cr |
| **Estimated glomerular filtration rate** | eGFR |
| **CD4 count** | CD4 |
| **Viral load (not uniformly performed)** | HIVVL |
| **Hepatitis B surface antigen** | HBsAg |
| **Hepatitis C antibody** | HCV |
| **Total Protein** | TProt |
| **Cholesterol** | Chol |
| **Haemoglobin** | Hb |
| **Complement factor 3** | C3 |
| **Complement factor 4** | C4 |
| **Antinuclear antibody** | ANA |
| **Anti-double stranded DNA** | Anti-dsDNA |
| **Serology for syphilis** | RPR/VDRL |
| **Antistreptolysin O titre** | ASOT |
| **AntiDNAse B** | AntiDNAse B |
| **Albumin** | Alb |

Estimated glomerular filtration rate (eGFR) in ml/min/1.73m$^2$ was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) formula:

$$eGFR\ (ml/min/1.73^2) = 175 + [serum\ creatinine\ (\mu mol/l)/88.4)^{-1.154}] * (Age)^{-0.203}$$

*** (0.742 if female)** *** (1.210 if black)**

(Levey et al., 2000) (Levey et al.,2006)

Table 7 shows those baseline ("within 2 weeks of biopsy") results obtained from the original database. In this study, looking at granulomatous interstitial nephritis and possible aetiologies thereof, additional information was obtained from record reviews and the National Health Laboratory Service database as outlined in table 8 below.

| Table 8. Additional information from record reviews and NHLS database | | |
|---|---|---|
| **Test** | **Abbreviation** | **Purpose** |
| White cell count | WCC | High or low WCC could indicate infection |
| Eosinophil count | Eos | Could indicate drug hypersensitivity reaction |
| Corrected calcium | Corr Ca | Hypercalcaemia can be seen in granulomatous diseases e.g. sarcoidosis or TB |
| Blood culture (within 2 weeks of biopsy) | | A positive blood culture would indicate infection |
| Urine dipsticks protein, blood, glucose and leukocytes | | To determine if there were any dipsticks findings in keeping with GIN other glomerular disease or urinary tract infection |
| Urine microscopy leukocytes, erythrocytes, casts | | To determine if there were any microscopy findings in keeping with GIN, other glomerular disease or infection. |
| Urine culture results (within 2 weeks of biopsy) | | Possible pathogens present in the urine that could cause GIN |
| TB specimens sent within 4 months of biopsy (excluding urine)** | | Evidence for possible disseminated TB as a cause of GIN |
| Urine specimens sent for TB culture and results | | Evidence for possible TB as a cause of GIN |
| CXR evidence of TB | CXR | Evidence for possible disseminated TB as a cause of GIN |
| Abdominal ultrasound evidence of TB | Abdo US | Evidence for possible disseminated TB as a cause of GIN |
| As comprehensive a drug history as possible at biopsy<br>• TB medication (and date of initiation)<br>• Streptomycin*<br>• Cotrimoxazole<br>• Diuretics<br>• Tenofovir*<br>• Antibiotics<br>• Non-steroidal anti-inflammatory drugs or aspirin | | Evidence of a drug cause of GIN |
| *: not implicated in the aetiology of GIN, but could contribute to kidney dysfunction<br>** sputum smear and microscopy, pleural, pericardial and ascitic fluid, fine needle aspirates, TB blood cultures, bone marrow biopsies and others | | |

## Follow up

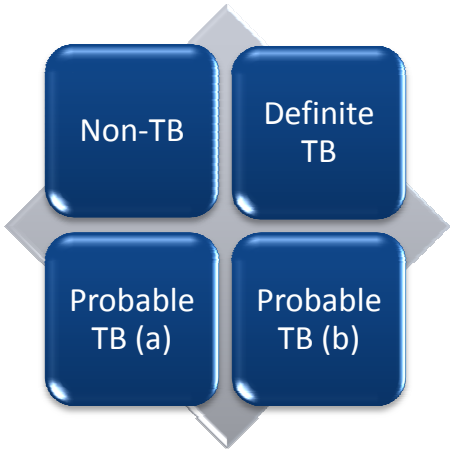
Most patients were followed up in the various clinics of the Division of Nephrology and Hypertension at Groote Schuur Hospital for at least one visit following biopsy, for the results of the biopsy, and to make a plan for further follow up. (See Appendix 3 for follow up visit sheet). Following this visit, many patients were referred back to the referring healthcare facility with a plan for further follow up, ART therapy and kidney function testing. If the severity of the kidney disease necessitated closer follow up, this was done at the Renal Clinic at GSH.

With regard to outcomes, Clinicom was used to ascertain subsequent healthcare visits (in those patients who were not followed up at GSH) as well as date of death. The NHLS database was also used to determine the last contact with the healthcare service (taken as the last date that investigations were performed). Those who had had contact with the healthcare service after 30 June 2013 were taken as “alive” for the purpose of analysis.

## Analysis

From the above information we divided patients into four groups (as previously done by Peter et al).(Peter, Theron, van Zyl-Smit et al. 2012:1213)

**Figure 6: Groups according to TB evidence**




| | Description |
|---|---|
| **Non-TB** | No microbiological proof of TB; CXR and US not suggestive of TB; not treated for TB. |
| **Definite TB** | At least one specimen positive for *M. Tuberculosis* (microbiological evidence) |
| **Probable TB (a)** | Fluid (pleural/pericardial/ascitic) ADA suggestive of TB in a patient with suggestive clinical picture; TB treatment started |
| **Probable TB (b)** | Clinical picture/CXR/abdominal US suggestive of TB; TB treatment started |

From the above investigations, we attempted to classify those patients with GIN into various categories according to most likely aetiology

| Table 9: Categories according to most likely cause of GIN | |
|---|---|
| **Category** | **Description with regard to possible cause of GIN** |
| **0** | No drug present*; non-TB; no evidence of ascending bacterial infection$. Cause uncertain |
| **1** | Drug present/absent; non-TB. Strong evidence of ascending bacterial infection$. |
| **2** | Drug present*; non-TB. Likely cause is drug |
| **3** | No drug present; non-TB but eosinophils on biopsy therefore likely to be drug |
| **4** | Drug present; probable or definite or no TB but eosinophils on biopsy therefore likely to be drug. |
| **5** | Drug present; probable TB. Could be either. |
| **6** | Drug present; definite TB. Could be either, but likely to be TB |
| **7** | Drug present; definite TB. More likely to be TB because kidney function improved despite continued use of drug or abnormal kidney function preceded use of drug |
| **8** | No drug present*; probable TB. Could be TB |
| **9** | No drug present*; probable TB. Could be TB, but additional evidence of pyelonephritis$. |
| **10** | No drug present*. Definite TB elsewhere. Likely to be TB |
| **11** | No drug present*; Definite TB. Likely to be TB but possibility of acute infection. |
| **12** | Evidence of TB on kidney histology/urine culture. Almost certainly TB |
| **13** | Evidence of TB on kidney histology/urine culture. Almost certainly TB, but additional evidence of ascending infection/pyelonephritis.$ |
| *: Drug information was obtained from folder review. Not necessarily all-inclusive – many patients may have been exposed to drugs prior to admission with no comprehensive drug history available. (e.g. analgesics, antibiotics).<br>$: Evidence of ascending infection on biopsy and not just positive urine culture. Acute inflammatory cells in tubules, neutrophil casts, peritubular acute inflammation | |

Descriptive statistics were performed using IBM SPSS Statistics version 21). Statistical analysis and survival curves were performed using Statistica, with the assistance of Kathryn Manning from the Department of Nephrology at Groote Schuur Hospital.

# Results

45 patients with granulomata on kidney biopsy were identified, the majority of which were in black patients (91.1%). 4 patients (8.9%) were of mixed race. There were no white patients in the study.

| **Results Table 1: Baseline characteristics at time of biopsy (n= 45)** | | | | |
|---|---|---|---|---|
| **Gender** | | | | |
| Male; *n* (%) | 19 (42.2%) | | | |
| Female; *n*(%) | 26 (57.8%) | | | |
| **Ethnic group** | | | | |
| Black; *n*(%) | 41 (91.1%) | | | |
| Mixed-race; *n*(%) | 4 (8.9%) | | | |
| **Age in years** | | | | |
| Median (IQR) | 33 (29-37%) | | | |
| **Kidney size *n* = 42** | | **Left kidney** | **Right Kidney** | |
| Median (IQR) | | 12.1 (10.6-13.5) | 11.9 (10.0-13.0) | |
| Large; *n* (%) | | 12 (28.6%) | 8 (19.0%) | |
| Discrepant kidney sizes; *n*(%) | 10 (23.8) | | | |
| **CD4 count (cells/mm$^3$)** | | | | |
| Median (IQR) | 126 (54-237) | | | |
| **Categories** | | **<50** | **50-200** | **>200** |
| *n* (%) | | 10 (22.2) | 19 (42.2%) | 16 (35.6) |
| **Estimated glomerular filtration rate (eGFR) (ml/min/1.73m$^2$)** | | | | |
| Median (IQR) | 24.6(9.6-70.3) | | | |
| **Dialysis at biopsy** | | | | |
| Yes *n*(%) | 12 (26.7) | | | |
| **Blood Pressure (mmHg)** | | | | |
| Median systolic (IQR) | 115 (109-128) | | | |
| Median diastolic (IQR) | 70 (60-79) | | | |
| **Antihypertensive medication** | | | | |
| No *n*(%) | 34 (75.6) | | | |
| **Oedema** | | | | |
| None *n*(%) | 33 (73.3) | | | |
| Anasarca *n*(%) | 2 (4.4) | | | |

Median CD4 count was 126 (range 6-645; IQR 54-237). Only one patient had a CD4 count >500. The median eGFR at biopsy was 24.6 ml/min/1.73m$^2$ (range 2.3-156.7-; IQR 9.6-70.3). 26.7% of patients required dialysis at the time of biopsy. Median systolic blood pressure was 115 mmHg (range 85-220; IQR 109-128) and median diastolic blood pressure was 70 mmHg

(range 45-135; IQR 60-79). Only 4 patients (8.8%) were on more than 2 antihypertensive agents. 73.3% of patients had no evidence of oedema at the time of biopsy and only 4.4% of patients had anasarca.

Kidney sizes were available in 42 patients, the majority of which were normal (9.1-13cm); 12 (26.7%). One patient was noted to have at least one large kidney (>13.1cm). Small kidneys (<9.0 cm) precludes kidney biopsy, and hence were not included in the study. 10 patients (23.8%) were noted to have discrepant kidney sizes (taken as >1.5cm difference between right and left kidney).

## Antiretroviral therapy

| Results Table 2: ART information | |
|---|---|
| On ART at biopsy | |
| **Yes *n*(%)** | 15 (33.3) |
| Duration | |
| **Median (IQR)** | 43 (6-438) |
| **Defaulted *n*(%)** | 2 (4.4) |
| ART initiated after biopsy | |
| **Yes (%)** | 19 (42.2) |
| Duration (days) | |
| **Median (IQR)** | 35 (14-91) |

15 patients (33.3%) were on ART at the time of biopsy (median duration 43 days; range 6-438; IQR 21-71). 2 patients had defaulted treatment prior to biopsy. ART was initiated after biopsy in 19 patients (median 35 days; range 3-447 days; IQR 14-91). 5 patients (11.1%), were never initiated on ART (4 of whom died, and 1 of whom was lost to follow up) and in 4 patients the data as to initiation of ART was missing.

## Biochemistry and serology results.

| Results Table 3: Haematology | |
|---|---|
| **Variable** | **Normal Range** |
| Haemoglobin (g/dl) *n*=45 | **13-17** |
| **Median (IQR)** | 8.2 (6.9-9.5) |
| White cell count (x$10^9$/l) *n*=45 | **4.0-10.0** |
| **Median (IQR)** | 6.06 (4.61-9.39) |
| **Range** | 2.34-13.2 |
| Eosinophil count (x$10^9$/l) *n*=28 | **0.0-0.45** |
| **Median (IQR)** | 0.07(0.04-0.22) |
| **Raised *n*(%)** | 2(4.4) |

Eosinophil count was raised in only 2 patients (4.4%) (although this information was missing in 17 patients).

| Results Table 4: Biochemistry | | |
|---|---|---|
| Variable | **Normal Range** | |
| Tprot (g/l) *n*=33 | **60-85** | |
| **Median (IQR)** | | 83 (67-88) |
| Corr Ca (mmol/l) *n* = 36 | **2.05-2.56** | |
| **Median (IQR)** | | 2.23 (2.08-2.3) |
| **Raised *n*(%)** | | 1(2.8) |
| Albumin (g/l) *n*=42 | **35-52** | |
| **Median (IQR)** | | 28 (21.7-34.0) |
| **Low *n*(%)** | | 34 (81) |
| Cholesterol (mmol/l) *n*= 25 | **<5** | |
| **Median (IQR)** | | 3.7(3.3-5.5) |
| **Raised** | | 9(36) |

Serum corrected calcium was high in only 1 of the 36 patients for whom a result was available. Serum angiotensin converting enzyme (ACE) was performed in none of the patients.

| Results Table 5: Serology | | | |
|---|---|---|---|
| **Variable** | ***N*** | | **% total *n*=45** |
| **Hep B sAg** | **42** | | |
| Positive *n*(%) | | 2(4.8) | 4.4 |
| **HCV Ab** | **34** | | |
| Positive n(%) | | 0 (0) | 0 |
| **C3** | **19** | | |
| Low *n*(%) | | 3 (15.8) | 6.7 |
| **C4** | **19** | | |
| Low n(%) | | 1 (5.3) | 2.2 |
| **ANA** | **27** | | |
| Positive n(%) | | 2 (7.4) | 4.4 |
| **AntidsDNA** | **20** | | |
| Positive n(%) | | 0 (0) | 0 |
| **ASOT** | **21** | | |
| Positive n(%) | | 3(14.3) | 6.7 |
| **AntiDNAseB** | **21** | | |
| Positive n(%) | | 9 (42.9) | 20 |
| **VDRL** | **40** | | |
| Positive n(%) | | 1 (2.5) | 2.2 |

Antistreptolysin O titre was reported as positive in 3 patients (using the laboratory reference range of 0-200 IU/ml), but taking a higher cut off of 500 IU/ml (as is done by the renal physicians at Groote Schuur Hospital) only 1 was positive. Similarly with antiDNAse B, 9 patients were reported as positive according to standard laboratory reference range, but with a practical cut off of 500 IU/ml, only 4 patients had a positive result. VDRL test for syphilis was positive in one patient, but confirmatory testing was negative.

## Urinary findings

**Results Table 6: Urinary findings**

| | | | | | |
|---|---|---|---|---|---|
| **Dipsticks *n*=40** | | | | | |
| Protein *n*(%) | 38(95) | | | | |
| Blood *n*(%) | 27 (67.5) | | | | |
| Leukocytes *n*(%) | 4 (10) | | | | |
| Glucose *n*(%) | 4 (10) | | | | |
| **Urine protein: creatinine ratio (g/mmol)** | | **Normal <0.02** | **0.02-0.1** | **0.1-0.3** | **>0.3 (Nephrotic)** |
| Median (IQR) | 0.19 (0.12-0.44) | | | | |
| *n*(%) | | 2 (4.4) | 6(13.3) | 20 (44.4) | 17(37.8) |
| **Microscopy *n*=41** | | | | | |
| Leukocytes *n*(%) | 39(95) | | | | |
| Erythrocytes *n*(%) | 27(65.8) | | | | |
| **Casts** | **Granular** | **Hyaline** | **Granular/ Hyaline** | **Granular/ White cell** | |
| *n* (%) | 14 (34.1) | 1(2.4) | 8(19.5) | 3(7.3) | |
| **Culture *n*=41** | **Mixed** | **Yeasts** | ***Ecoli*** | ***Enterococcus*** | **Coagulase negative staphylococcus** |
| n (%) | 6(14.6) | 6(14.6) | 8(19.5) | 2(4.9) | 1(2.4) |
| **Sterile pyuria*** | | | | | |
| n (%) | 16 (39) | | | | |

* Defined as positive urine microscopy with negative urine culture.

Urine dipstick information was available in 40 patients (89%). Median urine protein:creatinine ratio (UPCR) was 0.19g/mmol ( IQR 0.12-0.44), with 44.4% of patients having subnephrotic proteinuria and 37.8% of patients having nephrotic range proteinuria.

There was a high congruency between proteinuria recorded on dipstick testing (95% - unquantified) and the UPCR (95.6% had a UPCR >0.02g/mmol)

| Results Table 7: Performance of urine dipsticks versus urine microscopy | | |
|---|---|---|
| **Leukocytes** | **Urine microscopy positive** | **Urine microscopy negative** |
| **Urine dipsticks positive** | 4 | 0 |
| **Urine dipsticks negative** | 30 | 2 |
| **Haematuria** | | |
| **Urine dipsticks positive** | 20 | 5 |
| **Urine dipsticks negative** | 4 | 7 |

41 (91%) patients had urine sent for standard microscopy and culture. In those 36 patients in whom both dipstick testing and urine microscopy was performed, the sensitivity of dipstick testing with respect to leucocyturia was 11.8%. The sensitivity of dipstick testing for haematuria was much higher at 83.3%. Casts were seen in 26 patients (63.4%), the majority being granular and hyaline casts. Culture results are shown in the table with the most common organism grown was *Eschericiae Coli*. Only 2 patients with negative urine culture had no leucocytes seen on urine microscopy. 16 patients of the 41 who had urine sent for culture (39%) therefore had so-called “sterile pyuria”.

## TB data

| Results Table 8: TB information | | | | | |
|---|---|---|---|---|---|
| **TB treatment** | | | | | |
| **On treatment at biopsy** | | | | | |
| *n*(%) | 28(62.2) | | | | |
| Duration (days) Median (IQR) | 28(11-69) | | | | |
| **Started treatment after biopsy (*n*=17)** | | | | | |
| *n*(%) | 5(29.4) | | | | |
| Duration (days) Median (IQR) | 6.5(1-16.5) | | | | |
| **Not started *n*(%)** | 12(26.7) | | | | |
| | | | | | |
| **Evidence for TB** | | | | | |
| **CXR evidence of TB *n*(%)** | 27(60) | | | | |
| | | | | | |
| **Abdominal Ultrasound evidence of TB (*n*=25) *n*(%)** | 15(60) | | | | |
| | | | | | |
| **Total number of TB specimens sent (excluding urine)** | **0** | **1-4** | **>4** | | |
| *n*(%) | 12(26.7) | 31(68.9) | 2 (4.4) | | |
| **Sputum sent *n*(%)** | **31(68.9)** | | | | |
| **SSM positive *n*(%)** | 9(29.0) | | | | |
| **Culture positive *n*(%)** | 17(54.8) | | | | |
| **Other samples** | **Fluid[*] (*n*=7)** | **FNA[***] (*n*=2)** | **Blood/ BM (*n*=5)** | **Broncho scopy[**] (*n*=2)** | **Other biopsy[****] (*n*=2)** |
| **Smear positive n(%)** | 0(0) | 1(50%) | 1(20) | 0(0) | 0(0) |
| **Culture positive *n*(%)** | 4(57.1) | Not done | 0(0) | 1(50) | 0(0) |
| **Fluid ADA >30 U/l (*n*=5)** | 5 (100) | | | | |

[*]Fluid samples included 6 samples of pleural fluid and one pericardial fluid.
[**]Bronchoscopy specimens included bronchial washing/bronchial brushing and lung biopsy.
[***] FNA = Fine needle aspirate (of lymph node)
[****]Other biopsy included one each of oesophageal and skin biopsy.

Twenty eight patients (62.2%) were on TB treatment at the time of biopsy (median duration 28 days; range 1-140 days; IQR 11-69). 5 patients were started on TB treatment after biopsy (median 6.5 days; range 1-18 days; IQR 1-16.5).

