

**A TEN YEAR RETROSPECTIVE STUDY OF THE AETIOLOGY AND OUTCOME OF
CRESCENTIC GLOMERULONEPHRITIS IN CHILDREN PRESENTING TO THE RED
CROSS CHILDREN'S HOSPITAL, CAPE TOWN, SOUTH AFRICA.**

By

Dr CHISAMBO MWABA [Beit Scholar]

(MWBCHI001)

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE

MPhil (Paediatric Nephrology)

IN THE FACULTY OF HEALTH SCIENCES AT THE UNIVERSITY OF CAPE TOWN

Submitted 8 January 2016

SUPERVISORS:

Dr Priya Gajjar: School of Child and Adolescent Health, University of Cape Town

Dr Peter Nourse- School of Child and Adolescent Health, University of Cape Town

A/Prof Komala Pillay- Department of Pathology, University of Cape Town

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

CANDIDATE DECLARATION

I Chisambo Mwaba , hereby declare that this dissertation represents my own independent work (unless otherwise acknowledged) 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 another university. I empower the university to reproduce for the purpose of research any part or the whole of the contents in any manner whatsoever.

14.01.2016

Signed by candidate

Chisambo Mwaba

Signature removed

DEDICATION

To all the renal patients at the Red Cross War Memorial Children's Hospital, Cape Town

ACKNOWLEDGEMENTS

I owe the successful completion of this project to many people. My family- for insisting that they could still see the light at the end of the tunnel during the times when I would lose my 'eye glasses'.

Special thanks to my supervisors Dr Priya Gajjar, Dr Peter Nourse and associate professor Komala Pillay for their guidance. I wish to thank associate professor James Chipeta, and my colleagues Dr Francis Furia and Dr Adebowala Ademola for proof reading the final report and for their invaluable suggestions on the data analysis. Thanks to professor Musonda for graciously agreeing to help with the biostatistical analysis despite the very short notice.

I am grateful for the assistance of Mrs. Gail Vander Broeck, the ward E2 clerk and all the staff in the records department for their invaluable help in locating patient notes.

I remain indebted to the librarians at the Institute of Child Health library for assistance with navigating the world of searching databases and referencing.

Finally and certainly not the least, I wish to thank my sponsors, the Beit Trust, for the financial and moral support during the whole duration of my stay in Cape Town.

ABSTRACT

Background: Crescentic glomerulonephritis represents the extreme end on the spectrum of glomerular injury. It can result from a wide range of disease conditions and clinically is marked by a rapid deterioration in renal function over days, weeks or months. Although rare, crescentic glomerulonephritis is an important entity to recognize because prompt treatment can improve patient outcomes significantly. Literature on the prevalence, clinical presentation, aetiology and outcome of histologically proven crescentic glomerulonephritis among children, in Africa, is scanty. Most of what is known about this entity is extrapolated from adult studies and from paediatric studies that have for the most part been conducted outside the African continent.

Objective: This study was conducted in order to determine the incidence, clinical presentation, aetiology and outcome of histologically proven crescentic glomerulonephritis in children presenting to the Red Cross Children's Hospital, Cape Town, South Africa.

Methods: This was a retrospective folder review in which the renal biopsy records of children less than 18 years old who had had native kidney biopsies performed between 2004 and July 2015 at the Red Cross Children's Hospital were reviewed. The clinical notes of patients found to have been diagnosed with crescentic glomerulonephritis were traced so as to extract demographic and clinical information which was then recorded onto the study data sheet. No attempt to contact patients or their families was made. Data analysis with regard to the incidence, the clinical features and the outcome of crescentic glomerulonephritis was done using SPSS version 22.

Results: A total of 470 native kidney biopsies were performed in the period under review. Of these, 24 had crescentic glomerulonephritis, accounting for an incidence of 5.1 %. The sub-types of crescentic glomerulonephritis were immune-complex in 19 (80%), Pauci-immune in 2 (8 %), unspecified type in 3 (12 %) and no child had the anti-glomerular basement membrane subtype. The underlying aetiology of the immune complex sub-type was post-infectious in 11(57.9%), idiopathic in 4(21%), HSP/IgA nephropathy in 2 (10.5%), SLE in 1 (5.3%) and mesangiocapillary glomerulonephritis in 1(5.3%).

Fourteen of the subjects were male thus giving a male to female ratio of 1.4 while the mean age of the children was 8.3 [range- 1 to 14 years]. The commonest clinical features were hypertension (90%), nephrotic range proteinuria (80%), macroscopic haematuria (57%), oedema (94%) and anaemia (88%). None of these had a statistically significant association to the renal outcome. Ten (77%) out of the 13 children with crescentic glomerulonephritis who were followed up for more than a year were found to have either died, had residual renal dysfunction or been transplanted at the last clinical contact.

Conclusion: Crescentic glomerulonephritis was diagnosed in 5.1% of paediatric native renal biopsies which is consistent with what has been reported elsewhere. Unlike reports from other geographical areas the vast majority (80%) of the cases had immune-complex glomerulonephritis with a suspected

post-infectious aetiology in over half of these. Similar to earlier reports from South Africa the outcome was poor in most (77%) of the patients. Further research is required to characterise the factors that make post-infectious glomerulonephritis particularly severe in this population.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	13
1.0. Preamble	13
1.1 Structure of normal the glomerulus	13
1.3 Glomerular extra-capillary proliferation	15
1.4 Crescentic glomerulonephritis	16
1.6 statement of the problem	16
1.7 Study Question	17
1.8 Objectives	17
1.9 Overview of the Dissertation	18
CHAPTER 2: LITERATURE REVIEW	19
2.0 Introduction	19
2.1 Definition	19
2.2 Classification	20
2.3 Pathophysiology	21
2.4 Epidemiology	25
2.5. Treatment	27
2.6 Outcome	31
CHAPTER 3: MATERIALS AND METHODS	33
3.1 The study site	33
3.2 Study design and population	33
3.3 Selection of Subjects	33
3.4 Case definition	34
3.5 Inclusion criteria	34
3.6 Clinical Assessment	34
3.7 Data Management and Analysis	34
3.7.1. Dependent (outcome) variables:	34
3.7.2 Independent (predictor) variables	34
3.7.3 Data management and analysis	36
3.8 Ethical Considerations	36
CHAPTER 4: RESULTS	38
4.0 Recruitment of study subjects and incidence of crescentic glomerulonephritis	38
4.1 Demographic and Baseline Characteristics of the subjects	39
4.2 Clinical and Laboratory Characteristics of the subjects	41
4.3 Histopathology findings	47