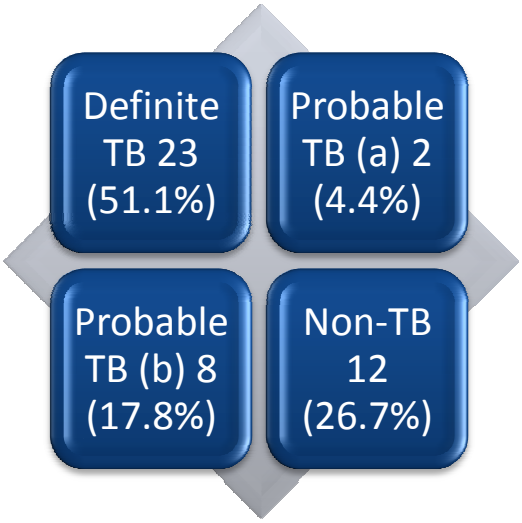
Thirty one patients had sputum sent for TB microscopy and culture. 9 (29%) had positive sputum smear, and 17 (54.8%) had positive sputum culture.

**Results Table 9: Sputum smear versus Culture**

| | Culture Positive | Culture Negative |
|---|---|---|
| SSM Positive | 7 | 2 |
| SSM Negative | 10 | 12 |

Two patients had a positive smear with negative culture (both were on treatment at the time the specimen was taken), while 12 patients who were sputum smear microscopy (SSM) negative, had a positive culture (sensitivity of SSM in this population 41.2%).

**Results Figure 1: Evidence of TB (see Methods section for explanation of categories)**



| Results Table 10: TB urine data | |
|---|---|
| **Number of patients with TB urine sent** | **26 (*n*)** |
| *n*(%) | 26 (57.8) |
| **Number of specimens sent** | **38** |
| **Maximum number of samples sent** | **4** |
| **Results *n*(%)** | |
| Microscopy positive | 1 (2.6) |
| Culture positive | 6 (18.4) |
| Contaminated | 4(15.4) |
| **Time to positivity (days)** | |
| Median (range) | 27 (21-29) |

Thirty eight urine samples from 26 (57.8%) patients were sent for TB microscopy and culture. Only one sample was positive on microscopy (2.6%) (also culture positive), with 6 samples being culture positive (18.4%). One patient had 3 positive cultures; therefore 4/26 patients had positive urine cultures (15.4%), with a median time to positivity of 27 days. All of the patients with positive urine culture results had at least one other specimen that was positive (1 positive on SSM and culture, 2 positive on sputum culture, and one positive on bone marrow biopsy).

## Biopsy findings

Granulomata were multiple in 16 patients (35.6%) and single in 29 (64.4%). Ziehl-Nielssen staining for acid-fast bacilli was performed in 23 patients, with only 2 being reported as positive (4.4%). None of the granulomata showed evidence of caseous necrosis. Eosinophils were present in 5 patients (11.1%).

HIVAN was present in addition to granulomas in 30 biopsy specimens (66.7%). 93% (42) of specimens had at least one other pathology in addition to granulomas ,with 18 (40%) having 2 additional pathologies and 1 (2%) having 3.

| Results Table 11: Biopsy diagnoses | |
|---|---|
| **Pathology** | ***n*(%)** |
| **GIN alone** | **3(6.7)** |
| **GIN + 1 other** | **23(51.1)** |
| HIVAN | 13(28.9) |
| Acute tubular necrosis (ATN) | 5(11.1) |
| ICGN | 2(4.4) |
| Mesangial proliferation | 1(2.2) |
| Hypertension | 1(2.2) |
| Diabetes | 1(2.2) |
| **GIN + HIVAN + 1 other** | **16(35.6)** |
| ATN | 5(11.1) |
| ICGN | 4(8.9) |
| Mesangial Proliferation | 1(2.2) |
| Pyelonephritis | 5(11.1) |
| **GIN + HIVAN + 2 other** | **1(2.2)** |
| **GIN + 2 other (No HIVAN)** | **2(4.4)** |

## HIVAN vs. Non-HIVAN patients

| Results Table 12: Comparison between HIVAN and Non-HIVAN patients | | |
|---|---|---|
| | **HIVAN *n*=30** | **Non-HIVAN *n*=15** |
| **eGFR (ml/min/1.73m$^2$)** | | |
| Median (IQR) | 39.1(10.8-80.3 | 22.8(22.0-29.9) |
| **CD4 (cells/mm$^3$)** | | |
| Median (IQR) | 135 (57-278) | 100 (38-228) |
| **UPCR (g/mmol)** | | |
| Median (IQR) | 0.22 (0.12-0.53) | 0.15(0.11-0.44) |
| **Died** | | |
| *n*(%) | 11(36.7) | 3 (20) |

## Possible causes of GIN.

As described in the Methods section, this grading is a spectrum of the possibility that a drug was responsible for the pathology seen, to the likelihood that the cause was TB-GIN, with other causes (ascending infection, IRIS and 'unknown') interspersed.

**Results Table 13: Possible causes of GIN**

| | Description | *n*(%) |
|---|---|---|
| 1 | Cause uncertain | 3(6.7) |
| 2 | Drug present/absent; non-TB. Strong evidence of ascending bacterial infection. | 2(4.4) |
| 3 | Drug present; non-TB. Likely cause is drug | 5(11.1) |
| 4 | Drug present; probable or definite or no TB but eosinophils on biopsy therefore likely to be drug. | 4(8.9) |
| 5 | Drug present; probable TB. Could be either. | 3(6.7) |
| 6 | Drug present; definite TB. Could be either, but likely to be TB | 6(13.3) |
| 7 | Drug present; definite TB. More likely to be TB because kidney function improved despite continued use of drug or abnormal kidney function preceded use of drug | 2(4.4) |
| 8 | No drug present*; probable TB. Could be TB | 5(11.1) |
| 9 | No drug present; probable TB. Could be TB, but evidence of pyelonephritis. | 1(2.2) |
| 10 | No drug present. Definite TB elsewhere. Likely to be TB | 8(17.8) |
| 11 | No drug present; Definite TB. Likely to be TB but possibility of acute infection. | 1(2.2) |
| 12 | Evidence of TB on kidney histology/urine culture. Almost certainly TB | 3(6.7) |
| 13 | Evidence of TB on kidney histology/urine culture. Almost certainly TB, but additional evidence of ascending infection/pyelonephritis. | 2(4.4) |

### *Drugs*

49% (n=22) of patients were found to be on a drug that has been implicated in GIN.

**Results Table 14: Drugs implicated in GIN**

| | *n*(%) |
|---|---|
| **Co-trimoxazole** | 11(24.4%) |
| **Non-steroidal anti-inflammatory drugs (NSAIDs)** | 7(15.6%) |
| **Antibiotics** | 7(15.6%) |
| **Diuretics** | 2(4.4%) |
| **>1 drug** | **5(11.1%)** |

### *Infection*

6 patients had evidence of ascending infection on kidney biopsy, i.e. acute inflammatory cells in tubules, neutrophil casts, and peritubular acute inflammation. Urine culture was negative in 50% of these cases, while one had mixed growth and 2 patients (33.3%) had

*E.Coli* present on urine culture. 2 patients had no evidence of TB elsewhere. Of these, only 1 patient was reported to be on a drug related to GIN (NSAIDs), making ascending infection the sole possible cause in only one patient.

| Results Table 15: Ascending Infection | |
|---|---|
| | ***n*(%)** |
| **Ascending infection total** | **6 (13.2%)** |
| Infection + TB | 4(8.8%) |
| Infection + drug | 1(2.2%) |
| Infection alone | 1(2.2%) |

## Paradoxical IRIS cases:

During retrospective analysis, 6 cases were identified which could have been due to paradoxical worsening of TB after initiation of ART (paradoxical TB-IRIS) These were included in the initial analysis, but were also analysed separately.

| Results Table 16: Paradoxical IRIS Cases | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **CD 4** | **Creat** | **UPCR** | ***M. tuberculosis* diagnosis** | **ART initiated** | **Onset of kidney disease** | **Outcome** |
| 9 | 57 | 319 | ND | SSM +; Sputum culture +; Pleural fluid and lung FNA +; CXR sugg. TB | 38 days after ATT | 8 days after ART | Died 3 months after ART |
| 11 | 21 | 310 | 0.56 | Fluid ADA 41; CXR and Abdo US sugg. TB | 27 days after ATT | 47 days after ART | Died: 3.5 years after ART of kidney failure (defaulted ART) |
| 18 | 191 | 96 | 0.05 | Pleural fluid culture + | 103 days after ATT | 37 days after ART | Alive |
| 19 | 92 | 126 | 0.15 | SSM +; PCR +; Culture -(on ATT for 2 months); CXR mediastinal nodes | 19 days after ATT | 35 days after ART | Alive |
| 22 | 163 | 498 | 0.19 | Sputum culture +; urine culture +; CXR and abdo US sugg. TB | 44 days after ATT | 20 days after ART | Alive |
| 26 | 41 | 183 | 0.42 | SSM and sputum culture +; abdo US sugg. TB | 24 days after ATT | 15 days after ART | Lost |

| Results Table 17: Descriptive data of IRIS cases (*n*=6) | | | | |
|---|---|---|---|---|
| **Gender** | | | | |
| Male *n*(%) | 5(83.5%) | | | |
| **Age (years)** | | | | |
| Median (IQR) | 33 (29-40%) | | | |
| **eGFR (ml/min/1.73m$^2$)** | | | | |
| Median (IQR) | 34.5(22.4-58.6) | | | |
| **UPCR (g/mmol)** | | **0.02-0.1** | **0.1-0.3** | **>0.3 Nephrotic** |
| Median (IQR) | 0.19 (0.1-0.49) | | | |
| *n*(%) | | 1(16.7%) | 2(33.3%) | 2(50%) |
| **CD4 (cells/mm$^3$)** | | **<50** | **50-200** | **>200** |
| Median (IQR) | 74 (36-170) | | | |
| *n*(%) | | 2(33.3%) | 4(66.7%) | 0(0%) |
| **Dialysis at biopsy** | | | | |
| *n*(%) | 2 (33.3%) | | | |

| Results Table 18: TB and ART information of IRIS cases (*n*=6) | |
|---|---|
| **Duration of TB treatment at biopsy (days)** | |
| Median (IQR) | 71.5 (63.2-95.7) |
| **Duration from ART to abnormal kidney function (days)** | |
| Median (IQR) | 27.5 (13.2-39.5) |
| **Duration from TB treatment to ART (days)** | |
| Median (IQR) | 32.5(24-44) |
| **Evidence for TB** | |
| Microbiological *n*(%) | 5 (83.3%) |
| Fluid ADA *n*(%) | 1 (16.7%) |
| CXR *n*(%) | 5 (83.3%) |
| Abdo US *n*=4 *n*(%) | 2 (33.3%) |

The median duration of TB treatment at the time of biopsy was 71.5 days (IQR 63.2-95.7).

The median duration from the initiation of ART to the first documented abnormal creatinine

was 27.5 days (IQR 13.2-39.5). The median duration of TB treatment prior to starting ART was 32.5 days. The majority of patients (83.3%) had chest XR findings in keeping with TB, and 50% of those who had an abdominal ultrasound performed had findings in keeping with abdominal TB. 3 patients were also on medication known to cause GIN: 1 patient on cotrimoxazole and antibiotics, and 2 patients on cotrimoxazole alone.

## Outcome (all patients)

14 patients were noted to have died on follow up while 25 patients were alive as of 30 June 2012. Comparative data on the 2 groups is shown in Results Table 19. 78.5% (11/14) of those noted to have died had kidney failure listed as their cause of death. The cause of death is not known for the remaining 3 patients. 6 patients were lost to follow up.

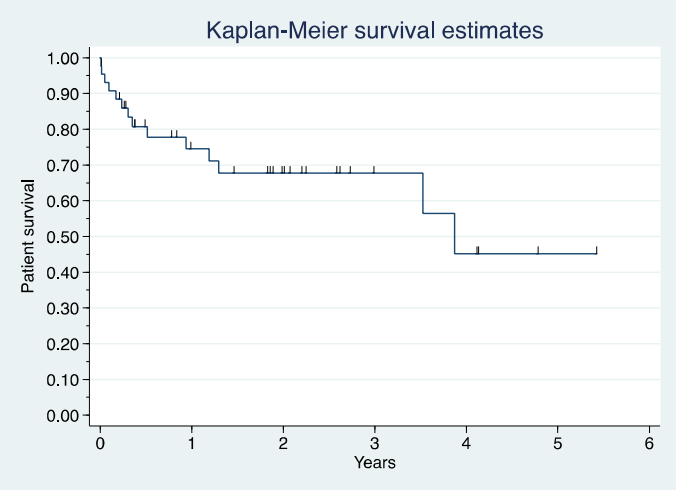
| **Results Table 19: Data on patients who died *n*=14 vs. those still alive *n*=25** | | | **p-value*** |
|---|---|---|---|
| **Time to death/follow up after biopsy (days)** | **Died** | **Alive** | |
| Median (IQR) | 117(40-404) | 718(296-939) | 0.019 |
| **CD4 count (cells/mm$^3$)** | | | |
| Median (IQR) | 147(52-251) | 92(45-237) | 0.558 |
| **eGFR (ml/min/1.73m$^2$)** | | | |
| Median (IQR) | 9.11(5.8-20.5) | 55.15(18.7-82.3) | 0.009 |
| **Gender** | | | |
| Male; *n*(%) | 6(42.9) | 12(48) | |
| Female;*n*(%) | 8(57.1) | 13(52) | 0.757 |
| **Age (years)** | | | |
| Median (IQR) | 36(31-46) | 33(29-36) | 0.333 |
| **UPCR (g/mmol)** | | | |
| Median (IQR) | 0.63(0.145-1.45) | 0.16 (0.09-0.27) | 0.004 |
| **TB evidence** | | | |
| Non-TB *n*(%) | 3(21.4) | 6(24) | |
| Probable TB(a)*n*(%) | 1(7.1) | 1(4) | |
| Probable TB(b)n(%) | 1(7.1) | 7(28) | |
| Definite TB | 9(64.3) | 11(44) | 0.411 |
| **ART** | | | |
| At biopsy *n*(%) | 4(28.6) | 10(40) | 0.729 |
| Started after biopsy *n*(%) | 6(42.9) | 11(44) | 0.626 |

*Note: p-value <0.05 was considered statistically significant

Time to death/follow up was significantly longer in those who were alive at the end of the study. At biopsy, the eGFR was significantly lower and the uPCR significantly higher (p=0.012 and p=0.004 respectively) in those who died compared to those who survived over the study period. There was no statistically significant difference between the two groups with respect to CD4 count, gender, age, TB evidence or ART.

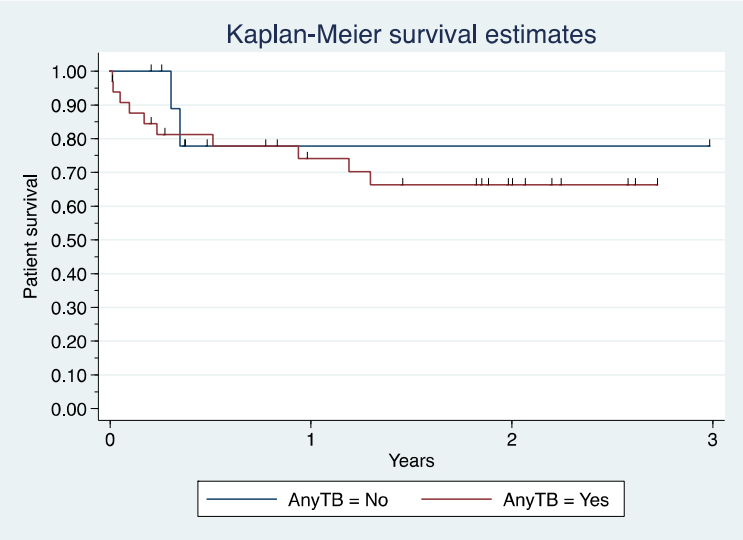
## Survival analysis

**Results Figure 2: Kaplan-Meier Curve for Overall Survival**




The above curve shows the survival estimates for the entire group of patients. Each vertical drop represents a death, with a tick showing date last seen. Those patients who were lost to follow up have been included, as each of these had a date available on which they were last seen. The overall cumulative probability of surviving 5.4 years in this sample is 45.2%.

**Results Figure 3: Kaplan-Meier Curve for Survival According to TB Status**




The survival estimates for the above comparison were curtailed at 3 years as it was felt that beyond this time it was unlikely that TB contributed towards cause of death. The cumulative probability of surviving 3 years in those without TB (blue line) was 77.8%. The corresponding survival probability for those with TB (red line) was 66.3%. The log rank test for equality of

survivor functions at 3 years showed that survival probabilities were not statistically different (p=0.837) despite patients with TB having lower probability of survival.

It was noted that the red “AnyTB” curve was particularly steep during the first 3-4 months and a log rank test showed that, in the first 6 months of the study, the survival probability in those patients with TB was significantly lower (p=0.0191).

# Discussion

The prevalence of GIN in our HIV-infected population is 9 to 24 times higher (12.2% versus 0.5-1.37%) than the rate of GIN observed in other studies of native kidney biopsies (all performed in developed countries with low HIV prevalence). (Bijol et al., 2006) (Javaud et al., 2007)There are numerous possible reasons for the high rate of GIN in our HIV-infected population:

i. South Africa, and Cape Town in particular, has a very high prevalence of tuberculosis, which is likely to be responsible for a large percentage of these cases.
ii. Patients with HIV-infection, particularly in the hospital setting, are exposed to multiple medications, many of which have been implicated in causing granulomatous interstitial nephritis.
iii. Patients with HIV-infection are at risk of numerous opportunistic infections (mycobacterial, fungal etc) which could be responsible for GIN.

Black patients made up the majority of participants in our study (91.1%) with 8.9% being patients of mixed race. There were no Caucasian patients in the study. This could be due to population sampling, with many of our patients coming from periurban townships around Cape Town. It is also likely that Caucasian patients, by virtue of their better socio-economic status, prefer to seek healthcare in the private sector as opposed to public hospitals. In addition, the racial prevalence of HIV infection in South Africa is highest amongst Black African people and those of mixed race (15% and 3.1% respectively) with a much lower prevalence amongst White and Asian people (0.3% and 0.8% respectively) (South Africa. Department of Health, 2012). It has also been found that HIVAN , hypertension and CKD (is more prevalent in those of African descent, with a genetic link established with MYH9 and APOL1 genes. (Atta et al., 2012) (Hays & Wyatt, 2012) (Kopp et al., 2008) (Tzur et al., 2010)

The median CD4 count was 126 cells/$mm^3$ . Although the range was quite wide (6-645), the majority of patients (64.5%) had CD4 counts below 200 cells/$mm^3$. In most of the biopsies, the granulomata were poorly formed, which could be a reflection of the low CD 4 counts and is in keeping with the observations that granuloma formation requires the presence of CD4 cells (Inoue et al., 2010) (Viero & Cavallo, 1995).

Kidney size estimations were reported as being within the normal range in 76.2% of patients. 23.8% of kidneys were reported as being large. One would expect increased kidney sizes in infiltrative conditions (such as GIN, and others), and also in HIVAN (which was

present in 66.7% of patients). Those patients with small kidneys were, by necessity, excluded from the study as kidney size <9cm is a contraindication to kidney biopsy. 23.8% of patients were noted to have discrepant kidney sizes (>1.5cm difference in size between the kidneys). This could be due to vascular causes but, it has previously been noted that in the "Classical" form of renal TB, the presentation is usually one of unilateral disease. (Eastwood, Corbishley & Grange, 2001) However, autopsy studies reveal that both kidneys are usually involved in the disease process. (Eastwood, Corbishley & Grange, 2001)

GIN has been documented as a cause of hypertension (together with active urine sediment and rapidly progressive kidney failure) (Kaul et al., 2011). However, the majority of our patients (75.6%) were on no antihypertensive therapy. This is in keeping with the observation made by Javaud et al.(2007), that 72.5% of patients with GIN in their series had a normal blood pressure.

In our study, only 4.4% of patients had peripheral blood eosinophilia, while 20% of patients were ascertained to have a probable drug-induced aetiology for their GIN. This discrepancy may be due to the low number of patients (62.2%) with an eosinophil count recorded as part of their workup., or it could reflect that the process is limited to the kidney, rather than a systemic response.

Serum calcium was raised in only one patient for whom a result was available (80% of patients). The usual pattern is for serum calcium concentration to decline in kidney disease. However, diffuse granulomatous diseases (including sarcoidosis, fungal and mycobacterial infections) may be associated with increased serum calcium levels. (Adams & Cook, 2007) No patients had a serum ACE performed likely because the presumed low rates of sarcoidosis in this HIV-infected population fail to justify the cost of the test.

Liver function tests (LFTs) were not captured in our database. Javaud et al. (2007) noted that 57% of patients with drug-induced GIN had evidence of cholestasis on liver function testing. However, the utility of LFTs in the workup of GIN in the study population is limited as abnormal results are seen in many conditions including, IRIS, mycobacterial and fungal infections, and sarcoidosis in addition to drug reactions.

ANCA testing was not routinely performed in this study, and was not warranted as the occurrence of ANCA-associated vasculitis in these patients is very rare, and there was no evidence of pauci-immune necrotising glomerulonephritis on any of the biopsies. (Cornely et al., 1999). The few positive ASOT and antiDNAse B results can similarly be explained by the

probable high rate of streptococcal infections in these patients, rather than indicating any evidence of post-infectious glomerulonephritis (which was not seen in any kidney biopsy).

Two patients (4.4%) were found to be co-infected with Hepatitis B virus (HBV) and HIV, based on serum positivity for Hepatitis B surface antigen (HBsAg). This is in keeping with the 4.8% HepBsAg-positivity seen amongst HIV-infected patients at an urban clinic in Johannesburg, South Africa. (Firnhaber et al., 2009) The absence of any Hepatitis C virus (HCV) and HIV co-infection was expected, as it has already been shown that this co-infection is rare in South Africa. (Amin et al., 2004)

## Urinary findings:

All patients had a urine protein:creatinine ratio (UPCR) , whereas information on dipstick testing was available in only 40 patients. The degree of proteinuria seen in our patients (median 0.19g/mmol or 1.9g/d) appears to be higher than those in other studies of GIN where proteinuria was described as mild (median 0.6g/d; range 0.08-3g/d) (Javaud et al., 2007), “minimal” (Joss et al., 2007) and “insignificant” (Bijol et al., 2006). Subnephrotic range proteinuria was described in one study of isolated GIN (Bijol et al., 2006)where the mean proteinuria at presentation for patients with TB-GIN was 1.31+/-0.83g/d (i.e. subnephrotic). (Chapagain et al., 2011) There are no reports indicating the occurrence of nephrotic range proteinuria with isolated GIN. Haematuria was observed on both dipstick and microscopy testing in 67.5 and 65.8% of our patients respectively. This is higher than reports of microscopic haematuria in GIN in literature reviews (15%) (Javaud et al., 2007) (Bijol et al., 2006), but similar to that seen in TB-GIN in case reports. (see Appendix 4). The higher proportion of both proteinuria and haematuria seen in our study could be related to pathologies in addition to GIN, particularly HIVAN and ICGN, observed in 93% of our patients.

UPCR was significantly higher in those patients who died on follow up (p=0.004). In addition the eGFR was significantly lower in those who died (p=0.009). This, coupled with the finding that 78.5% of deaths were attributed to renal failure, likely corresponds more severe kidney disease being present in those who died.

Fabian et al. (2009) performed a study of urinary screening abnormalities in HIV-infected outpatients and noted microscopic haematuria in 33%, and microalbuminuria/proteinuria in 44%. These figures are much lower than seen in our patient population. (Fabian et al., 2009)

It should, however, be noted that our study was based on hospitalised patients with demonstrable kidney pathology necessitating kidney biopsy, rather than outpatients. Our cut-off for overt proteinuria was also slightly lower (0.02g/mmol rather than 0.03g/mmol).

Urine dipstick testing for leucocytes was positive in only 10% of patients. In the outpatient study by Fabian et al. (2009), this percentage was much higher (30%), with a positive correlation between leucocyturia and TB (outside the urogenital tract) and sexually transmitted infections. (Fabian et al., 2009) 95% of the patients in our study had leucocytes documented on urine microscopy, and only those with 3+ or more leucocytes on microscopy had a positive urine dipstick for leucocyturia. The low sensitivity of dipstick testing for leucocyturia is of concern and may warrant further study as urine microscopy is not a routine point-of-care test performed in all patients (particularly outpatients). Possible reasons for this poor performance may include

1. Expired dipstick tests or incorrect storage.
2. Incorrect method of dipstick testing – either reading dipstick incorrectly, or not waiting the required amount of time (120 seconds on the dipstick tests currently in use at Groote Schuur Hospital) before reading the dipstick.

The frequency of sterile pyuria (negative urine culture in the presence of leukocytes on microscopy: 39%) was higher than that reported in the literature reviews in patients with GIN (22.5-25%). (Javaud et al., 2007) (Nzerue et al., 2000) However, in the study of patients with TB-GIN, 56.5% had sterile pyuria, (Chapagain et al., 2011) which is similar to the figure of 53.6% obtained in our study if those with leukocyturia and mixed growth on urine culture are added to the figure of true sterile pyuria. The reason for the high rate of mixed culture results (14.6%) is likely related to the manner in which the urine specimen is obtained (mid-stream urine versus clean catch versus catheter specimen), and likely represents contamination of the specimen. It is possible that many of our patients had been on antibiotic therapy which was undocumented prior to hospitalisation, and therefore so-called “sterile pyuria” may be due to a previously treated infection

In the outpatient study by Fabian et al. (2009), leucocytes were observed on dipstick testing in 30% of HIV-infected patients. (Fabian et al., 2009:S1-81)An infective organism was cultured in 29.1% of cases (*E.Coli* in 70% versus the 19.5% prevalence of *E.Coli* UTI in our population), with sterile pyuria in 70.9% (38.3% had negative culture and 32.6% had mixed organisms) (Fabian et al., 2009:S1-83). Our lower figure of 53.6% for sterile pyuria (negative

and mixed cultures combined) and the lower rate of *E.Coli* UTI could be due to the hospital based nature of our study, with 2 patients growing nosocomial organisms (*Enterococcus faecalis)*, and 6 patients with yeasts on urine culture, which could be related to urethral catheterisation.

There were no cases of confirmed drug resistant TB in our study. The rate of HIV-TB co-infection in our study was 73.3% (although in 17.8% the diagnosis was made on clinical grounds alone). The estimated prevalence of HIV-TB co-infection in South Africa is 60%. (South Africa. Department of Health, 2012:21) The higher rate of co-infection in our patient population is likely due to selection bias (many patients with GIN would have TB as a cause of this disease, and therefore those with co-infection are likely to have been over-represented). In the literature reviewed, amongst case reports of TB-GIN, 6/8 (75%) patients had evidence of active TB elsewhere and in the study by Chapagain et al. (2011), 9/17 (53%) of patients with TB-GIN who were biopsied had evidence of active TB elsewhere. (Chapagain et al., 2011) This is in keeping with observations that TB-GIN is often seen as a manifestation of disseminated infection, (Kaul et al., 2011) (Khilji et al., 2012) (Chapagain et al., 2011) (Nzerue et al., 2000) compared with the long latent period of 'classical renal TB'.

Sputum smear microscopy (SSM) sensitivity was 41.2% in this study. This is similar to the sensitivity of SSM (44%) seen in the study by Peter et al. (Peter, Theron, van Zyl-Smit et al., 2012). It should be noted, however, that with the widespread rollout of XPert MTB/Rif testing of sputum specimens at GSH, which has been shown to have a sensitivity of 63-92.% in smear negative patients and up to 100% in smear positive patients the yield of sputum investigations will likely increase. (Lawn et al., 2011) (Carriquiry et al., 2012)

All patients with positive urine cultures for TB, and AFBs observed on the kidney biopsy had microbiological evidence of TB elsewhere. This is in keeping with the disseminated nature of *M. tuberculosis* infection in HIV-infected individuals, particularly those with TB-GIN. (Kaul et al., 2011) (Khilji et al., 2012) (Chapagain et al., 2011) Although the likely cause of the GIN was thought to be TB in 28 patients (62%), the low yield of urine culture (15.4%) could be due to the fact that many patients were on TB treatment at the time of biopsy, which would decrease urine culture positivity. This poor performance of urine culture and, in particular, urine microscopy for TB has been noted in the literature. (Hemal et al., 2000:571) (Sun et al., 2010:343) (Colabawalla, 1990) In case reports of TB-GIN (see Appendix 4), urine culture again had a low yield, with 2/8 (25%) of patients having a positive urine culture for *M. tuberculosis.* With such documented poor performance of urine microscopy and culture for

TB, the fact that all patients had documented evidence of extrarenal TB, and the long delay to culture positivity of 27days, one may be justified in omitting these traditional investigations in the workup of GIN and replacing them with newer tests with improved sensitivity.

In light of the high proportion of extrarenal TB seen in our patients, one could argue against extensive investigation to prove that TB is, in fact, the cause of the GIN in a particular patient with documented TB elsewhere, as antituberculous chemotherapy would not be altered. However, consideration should be given to the mounting evidence that, in the absence of other infections or drugs being responsible for the GIN, corticosteroids may improve outcome in proven TB-GIN. (Kaul et al., 2011) (Sampathkumar et al., 2009) (Chapagain et al., 2011) It should also be noted that the presence of extrarenal TB does not preclude an aetiology other than TB for the GIN present in the kidney, and all cases should therefore be investigated fully.

## Biopsy findings:

No patients had evidence of caseation observed on kidney biopsy, regardless of the apparent high rate of TB-GIN. Granulomas were generally poorly formed (in keeping with the observation by Eastwood et al. (2001) in immunosuppressed patients) (Eastwood, Corbishley & Grange, 2001)and the low median CD4 count in our patients, as mentioned above. Non-necrotising granulomata are usually associated with sarcoidosis or drugs, however, TB-GIN can be a cause of non-caseating granulomata in the kidney. Caseation was observed in only 18.7% of patients with TB-GIN in the study by Chapagain et al. (Chapagain et al., 2011), although case reports suggest a higher frequency (see Appendix 4). ZN staining for AFBs was positive in only 2 of our patients (4.4%) which is in keeping with reports in the literature of low AFB positivity. (see Appendix 4) (Chapagain et al., 2011). Only one case report performed PCR on kidney biopsy tissue which was positive for *M. tuberculosis* DNA (Kaul et al., 2011) while Chapagain et al. (2011) performed PCR on 5 patients who were thought to have a high probability of *M. tuberculosis* DNA on kidney tissue, and the PCR was negative in all 5 cases. (Chapagain et al., 2011) This low positivity was thought to be related to the age of the specimens (average 4 years). (Chapagain et al., 2011) Javaud et al. identified 2 cases of TB-GIN upon culture of the kidney tissue. (Javaud et al., 2007) Unfortunately, neither *M. tuberculosis* PCR nor tissue culture was performed on the kidney biopsies in our study.

Eosinophils seen on kidney biopsy may be a feature of a drug reaction, but they are also seen in other settings such as TINU and TB-GIN. (Joss et al., 2007) (Chapagain et al., 2011) We did not find eosinophils on biopsy as often as Chapagain et al. (where all patients with TB-GIN had eosinophils on biopsy). (Chapagain et al., 2011) In our study, eosinophils were present in only 5 patients (11.1%), and were attributed to a drug reaction in 4 of those 5 patients. In only one patient was the cause of GIN with eosinophils thought to be TB alone. The reason for the lower rate of GIN with eosinophils in our patients with TB-GIN is unclear.

HIVAN was present in addition to granulomata in 30 biopsy specimens (66.7%). There is likely a selection bias here as HIVAN is more common in black patients and the majority of our patients fit into this population group. Of note, 93% of patients had at least one other pathology in addition to granulomata, and 2 patients (4.4%) had 3 additional pathologies. This is in keeping with the observation by Wearne et al that 34.8% of patients with HIVAN on kidney biopsy had an additional pathology. It also serve to highlight the point that, while HIVAN is thought to be the most common cause of kidney disease in HIV-infected patients, it is by no means the only cause. (Wearne et al., 2012) (Gerntholtz, Goetsch & Katz, 2006) Kidney biopsy is often essential to establish the cause of kidney dysfunction in these patients, as clinical diagnosis remains challenging and may be misleading. (Wearne et al., 2012) (Gerntholtz, Goetsch & Katz, 2006)

## Possible causes of granulomata:

It would have been preferable to divide the causes of GIN in our population into only a few groups i.e. unknown (idiopathic), drug-induced, TB, pyelonephritis +/- IRIS. However, this proved difficult to do, given the retrospective nature of our study. While 73.3% of patients had evidence of TB, 49% of patients were on drugs known to be associated with GIN. If one were to consider rifampicin as a possible cause of GIN as suggested by certain authors (Chung et al., 2009) (Javaud et al., 2007) (Rossert, 2001), an extra 15 patients could be added to the possible drug-induced GIN group. Therefore, up to 37 out of 45 patients (82.2%) were on at least one drug which could be implicated in GIN. Based on the information that was available through records reviews we came up with 13 categories ranging from unknown cause, to pyelonephritis, and then a spectrum from 2-13 ranging from the least likely to the most likely to be TB. The vast majority of cases of GIN (60%) were attributed to TB, while drugs were thought to be the cause in 20%. Pyelonephritis was

present on biopsy in 6 cases, but was thought to be the main cause in only 3 cases, with the other 3 having strong evidence of TB elsewhere.

The cause of the GIN could not be established in 3 cases. It is possible that these patients could have been on a drug associated with GIN that was not apparent on history. Other possibilities include sarcoidosis, TINU and idiopathic. With the low prevalence of sarcoidosis in this population, particularly at CD4 counts below 200cells/$mm^3$, it is reasonable, as a cost-saving measure, to defer investigation of possible sarcoidosis until the results of other investigations (e.g. TB cultures) become available, and to limit the use of these investigations to those patients in whom no other cause is apparent after extensive history and other investigation.

If one were to try to place our patients into fewer categories, the results based on our knowledge from record reviews would likely be inaccurate. However an attempt has been made to do this, for purposes of comparison with other studies.

**Discussion Table 1: Comparison of this study with existing reviews.**

| | **Bijol et al** | **(%)** | **Javaud et al** | **(%)** | **Mignon et al** | **(%)** | **Viero et al** | **(%)** | **Joss et al** | **(%)** | **Nel et al** | **(%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number | 46 | | 43 | | 32 | | 12 | | 18 | | 45 | |
| Frequency in kidney biopsies | 0.5% | | 1.37% | | 0.9% | | 5.9% * | | <1% | | 12% | |
| Sarcoidosis | 11 | 29 | 20 | 50 | 3 | 9.4 | 3 | 25 | 5 | 27 | 0 | 0 |
| Drug reaction | 17 | 45 | 7 | 18 | 10 | 31 | 3 | 25 | 2 | 11 | 9 | 20 |
| Mycobacterium | 0 | 0.0 | 5 | 13 | 3 | 9.4 | 0 | 0.0 | 0 | 0.0 | 27 | 60 |
| Drug/*M. tuberculosis* (unable to differentiate) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 6.6 |
| Miscellaneous infections | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 3 | 25 | 0 | 0.0 | 3 | 6.6 |
| BCG | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 |
| Wegener's | 2 | 5.3 | 2 | 5 | 8 | 25 | 1 | 8.3 | 0 | 0.0 | 0 | 0 |
| FBGCR | 2 | 5.3 | 0 | 0 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 0 | 0 |
| XPN | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 |
| TINU | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 2 | 11 | 0 | 0 |
| Crohn's | 0 | 0 | 1 | 2.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Idiopathic | 4 | 11 | 5 | 13 | 8 | 25 | 1 | 8.3 | 9 | 50 | 3 | 6.6 |

BCG**: intravesical *Bacille Calmette-Guerin* used to treat bladder carcinoma;** FBGCR**: Foreign body giant cell granuloma;** XPN**: xanthogranulomatous pyelonephritis;** TINU**: tubulointerstitial nephritis and uveitis syndrome.**

* **This figure represents the percentage of GIN among patients with acute interstitial nephritis (not total kidney biopsies performed)**

Paradoxical TB IRIS with GIN was thought to be a possibility in 6 cases. The median CD4 count was lower than in the other patients, with no patients having a CD4 count >200 cells/$mm^3$. This is in keeping with the observation that a low CD4 nadir on starting ART is a risk factor for developing IRIS. (Lawn, Bekker & Miller, 2005). The major risk factor for developing TB-IRIS is the initiation of ART within 2 months of starting TB treatment. (Lawn, Bekker & Miller, 2005) This was observed in our study, with a mean duration of TB treatment prior to ART initiation of 32.5 days in the IRIS cases. None of the patients in our study received corticosteroid therapy. IRIS has been shown to respond to steroids. (Meintjies et al., 2010) It is therefore possible that, had the diagnosis been considered, a percentage of these patients would have responded to treatment with steroids. In addition, if one were to make a case for treating those with TB-GIN and drug-induced GIN with corticosteroids, a further 33 patients would have been eligible for consideration of corticosteroids.

In addition to the aforementioned possible causes of GIN in HIV-infected patients, there is a possibility that the HIV virus itself could be responsible for the granulomata. This possibility could be examined in future, prospective, studies by determining the presence/absence of viral particles in kidney biopsy specimens.

## Survival Analysis

The overall cumulative probability of surviving 5.4 years for this cohort of patients was low (45.2%). This low rate of survival is likely multifactorial (kidney disease, HIV infection, and opportunistic infections (especially TB)). However, 78.5% of deaths were attributed to kidney disease, emphasising the point that GIN can result in severe kidney dysfunction. Therefore treating physicians should endeavour to diagnose, with a view to treating appropriately, the underlying cause of this pathological finding.

The survival probability of those patients with GIN and TB was significantly lower than those without TB in the first 6 months following presentation (p=0.191). This is in keeping with the high mortality rate of TB-HIV co-infection that has been noted in the literature (33% of incident HIV-TB cases in 2007). (Lawn and Churchyard, 2009)

## Diagnostic algorithm

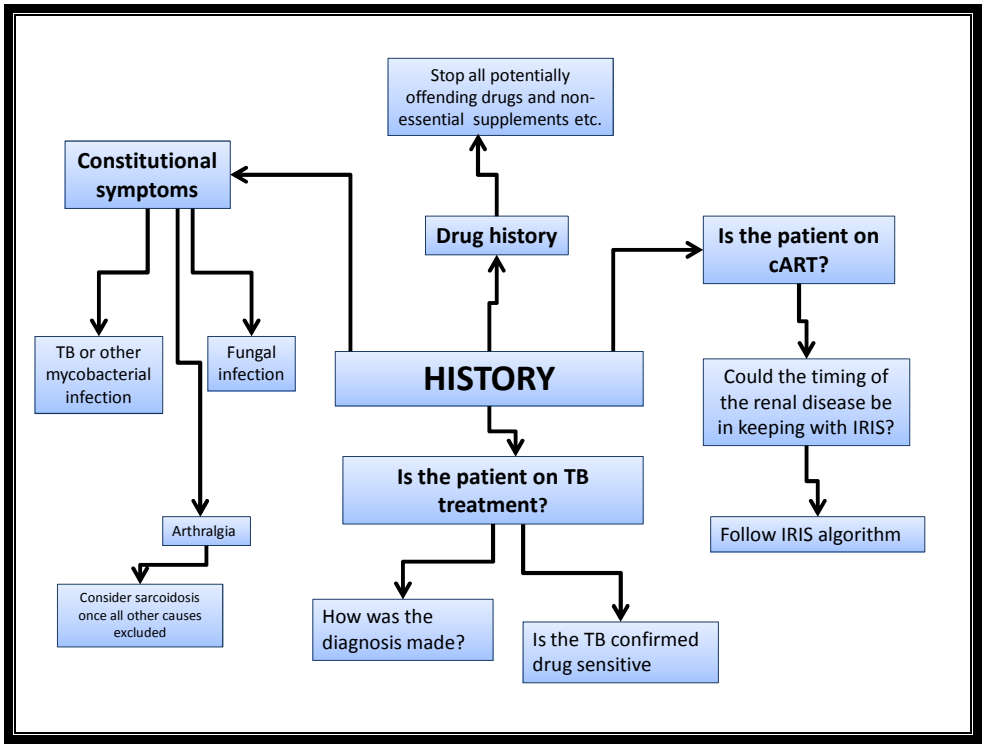
Although GIN is a relatively rare condition, the increased prevalence shown in our study suggests that HIV-infected patients are at an increased risk of GIN. A high index of suspicion

is required in this population which may lead to earlier diagnosis, and treatment of the various causes of this disease. (Chapagain et al., 2011) With earlier appropriate treatment one may be able to preserve kidney function and delay or obviate the need for renal replacement therapy. (Chapagain et al., 2011) When the kidney biopsy shows evidence of GIN, the differential diagnosis is large. (Nasr et al., 2003) The histological features may be relatively non-specific and identification of the underlying aetiology requires an extensive history, blood and urine investigations as well as the use of special stains, immunofluorescent, immunohistochemical and molecular methods. (Nasr et al., 2003). Familiarity with the most common causative agents, together with a high index of suspicion are key to establishing the correct diagnosis, particularly in the case of infectious causes. (Adams & Cook, 2007). Culture results may take days to weeks; it is therefore essential to initiate evaluation for bacterial, mycobacterial, fungal and viral infections as soon as possible to prevent any delay in definitive treatment. (Lapasia et al., 2010)

Based on the information from both the literature review, and that obtained in our study, a diagnostic and therapeutic algorithm for HIV-infected patients with GIN has been developed. For purposes of clarity these are presented in four parts.