4.5 Treatment	49
4.6 Outcome	50
4.7 Association of various patient characteristics to renal outcome	51
CHAPTER 5: DISCUSSION	53
5.0 Incidence of crescentic glomerulonephritis	53
5.1 Demographic and baseline characteristics of the subjects	53
5.2 Clinical and laboratory characteristics of the patients	54
5.3. Histopathology	55
5.4 Treatment	57
5.5 Renal outcome	58
5.6. Correlation of various patient characteristics with renal outcome	58
CHAPTER 6: CONCLUSION.....	59
6.0 Conclusion	59
6.1 Recommendations	59
6.2 Study limitations	59
LIST OF REFERENCES	60
APPENDIX A: STUDY PROFORMA.....	1
APPENDIX B: HOSPITAL APPROVAL OF RESEARCH	3
APPENDIX C: ETHICAL APPROVAL LETTER	5

LIST OF TABLES

Table	Page
Table 1. Categorization of crescentic glomerulonephritis	16
Table 2. Effect of age on the aetiology of crescentic glomerulonephritis	25
Table 3. Aetiology of immune –complex CGN from various series	26
Table 4. KDIGO recommended regimes for ANCA vasculitis with glomerulonephritis	28
Table 5. Renal outcome of crescentic glomerulonephritis from various series.	31
Table 6. K/DOQI staging of chronic kidney disease	36
Table 7. Demographic characteristics of the study subjects	40
Table 8. Anthropometric characteristics of the study subjects	42
Table 9. Patient clinical characteristics at initial presentation	43
Table 10. Summary of patient renal function at initial presentation	45
Table 11. Result of various serological immune markers in the subjects	46
Table 12. Aetiology of crescentic glomerulonephritis in RXH renal biopsy series	47
Table 13. Histology findings in study subject biopsies	49
Table 14. Renal outcome at one year	51
Table 15. Association of patient characteristics to renal outcome	52

LIST OF FIGURES

Figure		Page
Figure 1.	Micrograph of normal glomerulus	13
Figure 2.	Simplified illustration of components of the glomerular filtration barrier	14
Figure 3.	A glomerular crescent	15
Figure 4.	The final common pathway in the pathogenesis of CGN	22
Figure 5.	Flow chart showing selection of study subjects	38
Figure 6.	Seasonal distribution of CGN cases.	41
Figure 7.	Frequency of various clinical features in study subjects	44
Figure 8.	Number of CGN cases by year (2004-2014)	48

LIST OF ACRONYMS

1. **ANCA**- Anti Neutrophil Cytoplasmic Antibodies
2. **ASOT**- Anti-streptolysin O titres
3. **CKD**- Chronic Kidney Disease
4. **CGN**- Crescentic Glomerulonephritis
5. **eGFR**- Estimated Glomerular Filtration Rate
6. **EM**- Electron Microscopy
7. **GBM**- Glomerular basement membrane
8. **HIV**-human immune virus
9. **IF**- Immunofluorescence
10. **KDIGO**- Kidney Disease Improving global Outcomes
11. **KDOQI**- Kidney Disease Outcomes quality Initiative
12. **RPGN**- Rapidly Progressive Glomerulonephritis
13. **UK**- United Kingdom
14. **WHO**- World Health Organization

GLOSSARY OF TERMS

Anaemia - A reduction of red blood cell mass or blood haemoglobin concentration below an age appropriate level

Hyperphosphatemia- an abnormal blood phosphate level above age appropriate upper reference limit

Hyponatraemia- blood sodium level below 135 mille moles/L

Macroscopic Haematuria- blood in urine that is visible to the naked eye

Microscopic Haematuria-blood in urine that is detectable by dipstick or microscopy

CHAPTER 1: INTRODUCTION

1.0. Preamble

Crescentic glomerulonephritis is a severe form of glomerulonephritis that is marked on histology by an extra-capillary proliferation in Bowman's space, and can result from a vast array of disease conditions(1). It is a non-specific marker of severe glomerular injury and characteristically presents with a rapid deterioration in renal function over days, weeks or months(2). The ensuing renal damage is often severe, irreversible and unrelenting and will often result into chronic kidney disease and death particularly if left untreated(3). The resulting chronic kidney disease has a devastating effect on the growth and development of children and places a heavy drain on health system resources to provide the expensive therapies that are required for its management(4, 5). This burden is especially felt in regions of the world with limited resources such as Africa. Data on the aetiology, clinical features, outcome and factors that may modify outcome in crescentic glomerulonephritis in children, particularly from the African continent is scanty. Admittedly crescentic glomerulonephritis only affects a small proportion of children with estimates that it only comprises about eleven percent of unselected renal biopsies in children and that the accompanying clinical syndrome has an incidence of only 7 cases per million population per year (2, 6, 7). Yet because of the associated severe glomerular injury and the potential devastating renal dysfunction it becomes an important disease for clinicians to recognise. This is particularly so because it is known that the associated deterioration in renal function may be ameliorated by prompt and appropriate therapy administered in the early stages of the disease(8).

1.1 Structure of normal the glomerulus

The basic functional unit of the kidney is the nephron which consists of two parts: the glomerulus and the tubular segment. The glomerulus is the filtration unit and consists of a tuft of capillaries and matrix that are contained in the proximal part of the tubule: the Bowman's capsule. It consists of three main cell types: fenestrated endothelial cells, mesangial cells and podocytes. The endothelial cells and podocytes are separated by a basement membrane and together they form the filtration barrier. This filtration barrier acts to prevent cellular components of blood, proteins and other molecules from passing into the ultrafiltrate in Bowman's space. The passage of molecules is regulated in part due to limits set by charge and size(9). Various factors such as inflammation can disrupt the normal function of this barrier. Figure 1 below illustrates a microgram of a normal glomerulus while figure 2 is a schematic representation of the constituents of the filtration barrier.

Figure 1. Micrograph of Normal Glomerulus-courtesy of Dr Luchenga Muchelenganga, University of Zambia

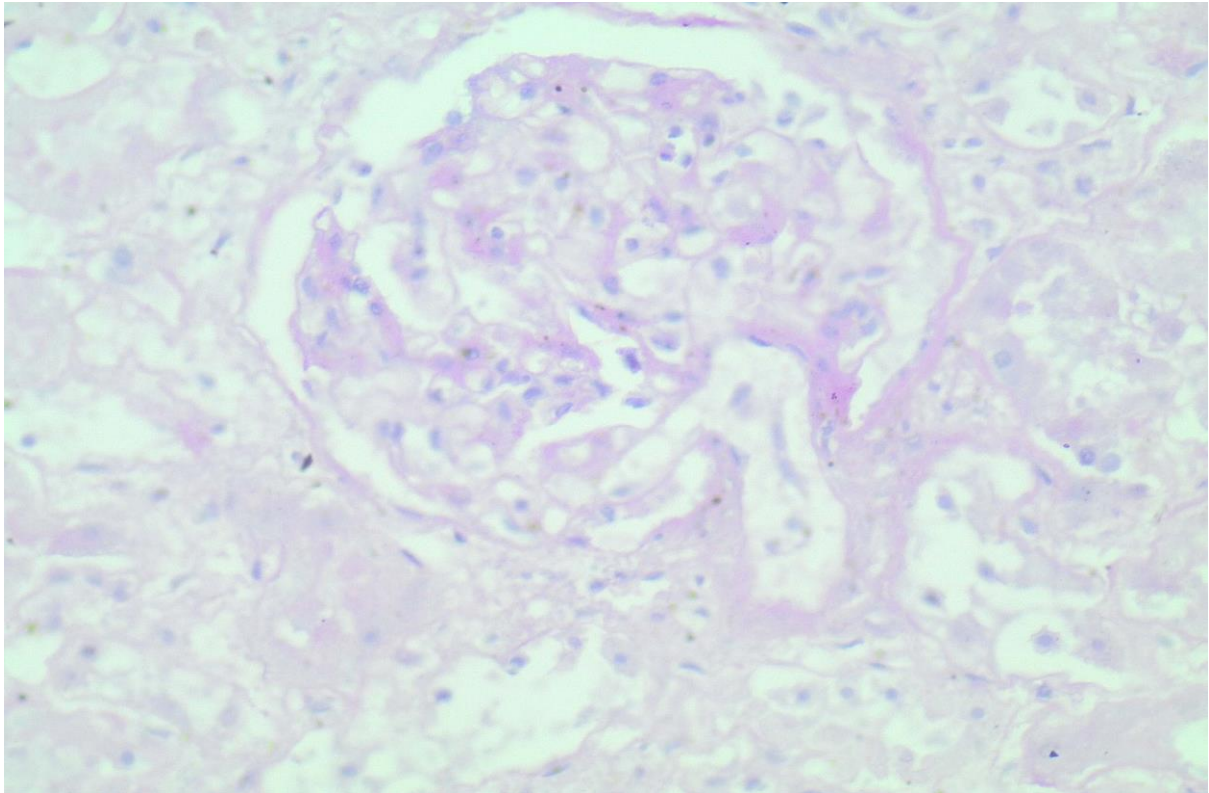
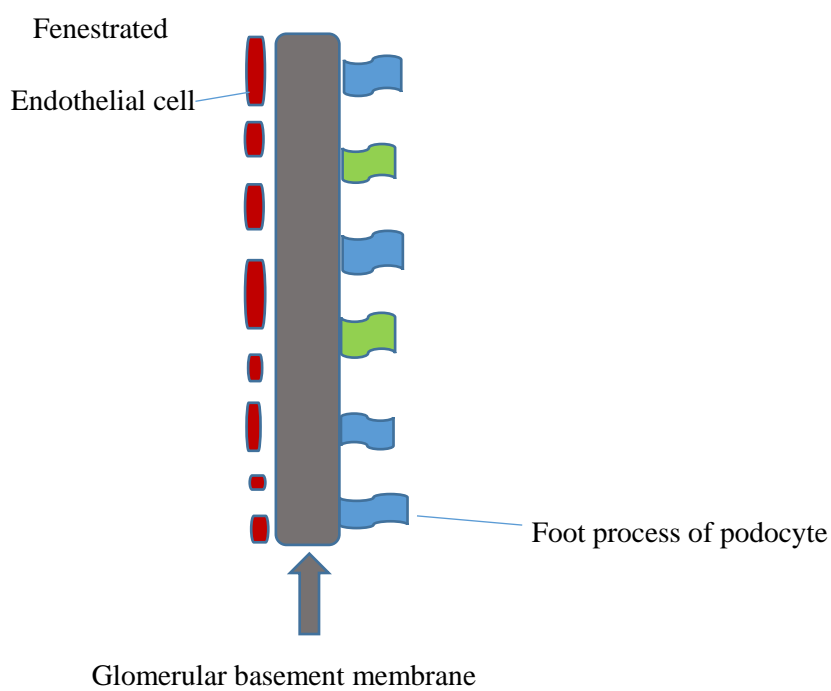


Figure 2. Simplified illustration of components of the glomerular filtration barrier