## History:

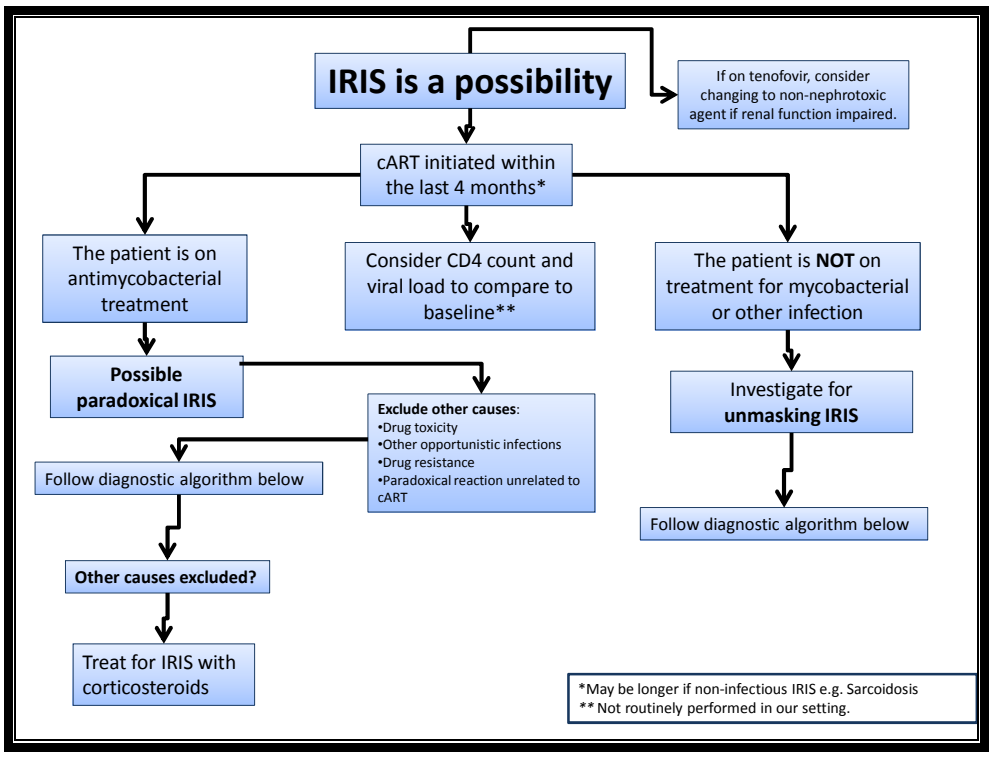
**Diagnostic algorithm 1: Points to consider on history**



- One should enquire about the presence of systemic symptoms including fever, nausea, vomiting, night sweats, weight loss. (Robson et al., 2003) (Chaudhari, Ranganath & Pavan, 2011) (Khilji et al., 2012) (El-Reshaid, Madda & Al-Saleh, 2001) (Chapagain et al., 2011) (Javaud et al., 2007) (Bijol et al., 2006) (Hong et al., 2007) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) (Vanhille et al., 1983) (Viero & Cavallo, 1995) (Larsen et al., 2008) (Kaul et al., 2011) (Fallouh et al., 2010) (Jehle et al., 2004) (Nasr et al., 2003) (Audimoolam & Bhandari, 2006) (Qian et al., 2011) (Larsen et al., 2008) (Colbert, Richey & Schwartz, 2012). These may indicate *M. tuberculosis* infection, but may also be present in fungal infection, other mycobacterial infection and sarcoidosis. 63.5% of patients with TB-GIN gave a history of systemic symptoms. (Chapagain et al., 2011)
    - Although rare in patients with HIV-infection, sarcoidosis may mimic TB with cough, hilar lymphadenopathy, loss of weight, and dyspnoea. (Foulon et al., 2004) In the study by Foulon et al. (2004) of patients who developed sarcoidosis after initiation of ART, 72.7% had a history of dyspnoea, cough, loss of weight, fever, night sweats and symptoms failed to improve, and in some cases worsened, after initiation of antituberculous therapy.
    - The presence of arthralgia may be suggestive of sarcoidosis. (Bijol et al., 2006) (Javaud et al., 2007)
- An extensive drug history should be obtained. (Nasr et al., 2003) (Bijol et al., 2006) (Inoue et al., 2010) (Khilji et al., 2012) (Colbert, Richey & Schwartz, 2012). Of particular importance are antibiotics, nonsteroidal anti-inflammatory drugs and analgesics, but should include all drugs including over-the-counter medication and traditional medication. The date that each drug was started should be recorded in order to establish a possible timeline.
    - Stop all potentially offending drugs, and all non-essential supplements (Lapasia et al., 2010)
- Enquire about co-morbid conditions. (Bijol et al., 2006) In particular, enquire about whether the patient is on TB treatment and if so, how the diagnosis was made, and if drug sensitivity testing was performed.
- It is important to establish if the patient is on ART and, if so, when this treatment was started, as well as the prescribed regimen. If ART was initiated within the last 4 months, there is a possibility that IRIS may be causing the kidney disease. (Izzedine et al., 2007) (Meintjies et al., 2008)

## Approach to possible IRIS-GIN

**Diagnostic algorithm 2: Approach to possible IRIS-GIN**



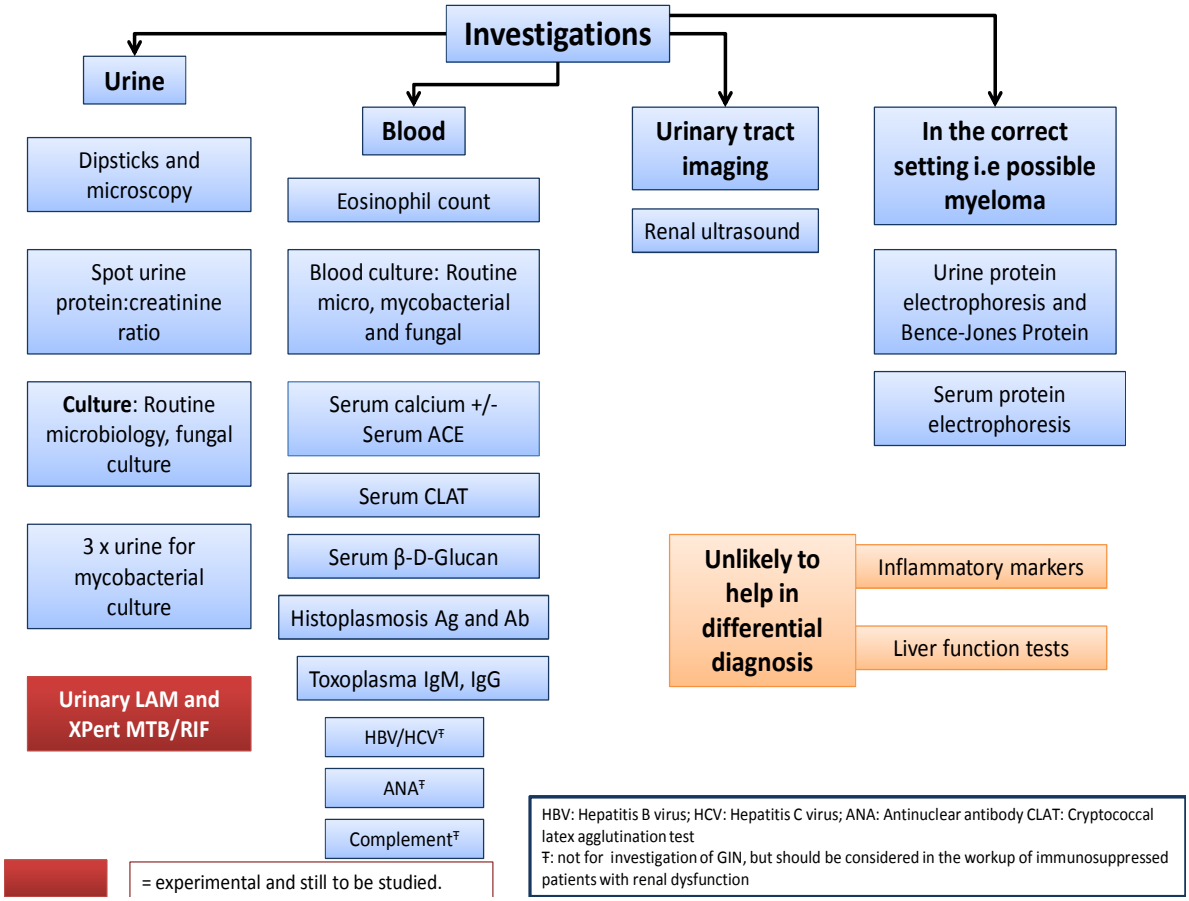

- Tenofovir has not been associated with GIN. However, if there is evidence of kidney dysfunction, consideration should be given to changing to a less nephrotoxic drug regimen.
- CD4 count and viral load testing may be performed at this stage to ascertain whether there has been a response to ART compared to baseline values, (Martin-Blondel et al., 2011) (Salliott et al., 2008) (Jehle et al., 2004) (Daugus et al., 2006) (Izzedine et al., 2007) (Croucher et al., 2010) although this is not routinely performed in a South African setting and is not required to establish a diagnosis of IRIS. (Meintjies et al., 2008)
- If the patient is on treatment for a mycobacterial infection, and particularly if ART was initiated within 2 months of starting that treatment, the patient is at risk of paradoxical IRIS reaction. (Lawn, Bekker & Miller, 2005).
    - One should exclude other causes of a similar presentation including
        - Drug reaction
        - Other opportunistic infection

        - Possibility of drug resistance. Check previous drug sensitivity testing, and enquire whether there was an initial response to antimycobacterial therapy. (Martin-Blondel et al., 2011)
        - Paradoxical reaction unrelated to ART initiation
        - (Lawn, Bekker & Miller, 2005) (Meintjies et al., 2008) (Martin-Blondel et al, 2011)
    - Although no cases have been described in the literature, it is possible that GIN could occur as part of an IRIS response to other opportunistic infections e.g. fungal.
- If the patient is not on treatment for mycobacterial or other infection, one should continue investigating as below with a view to diagnosing possible Unmasking IRIS.

## Investigations recommended in the workup of GIN in HIV-infected patients.

The investigations listed below are in addition to the standard workup of HIV-infected patients with kidney disease planned for kidney biopsy (including serum electrolytes, creatinine, full blood count and clotting profile and urine for haematuria and proteinuria). The list of possible investigations for causes of GIN is extensive, and likely to be very expensive to implement. For this reason, attention has been focussed on the most common causes of GIN in this population for purposes of cost-effective investigation. A more comprehensive investigation algorithm and explanation may be found in Appendix 5. This information can be used as a reference for diagnostic purposes, guided by the clinical context, in patients in whom more limited investigation fails to elucidate an aetiology for their GIN.

**Diagnostic algorithm 3: Proposed list of investigations for GIN in resource limited settings.**




***Urine:***

- Dipsticks and microscopy to assess for proteinuria, haematuria, leukocyturia and casts. (Chung et al., 2009) (Alsaad et al., 2007) (Adams & Cook, 2007) (Nasr et al., 2003) (Audimoolam & Bhandari, 2006) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Hong et al., 2007) (Javaud et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Tse et al., 2004) (Unal et al., 2007) (Vanhille et al., 1983) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011)

(Salliott et al., 2008) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Croucher et al., 2010) (Chun & Hale, 2004)

- Spot urine protein:creatinine ratio. (Chung et al., 2009) (Nasr et al., 2003) (Joss et al., 2007) (Ram et al., 2009) (Robson et al., 2003) (Singh & Colvin, 2003) (Unal et al, 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Salliott et al., 2008) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Daugus et al., 2006) (Croucher et al., 2010)
- Urine culture: routine microbiology (Kim & Sung, 2010) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Sampathkumar et al., 2009) (Larsen et al., 2008) (Kaul et al., 2011) (Fallouh et al., 2010) (Chun & Hale, 2004) and fungal culture (Chung et al., 2009) (David et al., 2009) (Alsaad et al., 2007) (Nasr et al., 2003) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Tse et al., 2004) (Chaudhari, Ranganath & Pavan, 2011) (Salliott et al., 2008)
- Send 3 early morning urine specimens for mycobacterial culture. (Bouzourene, Bouzourene & Francke, 1998) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Sung, 2010) (Ram et al., 2009) (Robson et al., 2003) (Tse et al., 2004) (Unal et al., 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al- Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Chun & Hale, 2004)
    - In light of this low sensitivity of urine culture for TB, together with the long delay to culture positivity one may make a case to omit this traditional investigations as highlighted in Discussion above.

### *Urinary tract imaging*

- Kidney ultrasound for kidney size estimation prior to biopsy and to assess gross anatomical abnormalities of the urinary tract (looking for evidence of classical renal TB). (Chung et al., 2009) (Nasr et al., 2003) (Korsten et al., 2010) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid,

Madda & Al- Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Salliott et al., 2008) (Daugus et al., 2006) (Croucher et al., 2010)

- Further imaging can be guided by these findings as needed.

### *Blood tests*

- Eosinophil count could assist in diagnosing possible allergic (drug) reaction. (Lapasia et al., 2010) (Hong et al., 2007) (Javaud et al., 2007) (Joss et al., 2007) (Kim & Hee Sung, 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Viero & Cavallo, 1995)
- Blood culture: (Chung et al., 2009) (Hong et al., 2007) routine microbiology, (Qian et al., 2011) (Singh & Colvin, 2003) (Tse et al., 2004) (Kaul et al., 2011), mycobacterial (Javaud et al., 2007) (Fallouh et al., 2010) and fungal. (David et al., 2009) (Alsaad et al., 2007) (Bijol et al., 2006) (Ramalakshmi, Bastacky & Johnson, 2003) (Larsen et al., 2008) (Izzedine et al., 2007)
  - The sensitivity of fungal culture in disseminated disease is 75-85%. (Adams & Cook, 2007)
- Serum calcium should be interpreted with caution. (Lapasia et al., 2010) (Adams & Cook, 2007) (Audimoolam & Bhandari, 2006) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Ram et al., 2009) (Robson et al., 2003) (Singh & Colvin, 2003) (Unal et al., 2008) (Viero & Cavallo, 1995) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Izzedine et al., 2007)
  - Serum calcium was raised in only one patient in our study of the 80% for whom a result was available.
  - Raised serum calcium may be seen in sarcoidosis, but many diffuse granulomatous diseases including fungal and mycobacterial infections may be associated with this finding. (Adams & Cook, 2007) This is due to increased absorption as a result of increased 1,25-dihydroxy vitamin D production within granulomas. (Adams & Cook, 2007).
  - Perhaps the combination of raised serum ACE and serum calcium could point towards sarcoidosis, once other causes are excluded.
- Serum Cryptococcal Latex Agglutination Test (CLAT) to assess for possible disseminated cryptococcal infection.

- Serum Beta-D-Glucan to assess for possible disseminated fungal infection.
- Serum histoplasmosis antigen and antibody (Adams & Cook, 2007) (Lapasia et al., 2010) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Qian et al., 2011)
    - False negative histoplasma antibody results can be seen in recent infection and immunocompromised patients. In disseminated disease, the sensitivity ranges from 63-81%. (Adams & Cook, 2007) (Qian et al., 2011) (Chung et al., 2009). False positive results may be found in patients with other fungal diseases. (Qian et al., 2011)
- Toxoplasma IgG and IgM (Pena de la Vega et al., 2005) (Khilji et al., 2012) (Fallouh et al., 2010) (Izzedine et al., 2007)
- Hepatitis B virus (HBV), Hepatitis C Virus (HCV) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Chung et al., 2009) (Qian et al., 2011) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Chaudhari, Ranganath & Pavan, 2011) (Kaul et al., 2011) (Fallouh et al., 2010) (Izzedine et al., 2007)
    - These tests are not for the investigation of GIN in particular, but should be performed in immunosuppressed patients with evidence of kidney dysfunction.
- Anti-nuclear antibody (ANA); (Nasr et al., 2003) (Chung et al., 2009) (Hong et al., 2007) (Audimoolam & Bhandari, 2006) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim Hee Sung, 2010) (Korsten et al., 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Chaudhari,Ranganath & Pavan, 2011) (Larsen et al, 2008) (Khilji et al., 2012) (Kaul et al., 2011) serum complement (Chung et al., 2009) (Hong et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Korsten et al., 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011)
    - These tests are not necessarily part of the workup of GIN, but are usually performed as part of the workup for acute kidney injury particularly rapidly progressive glomerulonephritise (Khilji et al., 2012)

***In the correct setting when there is suspicion of possible multiple myeloma***

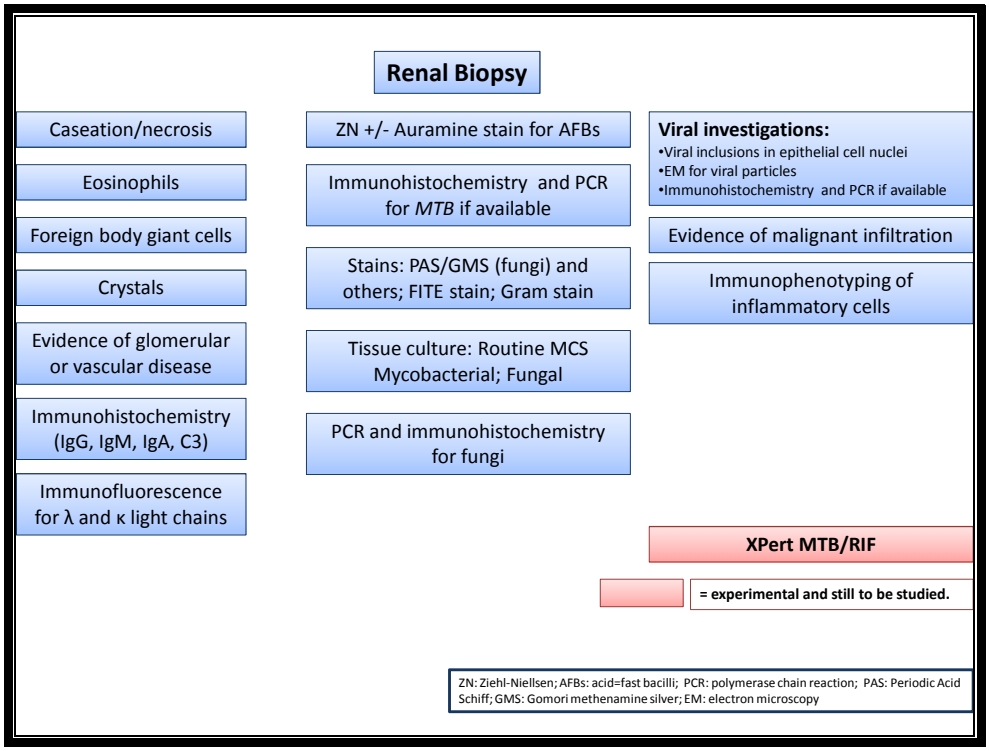
- Urine protein electrophoresis and Bence-Jones Protein to assess for possible paraproteinuria as a cause of GIN
- Serum protein electrophoresis (Chung et al., 2009) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Kim & Hee Sung, 2010) (Singh & Colvin, 2003) to assess for possible multiple myeloma as a cause of GIN

## The following investigations are unlikely to be helpful in determining the aetiology of GIN

- Inflammatory markers: erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) (Tse et al., 2004) (Sampathkumar et al., 2009) (Khilji et al., 2012) (Kaul et al., 2011) (El-Reshaid, Madda & Al- Saleh, 2001) (Chapagain et al., 2011)
    - Unhelpful in assessing infectious aetiology as they may also be raised in drug induced GIN, (Audimoolam & Bhandari, 2006) (Kennedy, Shrinkanth & Charlesworth, 2006) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) inflammatory conditions, (Unal et al., 2008) and IRIS. (Salliott et al., 2008) (Jehle et al., 2004) (Izzedine et al., 2007) They may also be low in TB. (Chaudhari, Ranganath & Pavan, 2011).
- Liver function tests (Nasr et al., 2003) (Montseney & Meyrier, 1998) (Javaud et al., 2007) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Viero & Cavallo, 1995) (Sampathkumar et al., 2009) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Izzedine et al., 2007) (Daugus et al., 2006)
    - These may be raised in many conditions associated with GIN including mycobacterial and fungal infections, IRIS, drug reactions and sarcoidosis.