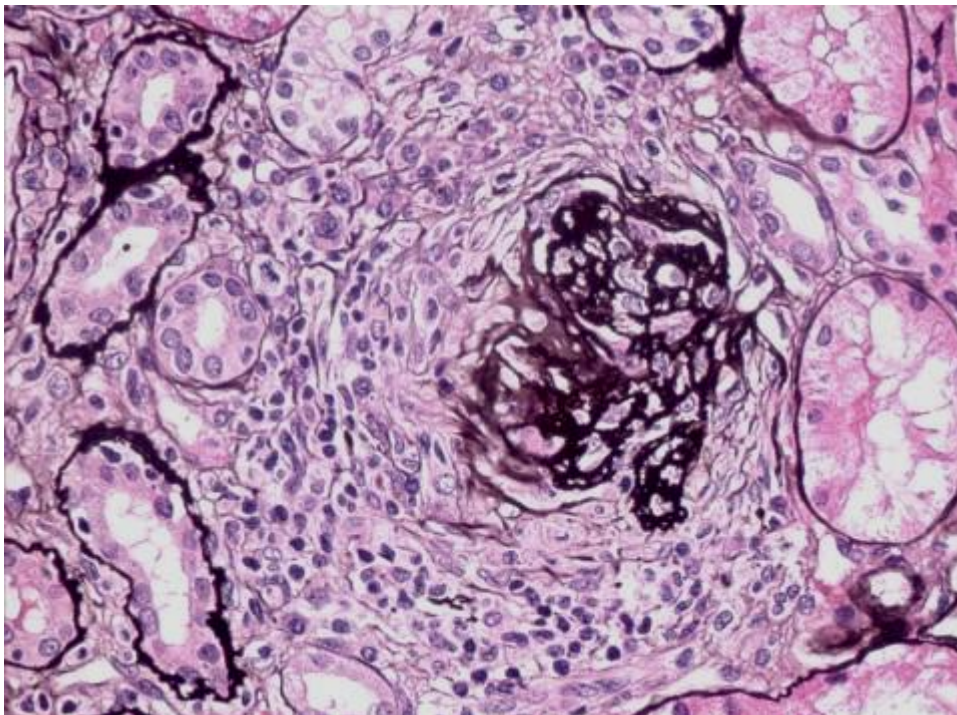


1.3 Glomerular extra-capillary proliferation

Many of the processes responsible for crescentic glomerulonephritis are immune based(1). These can result from many disease conditions. During inflammation leukocytes are attracted to the site of inflammation or injury by cytokines during the process of chemotaxis. Leukocytes undergo margination due to the interaction of various adhesion molecules expressed on their surfaces as well as on endothelial cell surfaces and eventually leave the capillary lumen by diapedesis. At the site of deposition of immune complexes the neutrophils are activated to go through the respiratory burst and to release the contents of their toxic granules such as proteinases. These together with reactive oxygen species cause damage to the extracellular matrix in the glomerular basement membrane thus breaching the filtration barrier. In addition there is further production of inflammatory cytokines which among other things enhance the migration of inflammatory cells as well as induce the adaptive arm of the immune system(10).

If the damage to the extracellular matrix is severe then a breach in the filtration barrier occurs with coagulation proteins, macrophages, neutrophils spilling into bowman's space. This exudate also contains factors that stimulate the visceral and parietal cells to begin to proliferate resulting into a multilayer of cells which encroach into bowman's space. This is referred to as an extra capillary proliferation. The resulting lesion is shaped like a crescent hence the term crescentic (1). By convention a crescent must have at least two layers of cells in Bowman's space. Figure 3 is a micrograph of a glomerular crescent.

Figure 3. A glomerular crescent-courtesy of A/prof Pillay, University of Cape Town (11)



1.4 Crescentic glomerulonephritis

By convention when more than 50% of glomeruli exhibit crescents this is referred to as crescentic glomerulonephritis(1). There are three sub-groups: type I is anti-glomerular basement disease, Type II is immune-complex crescentic glomerulonephritis and type III is Pauci-immune or Anti neutrophil cytoplasmic antibody associated glomerulonephritis(3). These are differentiated based on immunofluorescence findings on biopsy and also serological findings. This is illustrated in table 1 below.

Table 1. Categorization of crescentic glomerulonephritis

Sub-type	Immunofluorescence	Serology
I-Anti glomerular basement disease	Linear	Anti – glomerular basement antibodies
II- Immune- complex	Granular	Various depending on aetiology e.g. SLE markers
III- Pauci-immune	< 2+ immune globulin deposition ,scanty	80-90% ANCA

Various factors such as geography, sex, age, ethnicity and level of industrialization may influence the epidemiology of these subtypes(1). There is no clear indication from the literature on what the profile of crescentic glomerulonephritis in the paediatric age group is in the South African population but earlier series in adults predominantly and a few small series in children seem to suggest that a post infectious aetiology is predominant particularly in black children (12-14).

Crescentic glomerulonephritis usually presents with a rapid deterioration in renal function with children often presenting with haematuria, oliguria and fluid over load(3). This type of clinical presentation is called rapidly progressive glomerulonephritis. While it occurs often with crescentic glomerulonephritis it is not necessarily synonymous with it(2).

The outcome is reported as poor in about half of the cases particularly if post infectious. Treatment with immune suppression has been shown to improve outcome if instituted early. Thus one could say that potentially, crescentic glomerulonephritis is a preventable or treatable cause of chronic kidney disease albeit in only a small group of patients.

1.6 statement of the problem

As stated above, numerous studies have demonstrated that crescentic glomerulonephritis is not pathognomonic of any disease and can occur in a wide spectrum of disease conditions. It is known that the relative importance of each of these disease conditions varies with age, ethnicity and geography (2).

Most of these studies however have been conducted outside Africa and largely in small series. As such there is a paucity of data on the predominant clinical presentation, the spectrum of diseases associated with crescentic glomerulonephritis and also the patient outcomes with regards to renal function and patient survival in an African setting. This is particularly so in the paediatric age group.

We retrospectively reviewed the records of all children with a diagnosis of crescentic glomerulonephritis in their native kidney biopsies presenting to the Red Cross War Memorial Children's Hospital in the last ten years (2004-July 2015). The aetiology, presenting clinical features, and the association between these clinical features and patient and renal function outcomes were characterised. It is hoped that this information will be useful in shedding some light on these questions as well as help to provide clues for the direction of further research into crescentic glomerulonephritis in children.

1.7 Study Question

What are the incidence, clinical presentation, aetiology and outcome of crescentic glomerulonephritis in children presenting to the Red Cross Children's Hospital in Cape Town over the period 2004 and July 2015?

1.8 Objectives

Main objective

To determine the incidence, aetiology, clinical features and outcome of crescentic glomerulonephritis in children presenting to the Red Cross War Memorial Children's hospital for renal biopsy.

Specific objectives

1. To determine the incidence of crescentic glomerulonephritis in biopsies done over the last 10 years (2004-July 2015).
2. To document the various aetiological conditions presenting as crescentic glomerulonephritis.
3. To establish the renal and patient outcomes for children treated for crescentic glomerulonephritis in the last ten years.
4. To determine the correlation between various patient clinical features and the outcome.

1.9 Overview of the Dissertation

This monograph is arranged into 6 chapters. The first chapter seeks to give a backdrop to the study briefly highlighting the knowledge gaps and sets out the aims of the study. In the second chapter we present the results of a search of the literature relevant to the stated aims of the study. Chapter three presents the study methods and materials while the results are outlined in the fourth chapter. In chapter five we discuss the results in light of current literature and then in chapter six we conclude and provide recommendations. We used the Vancouver style of referencing and all the references are also presented in chapter six. Finally selected documents are presented in the appendices.

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

Crescentic glomerulonephritis is a medical emergency characterized by severe deterioration in renal function over a relatively short period of time that can quickly lead to irreversible kidney injury if not appropriately treated early in its course(8). The aetiology is quite varied ranging from primary and secondary kidney diseases, genetic conditions, drugs, infections, malignancy and autoimmune conditions (1, 12, 15-17). Though the outcome has been reported to be quite poor in many studies, what is clear is that early recognition and treatment regardless of the cause seems to improve this outcome. Most of this information on crescentic glomerulonephritis is extrapolated from adult series with a paucity of large published paediatric series in general and to our knowledge a paucity of published paediatric reports from Africa (13, 14, 18).

In this review we searched the literature for information with an attempt to:

1. Describe diagnostic criteria for crescentic glomerulonephritis
2. Describe current theories on the pathophysiology of crescentic glomerulonephritis
3. Describe what is known about the Epidemiology of crescentic glomerulonephritis in children
4. Describe the current treatments
5. Report on known outcomes of crescentic glomerulonephritis in children and factors that influence this outcome as described in the literature.

Databases were searched using the search terms “Rapidly Progressive Glomerulonephritis” , “ severe post-infectious glomerulonephritis” , “ANCA-associated glomerulonephritis”, “Good Pastures disease” , “anti- glomerular basement disease” , “ Pauci-immune glomerulonephritis” , “ crescentic glomerulonephritis” , “children” , “paediatric” , “outcome” , “aetiology”, “treatment”, “Therapy” , “Africa” , “developing countries/low resource setting” ,in various combinations. Only articles in English were included.

After the internet search, interesting articles were archived and later a manual search of some of their references lists was also carried out to try and locate more information. In addition reference nephrology text books were read and their references lists searched in a bid to locate more articles.

2.1 Definition

Crescentic Glomerulonephritis is a pathological diagnosis marked on histology by the presence of crescent shaped lesions in the glomeruli which consist of multiple layers of cells that partially or completely fill the urinary space (19). These crescents form as a result of proliferation of parietal and visceral epithelial cells and by migration of monocytes and macrophages into the urinary space, the so

called extra-capillary proliferation. By definition the presence of fifty percent or more of glomeruli exhibiting this lesion is necessary to make the diagnosis(2). The weakness in such a diagnostic criteria is that it depends on the renal biopsy sample being adequate so as to allow the inclusion of enough glomeruli for examination. But even when the specimen is considered adequate, biopsy may miss more extensive focal crescents particularly in the early stage of disease (20). This may perhaps be why a few studies are reported to have used an arbitrary percentage of glomeruli involved with crescents to make the diagnosis (19) (21, 22).

The clinical correlate is known as rapidly progressive glomerulonephritis (RPGN) and is characterised by a decline in renal function over days, weeks or months. It should be remembered however that CGN can have an insidious course and cases with only mild derangement of renal function have been described (23-25). Other entities such as HUS and proliferative glomerulonephritis without crescents can also present with a rapidly progressive course and so form an important differential diagnosis.

2.2 Classification

The most widely accepted classification of crescentic glomerulonephritis was proposed by Couser in 1988 (26). In this scheme there are three broad classes of Crescentic Glomerulonephritis. Patients are assigned to a sub-type based on renal biopsy immunofluorescence findings as well as on the presence of various serological markers. These classes are meant to reflect the possible mechanisms of the glomerular injury. These various mechanisms eventually lead to a final common inflammatory cascade which then results in the extra-capillary proliferation in Bowman's space. These groups are:

a) Anti -Glomerular Basement Membrane Crescentic Glomerulonephritis(Anti-GBM)(Type I)

This entity is uncommon in both the paediatric age group and adults(1). It is caused by the presence of auto antibodies directed against a component of the alpha 3 chain of type IV collagen that is referred to as Goodpasture antigen. It is a normally a hidden conformational epitopes(27). A post- transplant form of the disease occurs in some patients with Alports syndrome but the target of the GBM alloantibodies are conformational epitopes in the NC1 domain of alpha 5 collagen only(27). Anti-GBM nephritis can either occur as a renal-limited disease or as a pulmonary –renal vasculitic syndrome (Good pastures syndrome). On histology the distinguishing features of this sub-type of crescentic glomerulonephritis is the presence of linear sub epithelial deposits of Ig G or rarely IgA on immunofluorescence. The diagnosis is confirmed by detection of GBM allo-antibodies on serology. It has been observed however that some patients with GBM nephritis will either have undetectable anti-GBM antibodies or that they express both anti-GBM allo-antibodies and anti-neutrophil cytoplasmic antibodies (ANCA) (1). This is more likely to occur in the renal limited form of the disease.

b) Immune mediated Crescentic Glomerulonephritis (Type II)

This form of CGN accounts for a large proportion of crescentic glomerulonephritis in the paediatric age group(1). It is marked on immunofluorescence by granular deposits of immune complexes in the sub-endothelium of the capillary wall and also in the mesangium. There are various causes of this histological pattern ranging from post infectious conditions such as post-streptococcal glomerulonephritis, systemic conditions and primary glomerulonephritides. (26, 28, 29)

c) Pauci-immune crescentic glomerulonephritis(Type III)

In the Pauci -Immune Crescentic Glomerulonephritis sub-type, the crescents are often circumferential and there are minimal or no immune deposits demonstrated on immunofluorescence(1). There are also features of small vessel vasculitis with focal or diffuse necrosis(30). Most patients with Pauci-immune glomerulonephritis have a small vessel vasculitis such as microscopic polyangiitis or granulomatosis with polyangiitis (Wegener's). Occasionally though the vasculitis may be renal limited(31). Pauci immune glomerulonephritis is more common in Caucasians and adults and shows no sex predilection(1, 32).