## *Kidney biopsy*

**Diagnostic algorithm 4: Features on kidney biopsy**




- Look for caseation (Chung et al., 2009) (Lapasia et al., 2010) (Audimoolam & Bhandari, 2006) (Bouzourene, Bouzourene & Francke H, 1998) (Javaud et al., 2007) (Kim & Hee Sung, 2010) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Salliott et al., 2008) (Martin-Blondel et al., 2011) (Daugus et al., 2006) and necrosis (Bijol et al., 2006) (Magner, Sweet & Bear, 1986) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Jehle et al., 2004)
- Look for eosinophils (not necessarily suggestive of drug-induced injury). (Bijol et al., 2006) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Singh & Nickeleit, 2004) (Tong, Howell & Foreman, 2007) (Singh & Colvin, 2003) (Vanhille et al., 1983) (Chapagain et al., 2011) (Rossert, 2001)
- Look for foreign body type giant cells and polarisable crystals possibly indicating calcium oxalate as a cause of GIN. (Viero & Cavallo, 1995)

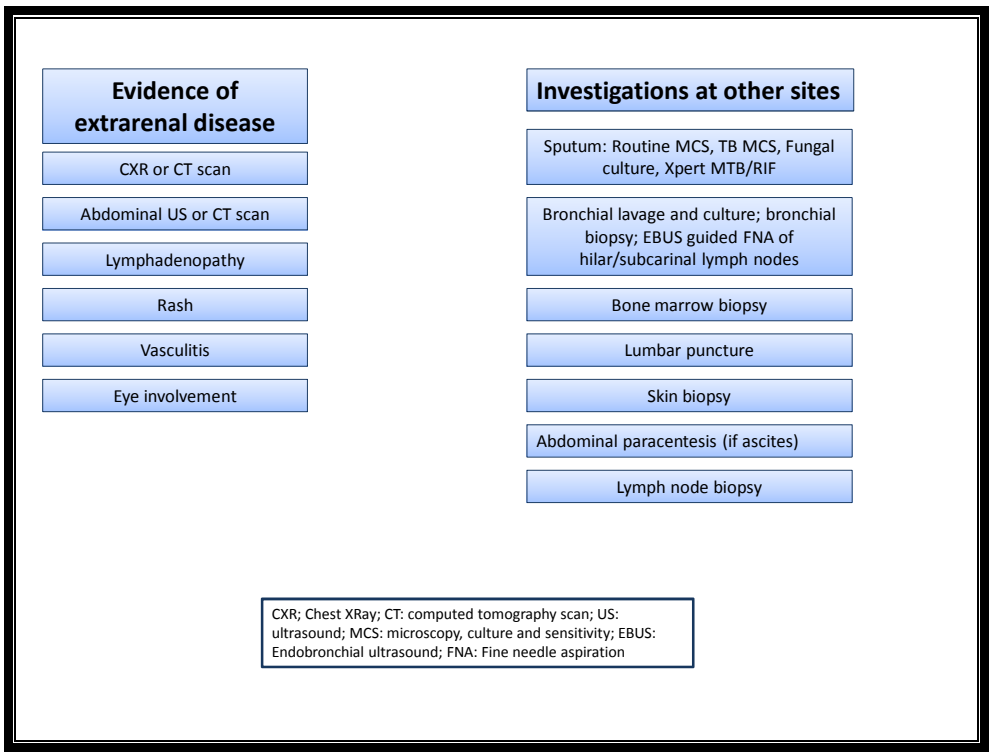
- Look for evidence of glomerular or vascular disease. (Nasr et al., 2003) (Bijol et al., 2006) (Ramalakshmi, Bastacky & Johnson, 2003) (Sampathkumar et al., 2009) (Salliott et al., 2008)
    - If granulomas are periglomerular (Nasr et al., 2003) look for evidence (including serology) of vasculitis. (Lapasia et al., 2010)
- Immunofluorescence/immunohistochemstry: staining for IgG, IgM, IgA, C3. (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Javaud et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Magner, Sweet & Bear, 1986) (Qian et al., 2011) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Viero & Cavallo, 1995) (Sampathkumar et al., 2009) (Larsen et al, 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al- Saleh, 2001) (Salliott et al., 2008) (Jehle et al., 2004) (Izzedine et al., 2007)
    - Look for immune-complex deposition in the tubulointerstitial compartment, (evidence of immune-complex mediated interstitial nephritis) which may be seen in autoimmune disease, and occasionally in drug reactions. (Nasr et al., 2003) (Tong, Howell & Foreman, 2007). Look for linear staining of tubular basement membrane to rule out anti-tubular basement membrane nephritis. (Nasr et al., 2003) (Rossert, 2001)
- Immunofluorescence for κ and λ light chains. (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Javaud et al., 2007) (Kim & Hee Sung, 2010) (Qian et al., 2011)
    - This is currently not available at GSH, but it is possible to screen for serum light chains.
- ZN staining for AFBs (Lapasia et al., 2010) (Alsaad et al., 2007). (Nasr et al., 2003) (Bijol et al., 2006) (Javaud et al., 2007) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Magner, Sweet & Bear, 1986) (Qian et al., 2011) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) (Tse et al., 2004) (Unal et al., 2008) (Viero & Cavallo, 1995) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Salliott et al., 2008) (Martin-Blondel et al., 2011) (Croucher et al., 2010)
- Auramine stain for mycobacteria. (Bouzourene, Bouzourene & Francke, 1998) (Pena de la Vega et al., 2005) (Magner, Sweet & Bear, 1986)

- Immunohistochemistry for mycobacterium (Kennedy, Shrinkanth & Charlesworth, 2006) and PCR for *M. tuberculosis* (Bouzourene, Bouzourene & Francke, 1998) (Kim & Hee Sung, 2010) (Sampathkumar et al., 2009) (Chapagain et al., 2011) if available
- Periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS) stains for fungi. (Chung et al., 2009) (Lapasia et al., 2010) (Alsaad et al., 2007) (Adams & Cook, 2007) (Nasr et al., 2003) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Javaud et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Magner, Sweet & Bear, 1986) (Qian et al., 2011) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) (Tse et al., 2004) (Viero & Cavallo, 1995) (Larsen et al., 2008) (Colbert, Richie & Schwartz, 2012) (Croucher et al., 2010)
- If these stains are positive they can be followed by Alcian Blue and mucicarmine stains. (Adams & Cook, 2007) (Chung et al., 2009) (Nasr et al., 2003). If these are negative: Fontana Masson stain for capsule-deficient Cryptococcus can be done (Chung et al., 2009) (Adams & Cook, 2007) (Nasr et al., 2003)
    - *Histoplasma* are small intracellular yeasts. (Adams & Cook, 2007). In the majority of cases of GIN secondary to *histoplasma* species, the organism was present in the kidney tissue examined. (Adams & Cook, 2007)
    - *Blastomyces* are larger and multinucleated with thick cell walls and broad based budding. (Adams & Cook, 2007) (Nasr et al., 2003)
    - Well encapsulated *Cryptococcus* are not usually associated with significant granulomatous inflammatory response and are seen as round yeast with a thick mucopolysaccharide capsule (Adams & Cook, 2007) on staining for mucin.
- FITE stain for *M. Leprae* (Alsaad et al., 2007) (Tse et al., 2004)
- Gram stain for bacteria (Alsaad et al., 2007) (Bijol et al., 2006)
- Tissue culture for routine microscopy culture and sensitivity, (Bouzourene, Bouzourene & Francke, 1998) mycobacterial culture, (Javaud et al., 2007) (Magner, Sweet & Bear, 1986) (Larsen et al., 2008) (Kaul et al., 2011) (Daugus et al., 2006) and fungal culture. (Magner, Sweet & Bear, 1986)
- PCR and immunohistochemistry for fungi (Qian et al., 2011) (Ogura et al., 2012)

- Viral investigations:
    - Look for evidence of viral infection:
        - Viral inclusions in epithelial cell nuclei (Singh & Nickeleit, 2004) (Alsaad et al., 2007) (Qian et al., 2011) (Tse et al., 2004)
        - Severe tubular destruction with ruptures of tubular basement membranes and necrosis. (Singh & Nickeleit, 2004)
        - Focal haemorrhage and intratubular red cell casts (Singh & Nickeleit, 2004)
    - Demonstration of crystalloid viral particles of about 80nm by electron microscopy. (adenovirus) (Singh & Nickeleit, 2004)
    - Immunohistochemistry stains for adenovirus. (Qian et al., 2011) (Lapasia et al., 2010) (Alsaad et al., 2007) This may be falsely negative if there is extensive necrosis of kidney tissue. (Alsaad et al., 2007)
    - PCR on kidney tissue for adenovirus. PCR for CMV and polyomavirus (PV) may be done, not for the workup of GIN in particular, but should be performed in immunosuppressed patients with kidney dysfunction. (Alsaad et al., 2007)
- Look for evidence of malignant infiltration. Cell surface markers may be needed to assess clonality.
- Immunophenotyping of inflammatory cells. Look for a high ratio of T cells to B cells as well as ratio of CD4 cells to CD8 cells and the presence of macrophages as clues of possible IRIS. (Martin-Blondel et al., 2011) (Inoue et al., 2010) (Viero & Cavallo, 1995) (Croucher et al., 2010)

### *Assessing for evidence of extrarenal disease*

**Diagnostic algorithm 5: Assessing for evidence of extrarenal disease, and investigations at other sites, as necessary.**




GIN may occur in isolation, but it is often seen as part of a systemic disease. With this in mind, it is useful to look for evidence of extrarenal disease in order to assist with decisions regarding further investigation and management.

- CXR (Lapasia et al., 2010) (Alsaad et al., 2007) (Adams & Cook, 2007) (Nasr et al., 2003) (Mitwalli et al., 1994) (Inoue et al., 2010) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Qian et al., 2011) (Ram et al., 2009) (Robson et al., 2003) (Tse et al., 2004) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Salliott et al., 2008) (Jehle et al., 2004) (Izzedine et al., 2007) (Daugus et al., 2006) (Croucher et al., 2010) (Chun & Hale, 2004) or CT scan of the chest (Qian et al., 2011) (Ram et al., 2009) (Singh & Colvin, 2003) (Tse et al., 2004) (Larsen et al., 2008) (Khilji et al., 2012) (El-Reshaid, Madda &

Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Martin-Blondel et al., 2011), (Izzedine et al., 2007) looking for evidence of pulmonary infiltrates, nodules, lymphadenopathy, pleural effusion etc.

  - Evidence of simultaneous pulmonary and kidney disease may be seen a number of conditions including TB, (Colbert, Richey & Schwartz, 2012) (Larsen et al., 2008) sarcoidosis, (Lapasia et al., 2010) (Korsten et al., 2010) (Larsen et al., 2008) fungal infection, (Adams & Cook, 2007) adenovirus infection (Alsaad et al., 2007) and rhodococcal infection, (Tse et al., 2004) as well as ANCA-associated vasculitides, Goodpasture syndrome and systemic lupus erythematosis (although the last three are usually associated with evidence of glomerulonephritis on kidney biopsy). (Larsen et al., 2008)
  - If pulmonary infiltrates have worsened since baseline in a patient with mycobacterial infection, consider IRIS. (Salliott et al., 2008) (Jehle et al., 2004) (Martin-Blondel et al., 2011)

- Abdominal ultrasound (Nasr et al., 2003). (Izzedine et al., 2007) or CT scan (Martin-Blondel et al., 2011) (Daugus et al., 2006) looking for evidence of hepatic/splenic involvement, ascites, lymphadenopathy, (Nasr et al., 2003) (Montseney & Meyrier, 1998) (Archimandritis & Weetch, 1993) (Salliott et al., 2008) (Izzedine et al., 2007) adrenal involvement (histoplasmosis). (Nasr et al., 2003)
- Lymphadenopathy (Bijol et al., 2006) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Chapagain et al., 2011) (Izzedine et al., 2007)
- Rash can be seen in drug reactions, sarcoidosis, TB and fungal infections. (Audimoolam & Bhandari, 2006) (Hong et al., 2007) (Vanhille et al., 1983) (Viero & Cavallo, 1995) (Khilji et al., 2012) (El-Reshaid, Madda & Al-Saleh, 2001) (Chapagain et al., 2011) (Salliott et al., 2008) (Izzedine et al., 2007)
- Vasculitis. (Sampathkumar et al., 2009)
- Eye involvement may be seen in sarcoidosis, TINU and mycobacterial infection. (Inoue et al., 2010) (Javaud et al., 2007) (Joss et al., 2007) (Kim & Hee Sung, 2010) (Sampathkumar et al., 2009) (Chapagain et al., 2011)

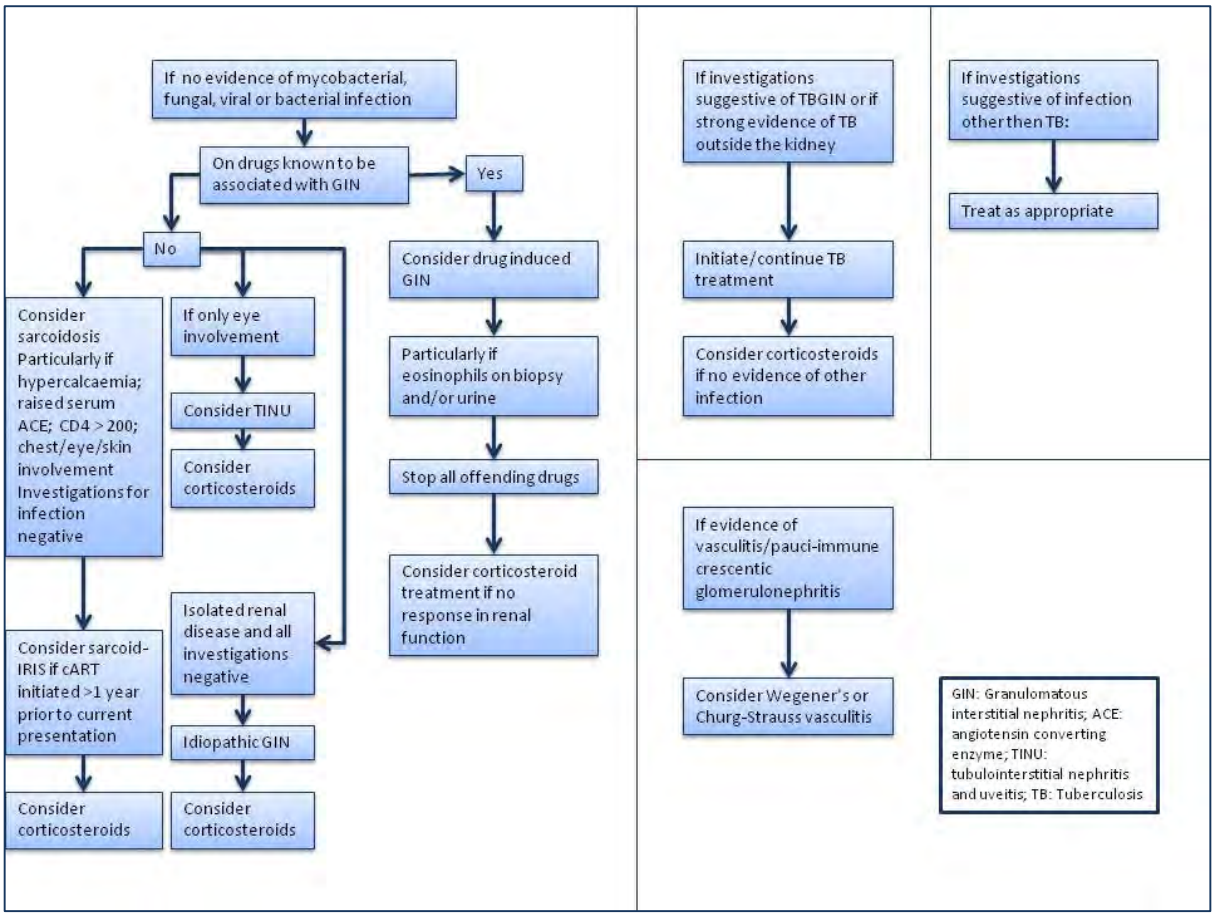
***Investigations at extrarenal sites:***

This should be guided by the clinical context, examination findings and results of special investigations and other evidence of extrarenal disease.