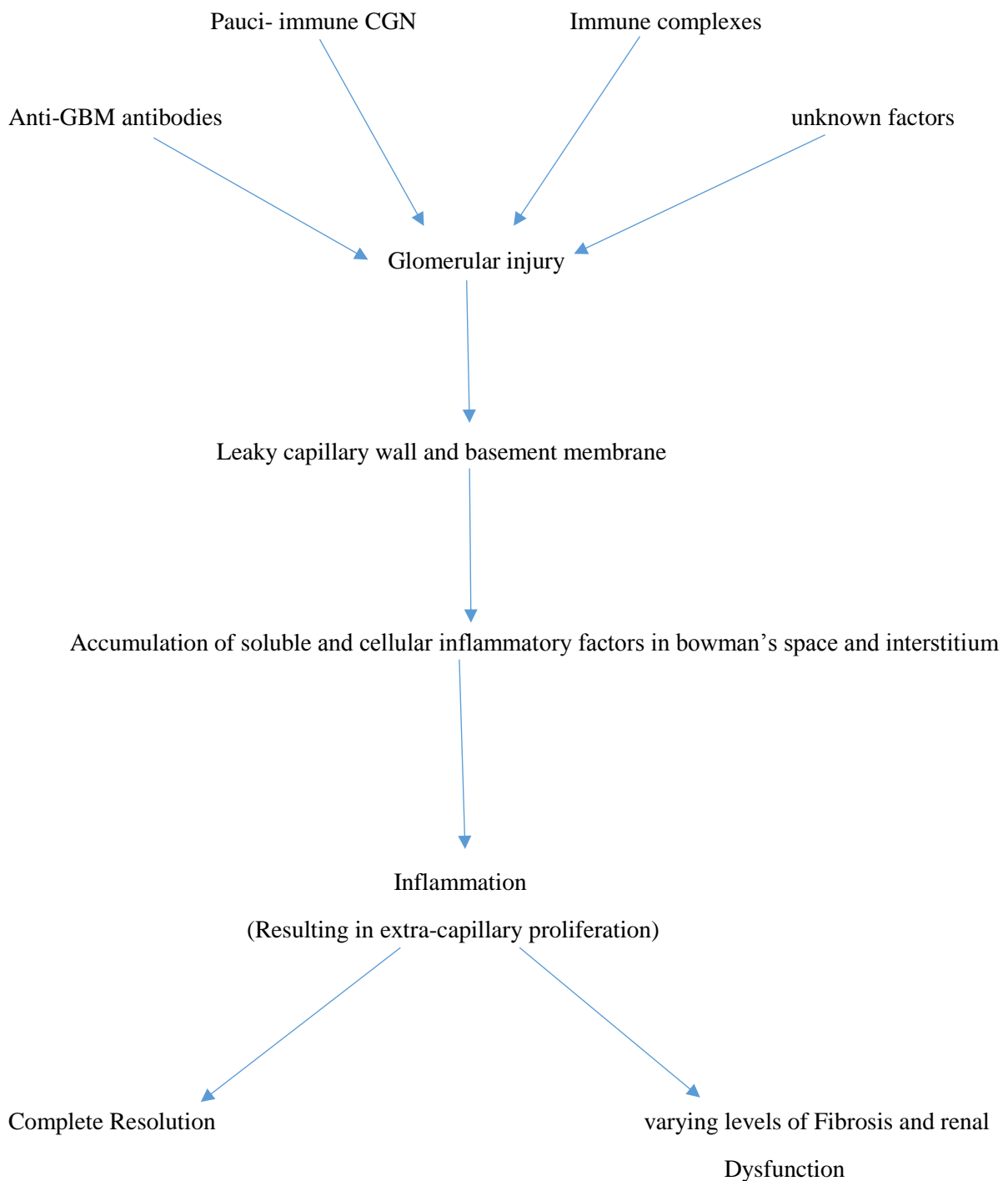
2.3 Pathophysiology

2.3.1 A final common pathway-mechanisms

It is postulated that the primary event that leads to crescentic glomerulonephritis is an alteration in the integrity of the capillary endothelial wall and the basement membrane which results in increased permeability. This allows both soluble and cellular pro-inflammatory elements to move into the urinary space where they become activated with resultant production of cytokines such as IL1 and TNF alpha. The release of chemokines results in the recruitment of further pro-inflammatory mediators such as macrophages and lymphocytes as well as stimulation of the proliferation of both visceral and parietal epithelial cells with subsequent formation of the characteristic crescent shaped lesions in the glomerulus. There is some evidence that renal progenitor cells may be involved in the formation of this hyperplastic lesion(33). The presence of these glomerular crescents is not specific to a particular disease but rather their presence is simply an indicator of the severity of the glomerular injury.

With time the inflammatory infiltrate may either resolve with resultant restoration of normal glomerular architecture or may progress to formation of fibrinous tissue which may lead to irreversible loss of renal function(10)

Fig. 4 The final common pathway in the pathogenesis of crescentic glomerulonephritis



2.3.2 Mechanism of glomerular injury in Type II /immune mediated crescentic glomerulonephritis

The causes of Type II crescentic glomerulonephritis are heterogeneous. What is common to all of them is that the mechanism of glomerular injury involves the localization of immune complexes in the capillary wall or mesangium. These immune complexes may be formed in-situ or by deposition of already formed circulating immune complexes. Once deposited the immune complexes cause activation of the inflammatory cascade via the intermediacy of various factors such as the soluble mediators of coagulation, blood cells (neutrophils, macrophages, platelets) and cytokines. If the resulting inflammatory damage breaches the capillary wall then an extra-capillary pattern of cellular proliferation results which then leads to formation of crescents(1, 10).

There are various theories over what the determinants of the severity of the glomerular injury might be in the various causes of immune complex crescentic glomerulonephritis (2, 34, 35). For example it has been reported that up to 25% of patients with immune complex crescentic glomerulonephritis have circulating ANCA as opposed to only less than 5% of patients with non-crescentic immune complex glomerulonephritis. This has led some investigators to postulate that ANCAs may predispose to more severe forms of glomerular injury in patients with immune complex mediated glomerulonephritis(2).

2.3.3 Mechanisms of glomerular injury in Pauci- immune crescentic glomerulonephritis

The exact mechanism of glomerular injury in Pauci-immune glomerulonephritis has not been completely elucidated but it is postulated that in most patients the Anti-neutrophil Cytoplasmic Antibodies (ANCA) plays a key role since they are detected in the serum of up to 80% of patients (1, 31, 35, 36)

Anti-neutrophil cytoplasmic antibodies (ANCAs) are autoantibodies, mainly of the IgG class that are directed against antigens in the granules of neutrophils and the lysosomes of monocytes. They mainly target the enzymes myeloperoxidase (MPO) and proteinase 3(PR3)(31).The pattern of distribution of antibodies on immunofluorescence is used to sub-classify ANCAs into two main groups.

- Cytoplasmic ANCA(c-ANCA)-these have a cytoplasmic staining pattern resulting from binding of ANCAs to antigen targets throughout the neutrophil cytoplasm.
- Peri-nuclear ANCA (p-ANCA)-these have a staining pattern around the region of the nucleus. Since the nucleus is negatively charged because of the Deoxyribonucleic acid

content while most of the antigens targeted by ANCAs are positively charged at neutral pH, during ethanol fixation, these positive antigens will migrate and surround the negatively charged nucleus. Antibody staining therefore results in fluorescence of the region around the nucleus giving rise to the observed pattern.

About 90 % of c-ANCA are targeted against PR3 while 70 % of p-ANCA are reactive against MPO. What causes the initial production of ANCA in the first place remains a mystery but numerous hypotheses have implicated various factors such as exposure to silica, *Staphylococcus aureus*, genetic predisposition, parvo B19 and anti-thyroid drugs (36, 37).

Though the exact mechanism of the initiating factors for ANCA formation and indeed for the resulting glomerular injury remain unknown, it is postulated that via stimulation by either cytokines or altered antigen gene expression, the antigen targets of ANCA which are normally inaccessible in the neutrophil cytoplasm become externalised and expressed at the cell surface. In the presence of circulating ANCA, there is an interaction between the autoantibody and the externalised antigens which leads to the activation of the neutrophil. Additionally there is evidence that the MPO and the PR3 antigens may be internalized via an active transport mechanism by endothelial cells. This results in a myriad things including the production of cytokines like interleukin 18 which is a pro-inflammatory factor.

The activation of neutrophils causes them to go through the respiratory burst which subsequently leads to the release of both primary and secondary granules and this ultimately causes the release of toxic oxygen radicals and other lytic substances at the site of the vessel wall thus producing a necrotizing inflammatory injury. Evidence against this hypothesis include the fact that up to 20 % of patients with Pauci-immune glomerulonephritis are ANCA negative and that often ANCA titres do not correlate with disease activity(22, 31). In addition ANCA occur in perfectly normal people who have no evidence of disease.

2.3.4 Mechanisms of glomerular injury anti glomerular basement antibody crescentic glomerulonephritis

It is postulated that by an as yet unknown mechanism, the usually hidden conformational epitopes of the type IV collagen (E_A and E_B) become exposed and that there is an induction of B cells to produce circulating anti-glomerular basement membrane autoantibodies (27, 38)

Some of the factors that are postulated to cause the unveiling of these cryptic epitopes include exposure to tobacco smoke, endogenous oxidants and hydrocarbons (39) (40) (41). The significance of these factors in paediatric patients has not been demonstrated though.

It is believed that these anti-GBM autoantibodies bind to the exposed epitopes and that this then results in the activation of neutrophils with the subsequent recruitment of other inflammatory factors. This

leads to the well described inflammatory cascade that leads to the glomerular injury that eventually results in crescent formation. It is important to remember though that despite being largely believed to be an antibody mediated glomerulonephritis, there has been some evidence for the role of T-lymphocytes in the initiation of the inflammatory cascade that results in anti-GBM disease((42) (43)

2.4 Epidemiology

The estimated incidence of crescentic glomerulonephritis is about 7 cases per million population per year and one study noted that it accounted for only 5 % of unselected renal biopsies in children (7, 44). The importance of each of the immune-pathologic sub groups varies depending on various factors. Generally anti-GBM crescentic glomerulonephritis is rare across all age groups but is more prevalent in adults(1) The Pauci-immune subtype tends to be more common in older individuals while immune-complex mediated crescentic glomerulonephritis is more likely to be diagnosed in the paediatric age group(44-46).

Table 1 illustrates findings from the North Carolina biopsy series. Pauci-immune glomerulonephritis was the most likely cause of RPGN in adults whereas in children the immune-mediated variety is more prevalent. In more recent times many paediatric series have reported an increased proportion of crescentic glomerulonephritis being due to the Pauci-immune subtype particularly in the industrialised countries for example Japan (15). This trend was however not described in a ten year retrospective study conducted in Wessex(47)

Table 2. Effect of age on sub-type of CGN-adapted from the North Carolina Series(1)

IMMUNOPATHOLOGIC CATEGORY(47)	ALL AGES (N=632)	1-20 YRS (N=73)	21-60 YR (N=303)	>60 YR (N=256)
	Anti-glomerular basement CGN	92 (15%)	9 (12%)	44 (15%)
Immune complex CGN	154 (24%)	33 (45%)	106 (35%)	15 (6%)
Pauci-immune CGN	377 (60%)	31 (42%)	145 (48%)	201 (79%)
Other	9 (1%)	0	8 (3%)	0

The data from studies conducted in different regions of the world show a striking difference in the aetiologies of immune-complex mediated crescentic glomerulonephritis (table 2). Generally speaking studies from less developed countries continue to show the relative importance of post-infectious glomerulonephritis whereas studies from more industrialized countries tend to show that this subgroup of causes has become less important (12-14, 25, 44, 46). In addition to this, an evaluation of cases from the more affluent countries has shown that with increased industrialisation and improved standards of living the trends over time have seen the proportion of post infectious glomerulonephritis dropping off (48-50).