- Sputum sample: routine microscopy, culture and sensitivity, TB microscopy, culture and sensitivity, (Kim & Hee Sung, 2010) (Larsen et al., 2008) (Khilji et al., 2012) (Fallouh et al., 2010) (Colbert, Richey & Schwartz, 2012) (Chapagain et al., 2011) (Croucher et al., 2010) fungal culture, and XPert MTB/RIF
- Bronchial lavage and culture (Adams & Cook, 2007) (Larsen et al., 2008) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Daugus et al., 2006), bronchial biopsy, (Javaud et al., 2007) or lung biopsy (Qian et al., 2011) if there is evidence of pulmonary involvement and sputum investigations are negative. Endobronchial ultrasound (EBUS) guided fine needle biopsy of hilar or subcarinal lymphadenopathy (Chapagain et al., 2011)
- Bone marrow biopsy to look for granulomas and culture for mycobacterium and fungi should be considered if there are unexplained cytopenias on the full blood count. (David et al., 2009) (Nasr et al., 2003) (Javaud et al., 2007) (Qian et al., 2011) (Larsen et al., 2008) (Kaul et al., 2011) (Martin-Blondel et al., 2011) (Izzedine et al., 2007)
- Lumbar puncture if any neurological signs and symptoms to look for evidence of chronic meningitis and perform CLAT on the cerebrospinal fluid. (David et al., 2009) (Larsen et al., 2008) (Martin-Blondel et al., 2011)
- Skin biopsy if a rash is present. May show evidence of allergic reaction, (Hong et al., 2007) fungal or mycobacterial infection, fungal infection, vasculitis or sarcoidosis. (Javaud et al., 2007) (Chapagain et al., 2011) (Salliott et al., 2008)
- Abdominal paracentesis (if ascites) looking particularly for disseminated mycobacterial infection. (Chapagain et al., 2011)
- Lymph node biopsy may reveal infection (mycobacterial or fungal) or sarcoidosis. (Javaud et al., 2007) (Joss et al., 2007) (Kaul et al., 2011) (Chapagain et al., 2011)

### *Diagnosis and management of GIN*

**Diagnostic algorithm 6: Approach to diagnosis and management of GIN after initial investigations.**




HIV-infected patients frequently present with respiratory complaints and bilateral pulmonary infiltrates and receive treatment for community acquired pneumonia, *Pneumocystis jirovicii* pneumonia or tuberculosis. (Lenner et al., 2001) It should be borne in mind that they may have sarcoidosis, particularly if their CD4 count is >200 cells/mm$^3$ or if ART has been initiated with a resulting increased CD4 count. (Lenner et al., 2001) The diagnosis of sarcoidosis may be difficult as many infections can be associated with granuloma formation, and additional evidence in the form of bronchoscopic examination and biopsy, serum calcium, serum ACE and extrathoracic involvement may be required. (Lenner et al., 2001) The percentage of patients with sarcoidosis and hypercalcaemia varies from 10-20% (Adams & Cook, 2007) to 29% in isolated sarcoid GIN, (Robson et al., 2003) to up to 80% in one study. (Joss et al., 2007) Serum ACE was elevated between 43 and 80% of patients in various studies of sarcoid-GIN. (Javaud et al., 2007) (Joss et al., 2007) (Robson et al., 2003)

## Conclusion

The diagnosis of drug-induced GIN in HIV-infected patients remains problematic. Patients may be on multiple drugs, many of which could cause GIN, and it may be difficult to decide which is the culprit drug. In the absence of evidence such as normal kidney function prior to initiation of a drug, and improvement on cessation of a drug, the diagnosis of drug-induced GIN is likely to be a diagnosis of exclusion. Stopping a particular drug in the absence of evidence of drug-induced GIN could be detrimental to the patient. To complicate matters, in cases of drug-induced GIN, there may be incomplete, or no, improvement after cessation of the drug, and corticosteroids may be required to improve outcomes. In the setting of drug-induced acute interstitial nephritis without granulomata, it has been established that a delay in steroid therapy of >7 days significantly increased the risk of incomplete recovery of kidney function. (Gonzalez et al., 2008) Ideally one would like to exclude TB and other infections prior to initiation of therapy, but with urine TB culture taking 6-8 weeks, this would result in unacceptable delays in initiation of definitive therapy. In this setting, tests with more rapid results (such as PCR for *M. tuberculosis* and fungi, fungal serology, and perhaps urinary LAM and XPert MTB/RIF performed on urine and kidney tissue) could be of assistance. In those instances where one is unable to decide whether the GIN is drug-induced or due to TB (in a patient with significant evidence of extrarenal TB), one could make a case for treating both conditions with antituberculous therapy and corticosteroids, with evidence that corticosteroids in the treatment of TB-GIN may be beneficial. (Sampathkumar et al., 2009) (Kaul et al., 2011) (Chapagain et al., 2011) In addition, one needs to be aware of the possibility of IRIS in the correct setting, which may also be responsive to corticosteroids.

## Limitations of this study.

The major limitation of this study is to do with its retrospective nature. Certain information is unavailable, especially with regard to medication history, and pre-hospitalisation course. For example antibiotics may have been given that could render urine cultures negative. It was therefore difficult to decide with any degree of certainty, what the exact cause for the GIN was in a particular patient as many patients were on drugs associated with GIN, but also had evidence of TB outside the genitourinary tract or evidence of acute infection on kidney biopsy. This led to 13 categories of possible causes of GIN in our population being formed, when 5 would have been preferable.

Another limitation is that no bacterial, mycobacterial or fungal cultures were performed on the kidney biopsy tissue, limiting our ability to diagnose or exclude infectious aetiologies. No molecular studies for *M. tuberculosis* or fungi were performed, with the same consequence. Many were also lost to follow up (13.3%) limiting our ability to comment meaningfully on survival and outcomes data.

# Conclusion

GIN appears to be more common in our HIV-infected patients than previously reported in developed countries (in predominantly HIV-uninfected patients). It is a condition that may contribute to the burden of chronic kidney disease in South Africa. Timely diagnosis of the condition, and its aetiology, with appropriate treatment has the potential to decrease the risk of chronicity. Corticosteroid therapy may be appropriate in certain circumstances e.g. drug-induced GIN and GIN related to IRIS. There have also been reports of TB-GIN treated with corticosteroids, together with appropriate antituberculous therapy. In order to make a decision regarding the use of corticosteroid therapy in these patients one should attempt, as far as possible, to determine the aetiology of the GIN (with reference to the diagnostic algorithms above), with particular focus on excluding possible infectious causes (other than TB). Once a diagnosis of a potentially treatable cause of GIN has been ascertained, one can assess the risks and benefits of implementing corticosteroid therapy on an **individual patient basis**, with the intention to reduce the chronicity of the kidney disease. This has thus far not been done at our institution, but is something that could be considered based on the evidence outlined in this study.

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# Appendix 1

**Renal Unit UCT/GSH**
**PATIENT INFORMATION SHEET**

---

I Dr N. Wearne/ Dr C Arendse wish to invite you to participate in the development EVALUATION AND FOLLOW UP OF HIV RELATED RENAL DISEASE **ESTABLISHED BY RENAL BIOPSY**

of a HIV positive renal biopsy registry. This data base will help the renal unit to determine the outcome of therapy of patients with HIV and renal disease diagnosed on renal biopsy.

What does this mean for you? You will be able to attend a specialized clinic, dedicated for HIV and renal disease that aims to provide you with ongoing care after your renal biopsy. All treatment provided will be that of standard clinic practice. We may recommend the initiation of HAART or other medication that aims to protect your kidney. The monitoring of your kidney disease will be done by means of a clinical evaluation with blood and urine tests at regular intervals at E13 renal clinic at GSH. Visits will be determined by the nature of your kidney disease.

The blood tests will enable us to monitor your kidney function.
The urine test will enable us to monitor for protein loss by the kidney.

The decision to participate in this study is entirely your own. You are also free to leave the study at any time, without penalty. Leaving the study will not affect your ability to get regular medical care at our clinic.

Throughout the study your privacy will be protected and nobody other than the doctors and nurses looking after you will know that you are participating. All data and results will be captured anonymously.

The Protocol of this clinical trial was submitted for approval to the University of Cape Town Research Ethics Committee, a research ethics committee registered with the National Health Research Ethics Council. The study has been structured in accordance with the Guidelines on Clinical Trials and Ethics in Health Research, published by the Department of Health and the Declaration of Helsinki 2008.

**If you experience any problems you can phone Dr Wearne or Dr Arendse at 404 3311 or Prof Blockman at the UC T FHS REC 021-4066492**

## HIV $^{+VE}$ RENAL BIOPSY – FOLLOW-UP CONSENT FORM

Please Tick if you agree:

I have read (or it has been read to me) the **PATIENT INFORMATION SHEET**

I have had the opportunity to discuss the study with_____

| I understand the following: | NO | YES |
|---|---|---|
| ▪ What kidney disease means ---------- | ☐ | ☐ |
| ▪ What HAART means ---------- | ☐ | ☐ |
| • What medication I need to take in this study ---- | ☐ | ☐ |
| ▪ That I will need to attend clinic for follow up ----- | ☐ | ☐ |
| ▪ I have had a chance to ask about other concerns | ☐ | ☐ |
| **I CONSENT TO PARTICIPATING IN THIS STUDY** | ☐ | ☐ |

**Name of Patient**_____

Signature _____ Date _____

**Name of person taking consent**_____

Signature _____ Date_____

**Witness if patient unable to write.**

**Name of Witness**_____

Signature _____ Date _____

# Appendix 2

## CLERKING SHEET FOR ALL HIV POSITIVE PATIENTS UNDERGOING A RENAL BIOPSY:

**STICKER : Or NAME/AGE AND FOLDER NUMBER**

**CONTACT NUMBERS: (please try to obtain 2 numbers for the patient)**

Most recent CD4: Date: Viral load (if available): Date:

Height: Weight:

**ARVs and cotrimoxazole**

| **Current ARV regimen** | **Date commenced** | **Was Tenofovir stopped Yes/No** |
|---|---|---|
| D4T/3TC/EFVor NVP | | **Is the patient on Bactrim: Yes/No** |
| 3TC/TDF/EFV or NVP | | |
| **Other** | | |

**Previous opportunistic infections (particularly TB) including the date if possible**

**Infection:** **Date:**

**HAS THE PATIENT BEEN DIAGNOSED WITH TB NOW? yes or no (circle)**

How was the diagnosis made?

*Presumptive* ☐ *Bacteriological evidence*: TB culture/Urine/Sputum(culture or AFB's)(circle)

**If presumptive:** give supporting evidence

**Symptoms of TB:**

Cough ☐

Loss of weight ☐

Fever ☐

Nightsweats ☐

**Urine dipstix:** pH Glucose protein Blood

**Microscopy :**

**Ultrasound report: (include kidney sizes)**

**CXR findings:**

**Is dialysis required? Yes or no (circle)**

**Check list of bloods and urine and diagnostic tests that need to be completed:**

| Specimen | Done | Result |
|---|---|---|
| **Urine (spot) dipstick** | | |
| **Urine (spot) protein:creatinine ratio** | | |
| **Urine (spot) TB MCS** | | |
| **Early morning urine: TB MCS** | | |
| **Urine for MTB/RIF assay*** | | |
| **Urine for LAM strip test*** | | |
| **Sputum #1** | | |
| **Sputum #2** | | |
| **FBC and diff (WCC/Hb/MCV/Plt)** | | |
| **CEU (Na/K/U/Cr)** | | |
| **CD4 count** | | |
| **Viral load** | | |
| **INR/aPTT** | | |
| **Bleeding time (if creat >200µmol/l)** | | |

*Details regarding these specimens to follow

**Result of Biopsy:**

**Patient needs to be followed up in 6 weeks from initial renal consult at the Renal HIV Clinic**

**6 WEEKS FROM BIOPSY**

Serum creatinine eGFR

Weight

Dipstix: Microscopy

UPCR:

1. Is patient still on TB treatment
2. Review of cultures
3. If not on ART and qualifies, refer

**Date: 3 months**

Serum creatinine eGFR

Weight:

Dipstix: Microscopy

UPCR:

1. Is patient still on TB treatment
2. Review of cultures
3. Is patient on ART?

**Date 6 months**

Serum creatinine eGFR

Weight:

Dipstix: Microscopy

UPCR:

1. Is patient still on TB treatment
2. Result of cultures
3. Is patient on ART?

# Appendix 3

## Follow up visit Information Sheet

Follow up visits : Patient Sticker:

Date of biopsy and Diagnosis on Biopsy: DATE Today:

Was patient on HAART at time of biopsy? Yes / No
HAS HAART BEEN INITIATED- if so give date / regimen & ARV clinic

*Clinical information*
BP WEIGHT

Clinical findings - including degree of oedema
Oedema =
Other

| Oedema: | |
|---|---|
| None | 0 |
| Minimal: foot oedema | 1+ |
| Moderate: → knees | 2+ |
| Severe: > knees | 3+ |
| Anasarca | 4+ |

*Current medications:*
*Antihypertensives* *Other* *HAART*

Renal Investigations today: → review flow sheet for all results
Urine Dipstix : Protein: Blood: Other
Microscopy: Urine Culture:

Assessment : ( Give date) Most recent CD4 VL
*GFR at biopsy* *Cr at Biopsy* *Pr/Cr ratio at Biopsy*
*GFR today* *Cr Today* *Pr/Cr ratio today*

1. *Same as Biopsy ( within 20% for Cr)*
2. *Improved*
3. *Deteriorated*

No.1-3

No.1-3

*Recommendations*

*NEXT APPOINTMENT*
*Signature*

GFR
CCG =
MDRD=

*Version 2a*

# Appendix 4

## Table of investigations into TB cases

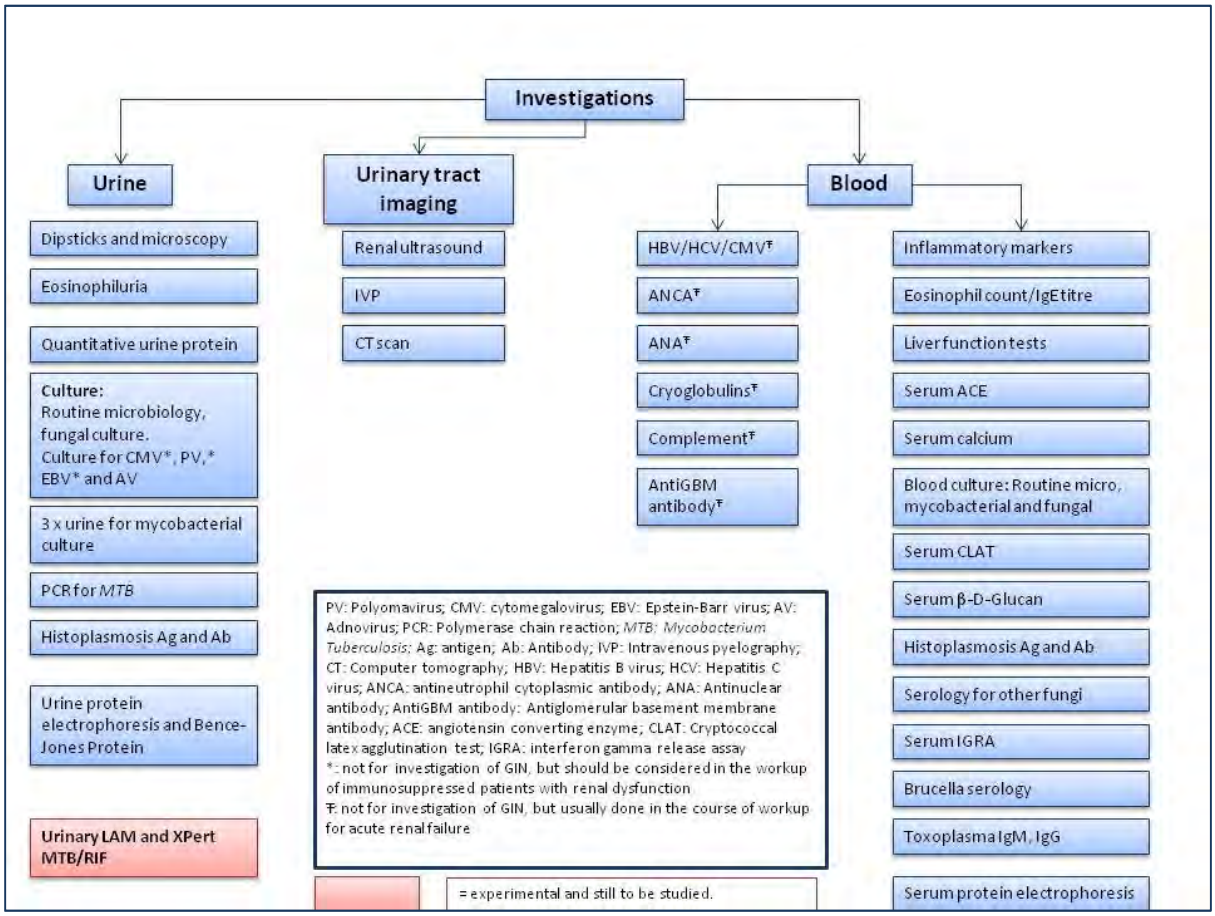
| | Chaudhari (Chaudhari AP, 2011) | Larsen (Larsen CP, 2008) | Kaul (Kaul S, 2011) | Sampathkumar (Sampathkumar K, 2009) |
|---|---|---|---|---|
| Country | India | African-American | India | India |
| Urine | Neg | Occasional | 0-1/hpf | 5-6/hpf |
| Leucocytes | Neg | 3+ | Pos | Neg |
| Blood | DS: Trace. UPCR: 0.3g/mmol | 2g/24 hrs | 3+; 3.2g/d | 2+/UPCR 2.8 |
| Protein | Neg | ND | Neg *3 | ND |
| AFB | Neg | ND | Neg*3 | ND |
| Culture | ND | ND | Pos | ND |
| PCR | | | | |
| HIV | Neg | Neg | ND | ND |
| Biopsy | Several granulomas; one caseous | Large areas of caseous necrosis.Macrophages; neutrophils, MNGC's, lymphs. AFB positive; culture positive | Well-formed epithelioid granulomas. No caseation. AFB and culture negative. | Tubules: hyaline casts; granular debris and neutrophils. Interstitium: multiple granulomas; one caseous. Lymphocytes, plasmas, eosinophils, neutrophils. PCR on renal biopsy pos |
| Urinary tract imaging | Normal | 2 cystic lesions in RK; LK atrophic | Normal | Normal |
| Extrarenal TB | No | Lung; bone marrow | Lymph node | No |
| TB treatment | ATT | ATT | ATT and steroids | ATT and steroids |
| Outcome | Renal function improved | Died | ESRD on HD | Complete recovery |

| | Khilji (Khilji et al., 2012) | Fallouh (Fallouh et al., 2010) | Colbert (Colbert, Richey & Schwartz, 2012) | El-Reshaid (El-Reshaid, Madda & Al-Saleh, 2001) |
|---|---|---|---|---|
| **Country** | Ireland (originally from Philippines) | Afro-Caribbean | Mexican in the US | Kuwait |
| **Urine** | | 98/µl | +; 50-100/hpf | Neg |
| **Leucocytes** | + | 126/µl | 2+/4/hpf | + |
| **Blood** | 3+; 1.2g/d | | 2+; 1.5g/d | 100mg/d |
| **Protein** | Neg | ND | Neg | Neg |
| **AFB** | Neg | Pos; INH resistant | 5 sent; 1 pos | Neg |
| **Culture** | ND | ND | ND | ND |
| **PCR** | | | | |
| **HIV** | Neg | Pos | ND | ND |
| **Biopsy** | Widespread caseating granulomas with Langerhan's cells. AFB neg. | Mixed infl. Infiltrate. Epithelioid granuloma; no caseation. AFB + | GIN with caseation; extensive tubular atrophy | Focal interstitial fibrosis; caseating granulomas. |
| **Urinary tract imaging** | Normal | ND | Asymmetric hydronephrosis; multilocular renal cysts; calcific deposits in prostate and seminal vesicles; irregular beaded ureters on retrograde pyelography | RK small with irregular outline; poorly functional RK with calicyectasis. LK normal |
| **Extrarenal TB** | Lymph node | PTB; Blood culture + | Lung nodules and retroperitoneal lymph nodes | Pulmonary fibrosis |
| **TB treatment** | ATT | ATT (Rif/EMB/moxifloacin) | ATT | ATT |
| **Outcome** | ESRD on HD; Rpt biopsy: ++ interstitial fibrosis; no GIN | Renal function moderately impaired; not on dialysis | Renal function stabilised; dialysis dependent | Complete recovery |

# Appendix 5

## List of comprehensive testing to determine aetiology of GIN.

For an abbreviated list more suited to resource limited settings please consult main text.



**Investigations**

Urine:

- Dipsticks and microscopy: look for proteinuria, haematuria, leukocyturia and casts. (Chung et al., 2009) (Alsaad et al., 2007) (Adams & Cook, 2007) (Nasr et al., 2003) (Audimoolam & Bhandari, 2006) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Hong et al., 2007) (Javaud et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Tse et al., 2004) (Unal et al., 2008) (Vanhille et al., 1983) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain, et al., 2011) (Salliott et al., 2008) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Croucher et al., 2010) (Chun & Hale, 2004)
- Urine eosinophils (Hong et al., 2007) (Korsten et al., 2010) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007).
    - Hansel's stain has a sensitivity of nearly 4 times that of the classic Wright's stain as it is less pH dependent. However, both stains have a relatively low sensitivity (+/-67%) and only moderately specificity (+/- 80%). (Singh & Colvin, 2003) (Rossert, 2001)
- Quantitative urine protein. (Chung et al., 2009) (Nasr et al., 2003) (Joss et al., 2007) (Ram et al., 2009) (Robson et al., 2003) (Singh & Colvin, 2003) (Unal et al., 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Salliott et al., 2008) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Daugus et al., 2006) (Croucher et al., 2010) This is most easily achieved by performing a spot urine protein: creatinine ratio. Serial measurements may be needed in order to monitor response to therapy.
- Urine culture: routine microbiology (Kim & Hee Sung, 2010) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Sampathkumar et al., 2009) (Larsen et al., 2008) (Kaul et al., 2011) (Fallouh et al., 2010) (Chun & Hale, 2004), fungal culture (Chung et al., 2009) (David et al., 2009) (Alsaad et al., 2007) (Nasr et al., 2003) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Tse et al., 2004) (Chaudhari, Ranganath & Pavan, 2011) (Salliott et al., 2008) and culture for adenovirus. (Alsaad et al., 2007)
    - Urine can also be sent for culture of cytomegalovirus (CMV), polyomavirus and Epstein-Barr Virus (EBV). (Javaud et al., 2007) (Alsaad et al., 2007) These tests are not for the investigation of GIN in particular, but should be considered in the workup of an immunosuppressed patient with renal dysfunction.
- Send 3 early morning urine specimens for mycobacterial culture. (Bouzourene, Bouzourene & Francke, 1998) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim &

Hee Sung, 2010) (Ram et al., 2009) (Robson et al., 2003) (Tse et al., 2004) (Unal et al., 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Khilji et al., 2012) (Kaul et al., 2011) (Fallouh et al., 2010) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chapagain, et al., 2011) (Martin-Blondel et al., 2011) (Jehle et al., 2004) (Izzedine et al., 2007) (Chun & Hale, 2004)

- Urine PCR for *M. tuberculosis* (Kaul et al., 2011)
- Urine histoplasmosis antigen and antibody (Lapasia et al., 2010) (Adams & Cook, 2007)
    - Testing for histoplasma antigen, particularly in the urine, is more sensitive than antibody testing (91-92%) (Adams & Cook, 2007), particularly in immunosuppressed patients and HIV-infected patients. (Qian et al., 2011)
- Urine protein electrophoresis and Ben-Jones Protein to assess for possible paraproteinuria as a cause of GIN.

Urinary tract imaging

- Kidney ultrasound for kidney size estimation prior to biopsy and to assess gross anatomical abnormalities of the urinary tract (looking for evidence of classical renal TB). (Chung et al., 2009) (Nasr et al., 2003) (Korsten et al., 2010) (Ram et al., 2009) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Salliott et al., 2008) (Daugus et al., 2006) (Croucher et al., 2010)+/- IVP (Archimandritis & Weetch, 1993) (Bouzourene, Bouzourene & Francke, 1998) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Chun & Hale, 2004)or CT scan (Nasr et al., 2003) (Hong et al., 2007) (Kim & Hee Sung, 2010) (Singh & Colvin, 2003) (Larsen et al., 2008) (Khilji et al., 2012) (El-Reshaid, Madda & Al-Saleh, 2001) (Colbert, Richey & Schwartz, 2012) (Martin-Blondel et al., 2011) (Izzedine et al., 2007) (Chun & Hale, 2004)

Blood tests:

- Hepatitis B virus (HBV), Hepatitis C Virus (HCV) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Chung et al., 2009) (Qian et al., 2011) (Ram et al., 2009) (Ramalakshmi, Bastacky & Johnson, 2003) (Chaudhari, Ranganath & Pavan, 2011) (Kaul et al., 2011) (Fallouh et al., 2010) (Izzedine et al., 2007), CMV (Ram et al., 2009)
    - These tests are not for the investigation of GIN in particular, but should be performed in immunosuppressed patients with evidence of renal dysfunction.
- Anti-nuclear antibody (ANA); (Nasr et al., 2003) (Chung et al., 2009) (Hong et al., 2007) (Audimoolam & Bhandari, 2006) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) Anti-neutrophil cytoplasmic antibody (ANCA); (Audimoolam & Bhandari, 2006) (Lapasia et al., 2010)

(Nasr et al., 2003) (Chung et al., 2009) (Pena de la Vega et al., 2005) (Hong et al., 2007) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Magner, Sweet & Bear, 1986) (Ram et al., 2009) (Singh & Colvin, 2003) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) Cryoglobulins; (Nasr et al., 2003) (Chung et al., 2009) (Hong et al., 2007) (Ram et al., 2009) (Salliott et al., 2008) serum complement (Chung et al., 2009) (Hong et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Korsten et al., 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Khilji et al., 2012) (Kaul et al., 2011) and antiglomerular basement membrane antibody (anti-GBM antibody). (Hong et al., 2007) (Korsten et al., 2010) (Khilji et al., 2012) (Kaul et al., 2011)

  - These tests are not necessarily part of the workup of GIN, but are usually performed as part of the workup for acute kidney failure. However, they should not be used to replace kidney biopsy; as false positives do occur. (Khilji et al., 2012)

- Inflammatory markers: erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) (Tse et al., 2004) (Sampathkumar et al., 2009) (Khilji et al., 2012) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Chapagain, et al., 2011)
  - Unhelpful in assessing infectious aetiology as they may also be raised in drug-induced GIN, (Audimoolam & Bhandari, 2006) (Kennedy, Shrinkanth & Charlesworth, 2006) (Ramalakshmi, Bastacky & Johnson, 2003) (Tong, Howell & Foreman, 2007) inflammatory conditions, (Unal et al., 2008) and IRIS. (Salliott et al., 2008) (Jehle et al., 2004) (Izzedine et al., 2007) They may also be low in TB. (Chaudhari, Ranganath & Pavan, 2011).
- Eosinophil count (Lapasia et al., 2010) (Hong et al., 2007) (Javaud et al., 2007) (Joss et al., 2007) (Kim & Hee Sung, 2010) (Ram et al., 2009) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Unal et al., 2008) (Viero & Cavallo, 1995) could assist in diagnosing possible allergic (drug) reaction.
- Serum IgE Titre: if raised could suggest an allergic (drug) reaction. (Hong et al., 2007) (Vanhille et al., 1983)
- Liver function tests (Nasr et al., 2003) (Montseny & Meyrier, 1998) (Javaud et al., 2007) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Robson et al., 2003) (Ramalakshmi, Bastacky & Johnson, 2003) (Singh & Colvin, 2003) (Tong, Howell & Foreman, 2007) (Viero & Cavallo, 1995) (Sampathkumar et al., 2009) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Izzedine et al., 2007) (Daugus et al., 2006)
- Serum angiotensin converting enzyme (ACE) (Audimoolam & Bhandari, 2006) (Javaud et al., 2007) (Robson et al., 2003) (Joss et al., 2007) (Kim & Hee Sung, 2010) (Korsten et al., 2010) (Ram et al., 2009) (Unal et al., 2008) (Chaudhari, Ranganath & Pavan, 2011) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Izzedine et al., 2007) to assess for possible sarcoidosis.
- Serum calcium (Lapasia et al., 2010) (Adams & Cook, 2007) (Audimoolam & Bhandari, 2006) (Bijol et al., 2006) (Pena de la Vega et al., 2005) (Joss et al., 2007) (Kennedy, Shrinkanth & Charlesworth, 2006) (Kim & Hee Sung, 2010) (Ram et al.,

2009) (Robson et al., 2003) (Singh & Colvin, 2003) (Unal et al., 2008) (Viero & Cavallo, 1995) (Sampathkumar et al., 2009) (Chaudhari, Ranganath & Pavan, 2011) (Larsen et al., 2008) (Kaul et al., 2011) (El-Reshaid, Madda & Al-Saleh, 2001) (Izzedine et al., 2007)

- Blood culture (Chung et al., 2009) (Hong et al., 2007): routine microbiology, (Qian et al., 2011) (Singh & Colvin, 2003) (Tse et al., 2004) (Kaul et al., 2011), mycobacterial (Javaud et al., 2007) (Fallouh et al., 2010) and fungal. (David et al., 2009) (Alsaad et al., 2007) (Bijol et al., 2006) (Ramalakshmi, Bastacky & Johnson, 2003) (Larsen et al., 2008) (Izzedine et al., 2007)
    - The sensitivity of fungal culture in disseminated disease is 75-85%. (Adams & Cook, 2007)
- Serum Cryptococcal Latex Agglutination Test (CLAT) to assess for possible disseminated cryptococcal infection.
- Serum Beta-D-Glucan to assess possible disseminated fungal infection.
- Serum histoplasmosis antigen and antibody (Adams & Cook, 2007) (Lapasia et al., 2010) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Qian et al., 2011)
    - False negative histoplasma antibody results can be seen in recent infection and immunocompromised patients. In disseminated disease, the sensitivity ranges from 63-81%. (Adams & Cook, 2007) (Qian et al., 2011) (Chung et al., 2009). False positive results may be found in patients with other fungal diseases. (Qian et al., 2011)
- Serological studies for other fungi including *Blastomyces, Cryptococcus, Coccidiomycosis,* and *Aspergillus*. (Adams & Cook, 2007)(Lapasia et al., 2010) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Qian et al., 2011)
- Serum Interferon Gamma Release Assay (IGRA) (Colbert, Richey & Schwartz, 2012) based on interferon release after T-cell stimulation by antigens specific to *M. tuberculosis.* It has a high specificity for *M. tuberculosis* and are unaffected by previous BCG vaccination. However, the test is unable to distinguish between active and latent TB, and its role is limited in low-income countries with a high TB burden. (Pai et al., 2009)
- Brucella serology (in multisystem disease) (Archimandritis & Weetch, 1993) (Unal et al., 2008) (Sampathkumar et al., 2009) (Khilji et al., 2012) (Izzedine et al., 2007)
- Toxoplasma IgG and IgM (Pena de la Vega et al., 2005) (Khilji et al., 2012) (Fallouh et al., 2010) (Izzedine et al., 2007)
- Serum protein electrophoresis (Chung et al., 2009) (Nasr et al., 2003) (Pena de la Vega et al., 2005) (Kim & Hee Sung, 2010) (Singh & Colvin, 2003) to assess for possible multiple myeloma as a cause of GIN.

# Appendix 6

## Protocol for:

**A retrospective review of Granulomatous Interstitial Nephritis (GIN) amongst HIV-infected patients at Groote Schuur Hospital Cape Town .**

**Principal Investigator:** Dr Debbie Nel

**Supervisor:** Dr Nicola Wearne: Division of Hypertension and Nephrology, Groote Schuur Hospital, University of Cape Town

**Contributors:**

- Dr Maureen Duffield, Division of Pathology, University of Cape Town
- Dr Andrew Boulle (statistician department of Public Health, University of Cape Town)
- Prof Bryan Rayner (Department of Nephrology and Hypertension, Groote Schuur Hospital)

## Table of contents:

## 1. Background and literature review

HIV-infected patients presenting with renal dysfunction have a wide differential diagnosis which includes HIV-associated nephropathy (HIVAN), immune complex glomerulonephropathies (ICGN) and many others. As survival has improved in the Highly Active Antiretroviral Therapy (HAART) era, the prevalence of kidney disease in the HIV-infected population is increasing. The incidence of HIVAN has remained stable, therefore the rising rates most likely represent increases in kidney disease seen in the general population e.g. due to hypertension and diabetes,[1] and others. Upon identification of renal dysfunction in this population group, the differential diagnosis is broad, and must include HIV-specific pathologies (HIVAN and ICGN), as well as those seen in the non-HIV-infected population[1] such as drug toxicities, acute tubular necrosis (ATN), lymphoma, tuberculosis (TB), glomerulonephritides unrelated to HIV infection, and others. Clinical diagnosis is possible, but renal biopsy is often necessary to determine the underlying diagnosis and guide treatment.[1] Regardless of the aetiology, early identification, accurate diagnosis and subsequent appropriate management most likely result in improved outcomes.[1]

The disease burden of renal dysfunction in HIV-infected patients is significant. HIV-infected patients are at an increased risk of developing renal failure compared with the general population.[2] Acute renal failure has been found to occur in up to 20% of hospitalised patients with HIV-infection, with a mortality of 18% at 2 months.[3] The prevalence of chronic kidney disease (CKD) in HIV-infected patients is approximately 17%.[4] A Kenyan study showed that, of 216 ART naive patients, 25% had a creatinine clearance (CrCl) of <90ml/min, 2% had a CrCl of <60ml/min and 8% had significant proteinuria of >1g/day.[5] In Uganda, 48.5% of patients with clinical stage 3 disease were found to have a CrCl <80ml/min.[6] Abnormal kidney function is seen in up to 30% of HIV-infected patients[7] and evidence of kidney dysfunction at the time of antiretroviral initiation has been found to be an independent predictor of mortality in this population.[8]

There is a paucity of information regarding renal biopsy histology in HIV-infected patients in South Africa, and much of what has been published tends to concentrate on HIVAN and related HIV-specific renal disease. Previous information regarding renal disease in this population was limited due to the poor prognosis of HIV-related renal disease without combined antiretroviral therapy (cART), which was previously denied to many South Africans.[9] There has also been a tendency to assume that kidney pathology in the setting of HIV is always related to HIVAN.[9]

**Biopsy series amongst HIV-infected individuals performed at Groote Schuur Hospital**[9]

At Groote Schuur Hospital, a study of all renal biopsies performed in HIV-infected individuals from January 2005 until December 2010 was done (retrospectively from 2005-September 2008 *n*=116, and prospectively thereafter until present *n*=105). A database has been set up with new patients being enrolled as renal biopsies are performed. Those biopsies which were in keeping with HIVAN were analysed and the results published by Wearne et al.[9] 221 biopsies were analysed of which 15 were excluded due to inadequate sampling or a diagnosis of "end-stage kidney disease" (ESKD) being made on histology. A further 14 were excluded due to a lack of information regarding cART and/or being lost to follow up.[9]

Of the remaining 192 biopsies, 110 had HIVAN seen on biopsy (57.3%). There was evidence of HIVAN together with immune complex glomerulonephritis (ICGN) in 42 patients and ICGN alone in 16 patients. 27 (14%) of the patients in this series had pathology that was unrelated to either HIVAN or ICGN (see table below). Of note, 34.8% of patients with any feature of HIVAN had an additional pathology present. This highlights the importance of renal biopsy in this group of patients, and the detrimental impact of the assumption that "all kidney disease in HIV is related to HIVAN", with possible reversible pathologies remaining undiagnosed. [9]

**Table 1:** Biopsy findings excluding HIVAN and ICGN in the study performed at Groote Schuur Hospital[9]

| Histology found on renal biopsy | Number = 27 |
|---|---|
| Drug reaction | 9 |
| Acute tubular necrosis | 5 |
| Diabetic glomerulosclerosis | 4 |
| Lymphoma | 3 |
| Tuberculoma | 2 |
| Myeloma | 1 |
| Malignant hypertension | 1 |
| Amyloidosis | 1 |
| Microangiopathic haemolytic anaemia | 1 |

**Causes of renal dysfunction in HIV-infected individuals**

As the aforementioned study highlights, in a South African context there are numerous causes of renal disease in HIV-infected individuals, aside from HIV-specific renal disease. This has been borne out in numerous analyses worldwide.[1,2,10,11] A further consideration is that as cART has improved survival, the HIV-infected population has aged[1] (although this is more relevant in developed countries rather than in South Africa). There has therefore been increasing interest in so-called 'non-communicable diseases' including glucose intolerance or diabetes mellitus (which may be worsened by certain antiretrovirals),

hypertension and kidney disease. Hypertension appears to be an important co-factor, with 40.7% of HIV-infected patients with renal dysfunction in an Indian study having co-morbid hypertension.[11]

Kidney disease in HIV-infected patients can be divided into those which are a primary manifestation of the HIV infection and secondary causes which are similar to those affecting the general population.[2] Table 2 shows possible causes of renal disease in HIV-infected individuals, but this list is by no means exhaustive. Primary causes include HIVAN, HIV-associated immune-complex disease (HIV-IC/ICGN) and HIV associated thrombotic thrombocytopaenic purpura/haemolytic uraemia syndrome (TTP/HUS). [12] Diffuse Infiltrative Lymphocytosis Syndrome (DILS) is another pathology that has been seen exclusively in HIV-infected individuals at Groote Schuur Hospital, and should probably be classified in this group.[9] These may present as acute or chronic renal failure, but are primarily implicated in the burden of chronic kidney disease (CKD) in the HIV infected population.[12]

## Table 3: Breakdown of the aetiology of GIN found in 5 retrospective reviews

| | **Bijol et al[14]** | **(%)** | **Javaud et al[15]** | **(%)** | **Mignon et al[16]** | **(%)** | **Viero et al[17]** | **(%)** | **Joss et al[18]** | **(%)** |
|---|---|---|---|---|---|---|---|---|---|---|
| Total number | 46 | | 43 | | 32 | | 12 | | 18 | |
| Excluded | 8 | | 3 | | 0 | | 0 | | 2 | |
| Frequency | 0.5% | | 1.37% | | 0.9% | | 5.9%* | | <1% | |
| Sarcoidosis | 11 | 28.9 | 20 | 50 | 3 | 9.4 | 3 | 25.0 | 5 | 27.8 |
| Drug reaction | 17 | 44.7 | 7 | 17.5 | 10 | 31.3 | 3 | 25.0 | 2 | 11.1 |
| Mycobacterium | 0 | 0.0 | 5 | 12.5 | 3 | 9.4 | 0 | 0.0 | 0 | 0.0 |
| Miscellaneous infections | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 3 | 25.0 | 0 | 0.0 |
| BCG | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Wegener's | 2 | 5.3 | 2 | 5 | 8 | 25.0 | 1 | 8.3 | 0 | 0.0 |
| FBGCR | 2 | 5.3 | 0 | 0 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 |
| XPN | 1 | 2.6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| TINU | 0 | 0.0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 2 | 11.1 |
| Crohn's | 0 | 0 | 1 | 2.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Idiopathic | 4 | 10.5 | 5 | 12.5 | 8 | 25.0 | 1 | 8.3 | 9 | 50.0 |

BCG: **intravesical *Bacille Calmette-Guerin* used to treat bladder carcinoma;** FBGCR: **Foreign body giant cell granuloma;** XPN: **xanthogranulomatous pyelonephritis;** TINU: **tubulointerstitial nephritis and uveitis syndrome.**

* **This figure represents the percentage of GIN among patients with acute interstitial nephritis (not total renal biopsies performed)**

Secondary causes can be further divided into prerenal, intrarenal and postrenal (obstructive causes).[13] This group includes those renal diseases causing potentially reversible acute kidney injury (AKI) as well as intrinsic renal disease unrelated to HIV (e.g. co-morbid diabetes mellitus or hypertension) more commonly associated with CKD.[12] Iatrogenic renal injury may result from the numerous drugs used to treat HIV infection as well as the many opportunistic infections (OI's) associated with immunosuppression. Moreover, the kidney can, itself, also be affected by infections, many of which may be classified as opportunistic.[2] There are also numerous electrolyte and acid-base disorders which have been documented in HIV infection. These may arise from HIV infection itself, from opportunistic infections or malignancies, or from the many drugs used in the treatment of these patients. [12]

**Granulomatous interstitial nephritis (GIN)**

Acute interstitial nephritis (AIN) is commonly seen in patients with acute renal failure. A unique variant of this, granulomatous interstitial nephritis (GIN), has been described, in which granulomas are found in the renal interstitium, often surrounded by inflammatory infiltrate.[14] The granulomas may be isolated, or extensive; necrotising or non-necrotising, and in the study by Bijol et al were found to be accompanied by significant other renal disease in 30.4% of cases. There may be isolated renal involvement, but GIN can be associated with granulomas elsewhere.[15] Rarely, there may be glomerular involvement. [14]

There are many causes of GIN (see table 4). Several reviews have been done to document the various causes of GIN. We have selected the five largest reviews and tabulated their findings (table 3). [14-18]

As can be seen, drug reactions and sarcoidosis accounted for the majority of cases, with numerous cases being documented as 'idiopathic' (i.e. no cause was found after extensive investigation). Wegener's granulomatosis was listed as a leading cause in one series,[16] and this has been documented to occur in the absence of glomerular lesions or systemic evidence of the disease.[19] Infections (mycobacterial and other) accounted for only 5% of the cases of GIN in these reviews. 2 of the studies identified tuberculosis as a cause.[15,16] In the Paris series, 2 out of 3 patients had concomitant pulmonary tuberculosis, and all had extrathoracic tuberculosis (lymph node and liver involvement).[15] Other mycobacterial infections identified in relation to GIN were leprosy, and *Mycobacterium avium-intracellulare* infection.[15] GIN has also been described as part of the immune reconstitution inflammatory syndrome (IRIS), shortly after commencement of cART, and is most likely related to opportunistic infections.[20] Of note, only one of the 5 patients in whom Joss identified sarcoidosis as a cause of GIN had a pre-biopsy diagnosis of sarcoidosis, although 3 of the patients subsequently developed features of the disease.[18] This has been noted previously, with reports of isolated GIN being the only manifestation of sarcoidosis, with no extrarenal involvement at the time of renal biopsy. [21,22] Javaud et al noted that, in 25% of the patients with sarcoidosis, GIN occurred after cessation of steroid therapy (median 18 months).

| Table 4: Possible causes of Granulomatous Interstitial Nephritis (GIN) | |
|---|---|
| Drugs | BCG, intravesical[14,23]<br>Antibiotics<br>• Ampicillin; penicillin;[15,17] Vancomycin;[24] Cefuroxime;[25] Fluoroquinolones;[26,27] Clarithromycin;[28] Nitrofurantoin[29]<br>TNF-α blockers (associated with sarcoidosis) [30]<br>NSAIDs[31]<br>Diuretics[32]<br>Allopurinol[33]<br>Anticonvulsant (lamotrigine; carbamazepine) [34,35]<br>Bisphosphonates (alendronate) [36]<br>All-*trans* retinoic acid[37]<br>Heroin[38]<br>Omeprazole[39] |
| Infection | Bacteria[17]<br>Mycobacteria<br>• Tuberculosis;[16,40,41] Nontuberculous mycobacteria;[15] Leprosy[15]<br>Viral (adenovirus) [42,43]<br>Fungi<br>• Histoplasma;[44,45] Candida;[46] Mucormycosis;[47] Trichosporon[46] Cryptococcus[48] |
| IRIS | Most likely related to opportunisticinfections[20] |
| Inflammatory conditions | Sarcoidosis[14-18]<br>Wegener's granulomatosis[14-17]<br>TINU[18]<br>Crohn's disease[14,49] |
| Malignancies | Chronic lymphocytic leukaemia[50]<br>Multiple myeloma |
| Idiopathic | |

**BCG**: *Bacille Calmette-Guérin* used to treat bladder carcinoma; TNF-α: **Tumour necrosis factor-α;** NSAIDs**: Non-steroidal anti-inflammatory drugs;** IRIS**: Immune reconstitution inflammatory syndrome;** TINU**: Tubulointerstitial nephritis and uveitis syndrome**

It has been estimated that acute interstitial nephritis (AIN) accounts for 4.5% of cases of drug-induced renal insufficiency. This may be an under-estimate as renal biopsies are not routinely performed in this clinical setting.[15] These drug reactions may be accompanied by fever, rash, eosinophilia and arthralgia (33% in the series by Javaud et al[15]), but these symptoms appear to be less frequent in those patients in whom granulomas are seen on biopsy than in those with regular AIN.[15] The other causes listed in table 4 are largely from case reports.

There have been no studies done related to the aetiology of GIN in HIV-infected individuals, particularly in a developing country such as South Africa. One might expect, particularly with the high tuberculosis (TB) burden in this country, that infectious causes might account for an increased number of cases in this immunosuppressed population when compared to developed countries. This is a phenomenon that has already been

noted amongst renal transplant recipients, with mycobacterial and fungal infections being a main causative factor of GIN in these patients.[48] Only 2 patients in the aforementioned studies were noted to be HIV-infected.[15] The aetiology of the GIN in both of these patients was found to be infective (Tuberculosis and *Mycobacterium avium interacellulare).*[15]

GIN appears to be a rare entity. Bijol et al assessed 9779 kidney biopsies performed between January 1987 and July 2004. 46 cases of GIN were identified (0.47%).[14] *Mignon et al* found GIN in 0.9% of native kidney biopsies,[16] while Viero and Cavallo found granulomas in 6% of all cases with a diagnosis of interstitial nephritis (12/203).[17] Javaud et al identified granulomas in 43 of the 3132 (1.37%) renal biopsies performed between January 1991 and February 2004. [15]

Preliminary assessment of the biopsy data among HIV-infected individuals at Groote Schuur Hospital reveals that 43 patients out of 370 (11.6%) were found to have granulomas on renal biopsy. As can be seen, this rate is much higher than those previously reported. There are numerous possible reasons for the high prevalence of GIN in this population:

iv. Patients with HIV-infection, particularly in the hospital setting, are exposed to an ever-increasing number of medications, many of which have been implicated in causing granulomatous interstitial nephritis.
v. Patients with HIV-infection are at risk of numerous opportunistic infections (mycobacterial, fungal etc) which could be responsible for GIN.
vi. South Africa, and Cape Town in particular, has a very high prevalence of tuberculosis, which could be responsible for at least a percentage of these cases.

To date, this high rate of GIN in HIV-infected patients has not been noted in the literature, and no studies have been performed in this particular group of patients. As mentioned above, the causes of GIN are myriad, and the possible aetiologies may be different in this group of patients. A review of possible causes of GIN in this population group would be important in guiding diagnostic methods. For example, should renal biopsy tissue be sent routinely for mycobacterial and fungal culture? (2/3 of the cases of *Mycobacterium Tuberculosis* in the series by Javaud et al were identified in this manner).[15] This is currently not standard practice at Groote Schuur Hospital. Should special fungal stains be performed on all renal biopsies with evidence of granulomas? Should we be extending our workup of HIV-infected individuals with renal dysfunction to include special tests, for example Histoplasma antigen/antibody testing? Should we be assessing all of these patients with GIN for possible sarcoidosis or Wegener's granulomatosis?

Concerning treatment of these patients with GIN, possible infections should be sought and treated appropriately. In the case of drug reactions, there is evidence to suggest that the use of corticosteroids in drug induced AIN may improve outcome, although this is still controversial and large prospective studies are needed. [25] Javaud et al treated all patients with drug-induced GIN with oral corticosteroids, with favourable outcomes. Corticosteroids were also given to 3/5 patients with idiopathic GIN, with improvement of renal function.[15] This should perhaps be a consideration in our patient population once infective aetiologies are excluded. Regardless, identification of a possible causative drug would be of importance in order to prevent subsequent exposure to the drug, with

possible disastrous consequences (although no studies have been done on re-exposure to drugs after GIN). [18]

**Outcome after renal injury**

Arendse et al reported good survival in HIV-infected patients with acute renal failure who required dialysis for ATN. [51] With regard to chronic renal disease, those with HIVAN who required acute dialysis were found to have a good in-hospital prognosis, but long-term outcome was found to be dictated by availability of cART and renal replacement therapeutic options. Szczech et al stated that 'among patients with renal disease other than HIVAN, initiation of cART was not associated with a beneficial effect on renal function'. [52] Wearne et al demonstrated an improvement in proteinuria, stabilisation in renal function and mortality with cART initiation in the ICGN group although this was not found to be statistically significant. [9] The impact of cART in patients with renal disease unrelated to HIV has not yet been assessed. Where available, we will collect this data to assess whether cART is related to improved outcome in these patients. The outcomes of HIV-infected patients with GIN on biopsy (of which this is the largest group) has not been assessed.

## 2. Aims

To analyse all biopsies in HIV-infected patients done at Groote Schuur Hospital from January 2005 until October 2012 to examine the non-HIVAN/ICGN pathologies and assess the clinical correlations and outcomes. All cases that showed granulomatous inflammation on renal biopsy will be reviewed in an attempt to correlate histological evidence with clinical, microbiological or radiological evidence of tuberculosis or other conditions/drugs known to cause granulomatous inflammation.

## 3. Methods

As mentioned above, previous investigators have generated a database of information pertaining to all HIV-infected individuals who underwent renal biopsy during the period from January 2005 until the present. Information regarding patient demographics, renal function, cART at the time of biopsy, other drugs, kidney size, CD4 count, blood pressure and presence/absence of oedema was obtained, together with numerous serological, biochemical and haematological tests (see below). Information relating to microbiological specimens (urine and blood cultures for bacteria, fungi and mycobacteria, and sputum/other specimens for mycobacterial investigation) was also recorded. All of the above information was documented, together with the results of renal biopsy (see Appendix 2: Clerking sheet).

| Blood tests recorded | Abbreviation |
|---|---|
| Hepatitis B surface antigen | HBsAg |
| Hepatitis C antibodies | HCV |
| Complement 3 | C3 |
| Complement 4 | C4 |
| Anti-nuclear antigen | ANA |
| Anti-double stranded DNA | AntidsDNA |
| Syphilis serology | RPR |
| Confirmatory test for syphilis | TPHA |
| Antistreptolysin O titre | ASOT |
| AntiDNAse B antibody | AntiDNAse |
| Electrolytes and creatinine | CEU |
| CD4 count | CD4 |
| Viral load (where available) | HIVVL |
| Haemoglobin | Hb |
| Albumin | Alb |
| Total protein | Tprot |
| Cholesterol | Chol |

Patients have been followed up for various time intervals, and information regarding death (and cause thereof) and subsequent renal function was also recorded.

Wearne et al have already analysed the data pertaining to those patients whose renal biopsies were in keeping with a diagnosis of HIVAN (with or without other pathology). The data pertaining to those with evidence of ICGN on biopsy is the focus of another paper.

In this study, we will be analysing the data pertaining to 2 groups of patients:

I. Those who did not have a diagnosis of HIVAN or ICGN made on renal biopsy ('non-HIVAN/ICGN group'). We will be using the existing database to retrieve most of the data. In addition, medical record reviews will be performed to further elucidate information regarding presentation, concurrent illness and medication history where this information is missing.
II. 'Granuloma group': those patients with granulomas present on renal biopsy, with or without other pathology. As mentioned, a significant number of patients had granulomas seen on renal biopsy. As a specific focus in this study we will be focusing on the 'granuloma group' and assessing their clinical correlates and outcomes, as well as possible causes of the granulomatous interstitial nephritis. A large focus will be placed on whether a diagnosis of tuberculosis was made during the time period surrounding the renal biopsy, whether there was a clinical suspicion of tuberculosis (symptoms/chest XRay/abdominal ultrasound etc), as well as medication history and the presence of other infections In order to do this, a medical records review will be necessary together with a review of the microbiological samples sent for TB during this time period. The National Health Laboratory Services (NHLS) Database would be used for this purpose, as not all of the specimens are analysed at Groote Schuur Hospital.

The NHLS database will also be used to obtain data pertaining to follow up renal function, CD4 count and viral load in patients who are no longer being followed up at Groote Schuur Hospital, as well as any 'missing' data in the original database.

## 4. Scientific Design

This is to be a descriptive study focusing on the 'non-HIVAN/ICGN' pathologies seen on kidney biopsies done in HIV-infected individuals at Groote Schuur Hospital from 2005 to October 2012 to assess clinical correlates and outcomes. There will be particular focus on the group of patients with granulomas seen on histology ('Granuloma group'), in an attempt to correlate histological findings with clinical evidence of conditions or drugs known to cause granulomatous interstitial nephritis.

## 5. Study population, recruitment and enrolment.

Groote Schuur Hospital is a tertiary hospital in Cape Town, South Africa. The renal unit receives referrals from the Department of General Medicine at this hospital, from three secondary level hospitals (New Somerset Hospital, GF Jooste Hospital and Victoria

Hospital) as well as many day hospitals and community health centres in the Cape Town Metropole. All HIV-infected patients with renal dysfunction w-ho qualified for, and subsequently underwent, a renal biopsy during the period of January 2005 to October 2012 were included in the study population (370 patients in total) to generate the abovementioned database. The decision to proceed to renal biopsy was taken by the nephrologists working in the Department of Hypertension and Nephrology at Groote Schuur Hospital, and was made on clinical grounds. No biopsy was performed for research purposes alone.

In this study, all of the above biopsy data was reviewed. Those with HIVAN or ICGN (with or without other pathologies) were excluded, leaving a total of 65 biopsies (non-HIVAN/ICGN group). All patients found to have granulomas on biopsy (with or without HIVAN/ICGN) have been included in the 'granuloma group'. This is a total of 43 patients.

## 6. Inclusion and exclusion criteria

**Inclusion criteria:**

- HIV positive.
- Older than 18 years.
- Meeting criteria for renal biopsy, including, but not limited to
    - Unexplained renal dysfunction.
    - Unexplained proteinuria or haematuria.
        - Renal biopsy was only offered to patients if deemed clinically relevant and part of standard practice.
- Able and willing to provide informed consent for renal biopsy and inclusion in the study.

**Exclusion criteria**

- HIV negative.
- Younger than 18 years.
- Unwilling or unable to provide informed consent.
- Not fulfilling criteria for renal biopsy.
- "Inadequate" specimen to assess for renal pathology.

## 7. Informed consent

Informed consent was taken from all participants prior to renal biopsy (as per standard clinical practice). The risks of the procedure were clearly communicated, and patients informed that they were under no obligation to proceed with the biopsy if they so chose.

Further informed consent for inclusion into the study was taken in the patient's own language. Consent forms (see Appendix 1) outlined the procedures involved as well as the inclusion of information regarding the biopsy and related investigations into the database.

## 8. Follow up

All patients were followed up at the E13 renal clinic at Groote Schuur Hospital to receive their final biopsy results and to plan for further follow up, especially with regard to cART initiation in those patients who were not yet on antiretroviral therapy. Based on the pathology seen, and its reversibility, further follow up was arranged, either at Groote Schuur Hospital, or the referring health care facility (see Appendix 3: Follow up Data Sheet).

## 9. Privacy and confidentiality

During initial hospitalisation and subsequent clinic visits, patients were afforded privacy and confidentiality as per standard clinical practice. The names of patients included in the study, as well as those who refused, were known only to investigators.

For the purposes of this study, we will be accessing the existing database of HIV-infected patients who underwent renal biopsy between January 2005 and October 2012. This database is only made available to those directly involved in research surrounding the abovementioned patients.

From this main database, we will be generating two further databases (one each for 'Non-HIVAN/ICGN group and the 'Granuloma Group'). In these databases, there will be no patient identifiers. Patients will be allocated a number only. The identifying data pertaining to the participant will be stored in a separate location and will only be available to Dr Debbie Nel and Dr Nicola Wearne. No personal identification data will be listed in the final publication.

## 10. Conflicts of interest

None to declare

## 11. Authorship

Dr Debbie Nel will be the main author and will submit a completed thesis for MMed degree purposes. Subsequent publications related to the research will be co-authored with collaborators.

## 12. Ethical and Regulatory compliance

The Human Research Ethics Committee (HREC) at the University of Cape Town approved the original study to develop the database of HIV-infected patients who underwent renal biopsy and subsequent analysis based on the biopsy (see Appendix 4). This proposal to

further analyse certain aspects of that database will be submitted to the HREC for assessment.

Permission regarding medical folder review has been obtained from the Medical Superintendent of Groote Schuur Hospital.

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# Appendix 7

**Table 5. Enlarged. Case reports of AKI thought to be due to IRIS related tubulointerstitial nephritis.**

| Case report | CD4 count[c] | HIVVL[c] (copies/ml) | Mycobacterial diagnosis | cART initiated | Onset of renal disease | Other manifestations | CD4 count[β] | HIVVL[β] (Copies/ml) | Renal biopsy | Drug changes | Treatment | Renal function improvement | Follow up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jehle 2004 | 69 cells/µl | 1247786 | There are no sources in the current document. Miliary TB and urinary TB. Culture +, drug sensitive | 2 weeks after ATT | 6 weeks after cART | New pulmonary infiltrates | 82 cells/µl | 104 (↓4 $log_{10}$) | Severe GIN with interstitial infiltrates | Cotrimoxazole stopped[ε] | Prednisone 1mg/kg | Within 10 days | Healthy at 1 year |
| Daugus 2006 | 26 cells/µl | 56732 | Pleural effusion (Culture + MTB) | 1 month after ATT | 4 weeks after cART (during steroid withdrawal) | Abdominal TB IRIS* | 127 cells/µl | Undetectable | Diffuse interstitial inflammation. No granulomas. | TDF dose adjusted | Steroids reintroduced at 1mg/kg | Within 1 week | Normal renal function after 1 year |
| Izzedine et al 2007 | 37 cells/µl | 3750000 | Symptoms: bone and liver granulomas | 3 months BEFORE ATT | 8 weeks after ATT 5 months after cART | Erythematous skin lesions: lymphadenopathy; splenomegaly; cholestasis | 148 cells/µl | > 200 | Acute GIN | | Prednisone 1 mg/kg. | Over 2 weeks. | Normal renal function at 10 months |
| Salliot et al 2008 | 88/mm³ | 177504 | Pulmonary, hepatic and cutaneous TB | 45 days after ATT | 15 days after cART | None | 326 cells/mm³ | 494 | Intense GIN | EMB, PZA and cART stopped; cART restarted after 3.5 months | Prednisone 1mg/kg initially; ↑ to 1.5mg/kg. | Slow (needed higher dose of prednisone). | Normal renal function at 8 months. |
| Croucher et al 2010a | 79 cell/µl | 61573 | Pulmonary TB | 2 days before ATT (previously defaulted cART) | 50 days after restarting cART (after withdrawal of steroids)[γ] | Fever | 320 cells/µl | 333 | Lymphocytic AIN (CD4+ predominant); No granulomas | TDF changed to ABC | Prednisone restarted. | Within 24 hours; normalised after 2 weeks. | Normal renal function at 18 months |
| Martin-Blondel et al 2011 | 25 cells/mm³ | 6.5 $log_{10}$ | MAC (diagnosed 34 days AFTER ART) | 34 days prior to MAC diagnosis and treatment | 103 days after cART (69 days after MAC treatment) | Fever; cognitive impairment; interstitial pulmonary infiltrate; progression of retroperitoneal lymphadenopathy | 88 cell/mm³ | 2.4 $log_{10}$ | Severe acute GIN Macrophage predominant (60%); T cells 40% (CD8+: 80%) | ART regimen changed | Prednisone 1mg/kg. | Over 1 month | Normal renal function at 10 months |

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**04 December 2012**

**HREC REF: 633/2012**

**Dr N Wearne**
Division of Nephrology & Hypertension
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Dear Dr Wearne

**PROJECT TITLE: A DESCRIPTIVE STUDY OF RENAL BIOPSIES IN HIV-INFECTED PATIENTS WITH RENAL DYSFUNCTION WITH FOCUS ON:**
**1: CLINICAL CORRELATIONS AND OUTCOMES OF THOSE PATIENTS WITH KIDNEY DISEASE EXCLUDING HIV ASSOCIATED NEPHROPATHY (HIVAN) AND IMMUNE COMPLEX GLOMERULONEPHROPATHY (ICGN)**
**2: CLINICAL CORRELATIONS AND OUTCOMES OF PATIENTS WITH GRANULOMAS SEEN ON RENAL BIOPSY.**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

**Approval is granted for one year till the 28 December 2013.**

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form, if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the HREC. REF in all your correspondence.**

Yours sincerely

**PROFESSOR M BLOCKMAN**
**CHAIRPERSON, HSF HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
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