Table 3. Aetiology of immune-complex CGN from various series

	Jardim(51)UK (N=30)	Srivastava, <i>et,al</i> (25) New Delhi (N=43)	Dewan <i>et al</i> (44) (N=22)	Parag Natal al(14) (N=27)	Zent <i>et al</i> , cape town (12) (N=73)	Sinha <i>et al</i> (45) India (N=36)
Post infectious GN	6.6%	25.5%	31.8	29.2%	28.7%	8.3%
Systemic Lupus Erythematosus	3.3%	2.3%	9.1%	3.7%	20.5%	11%
HSP, IgA nephropathy	30%	6.9%	13.6%	-	-	11%
Membranoproliferative GN	23.3%	-	13.6%	11.1%	-	5.5%
Others			18.2	26%	-	
Others causes of CGN In the series	34%	65%	15%	32%	-	-

Table 2 above shows the various aetiologies of immune-complex crescentic glomerulonephritis as reported in various series that included children from various parts of the world. The study by Zent *et al* was conducted at Groote Schuur Hospital, in Cape Town. Although the subjects were adults we included this series because it was done in a population very similar to our study population in many other respects. What was striking about the results from this study was the high prevalence of crescentic lupus nephritis as well as post-infectious crescentic glomerulonephritis particularly since the subjects were adults. (14, 44).

Ethnicity is known to influence the epidemiology of crescentic glomerulonephritis. For example numerous studies continue to report a higher prevalence of post-infectious crescentic glomerulonephritis among black patients and indigenous racial groups on other continents (12-14, 52).

This is probably a reflection of the disadvantaged socio-economic circumstances of these groups of people (48).

Pauci-immune crescentic glomerulonephritis on the other hand is more common in Caucasians and has no sex predilection(1). A study conducted exclusively in a paediatric cohort in Japan however showed a higher prevalence in girls(53). In a nationwide survey also in Japan there was a noted higher prevalence (64%) of this subtype as a cause of rapidly progressive glomerulonephritis(15). In a study examining ANCA associated glomerulonephritis in African –American, the outcome was noted to be similar to that in Caucasians. It is worth noting that many of these studies were done in studies that included adults and thus the same may not necessarily be true in the paediatric population.

2.5. Treatment

The treatment of crescentic glomerulonephritis can be divided into supportive and specific(3). Supportive management includes correction of fluid overload, correction of electrolyte imbalances, treatment of infections and any other symptoms associated with renal dysfunction. Specific treatment on the other hand is directed at mitigating against the immune mechanisms causing renal dysfunction. Most of the evidence concerning the efficacy of various therapeutic strategies directed at childhood crescentic glomerulonephritis is extrapolated from case series and adult data (31). We shall briefly review two of the treatment guidelines for crescentic glomerulonephritis from two international kidney organisations: The kidney disease improving global outcomes (KDIGO) and the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI).

The kidney disease improving global outcomes (KDIGO) group is the major organisation that formulates evidence based clinical practice guidelines for kidney diseases(54). These guidelines are meant to be applicable all over the world. National kidney organisations such as NKF KDOQI, will often review these guidelines in order to make them more applicable to their local conditions(54).

2.5.1. Treatment ANCA associated vasculitis

Table 5 is a summary of the KDIGO recommended treatment guidelines in Pauci-immune crescentic glomerulonephritis in various patient populations along with some of the evidence supporting these recommendations(31). The recommendation that all patients with necrotizing and crescentic glomerulonephritis be treated with corticosteroids and cyclophosphamide was based on the fact that the outcome without therapy is universally poor and also due to the fact that many studies clearly show an improvement in renal outcomes with treatment. They recommend that all patients with extra-renal manifestations of disease receive immunosuppression regardless of the level of renal dysfunction. Prudence is advised when it comes to patients already requiring dialysis but with no extra renal

manifestations of disease. The guideline cites evidence which shows that close to half of the patients with an estimated GFR below 10 ml/min/1.73 m² at initial presentation, who received immunosuppression were noted to have regained function a year later. However for patients showing obsolescence of all glomeruli and severe tubular atrophy the benefit of treatment is said to be doubtful (55, 56). Caution is also advised in the case of patients who remain dialysis dependant after 3 months of cyclophosphamide. In this case the guidelines state that the cyclophosphamide be stopped. This is because of evidence that shows that the majority of patients who enter remission do so within the first three months of treatment with cyclophosphamide.

Table 4:KDIGO recommended treatment regimens for induction of remission in ANCA vasculitis with glomerulonephritis-adapted from(31)

Agent	Route	Indication
Cyclophosphamide	I.V or P.O for 3-6 months	All patients- unless contraindicated
Corticosteroids for 3 days	I.V	All patients
Corticosteroids	P.O	All patients
Rituximab	I.V weekly x4	Alternative initial treatment in patients without severe disease or in whom cyclophosphamide is contraindicated
Plasmapheresis	60 ml/kg volume replacement	-patients requiring dialysis or with rapidly increasing serum creatinine -patients with overlap syndrome of ANCA vasculitis and anti-GBM GN -patients with diffuse pulmonary haemorrhage -

The decision as to whether the cyclophosphamide should be given as oral or intravenous pulses depends upon the treating physicians/patient/family assessment of potential for compliance, cost implications, cumulative dose of cyclophosphamide and risk of infection(31). Otherwise the oral and intravenous routes are associated with similar rates of remission and relapse(57).

In addition the guidelines provide evidence that indicates that Rituximab is just as efficacious as cyclophosphamide but questions the wider applicability of its use globally due to the cost of the drug(58).

Furthermore, plasmapheresis is advocated for severe necrotizing crescentic glomerulonephritis. Evidence is quoted from a multicentre trial which showed improved renal outcomes in patients presenting with advanced kidney dysfunction which was defined as a serum creatinine greater than 500 micromoles/litre(59). Evidence for use of plasmapheresis in patients with serum creatinine less than 500 micromoles/litre was said not to be as strong(60).No equivalent threshold is provided for the paediatric population. Since the range of body mass in paediatric patients is widely ranging perhaps use of a threshold based on glomerular filtration rate would be more appropriate for this age group. The guideline also brings attention to the fact that most evidence for the effectiveness of plasmapheresis in the setting of severe diffuse pulmonary haemorrhage is based on retrospective case series but recommends its use due to the associated reduction in mortality in these series (61-63).

In commenting on the fore-going recommendations the kidney disease outcome quality initiative (KDOQI) group raises a few questions(54). While concurring with KDIGO guidance on the use of corticosteroids and cyclophosphamide in the initial management, they point out the fact that the evidence on the dose of intravenous cyclophosphamide is scant and that it is based on lupus nephritis treatment protocols. In addition they contend that rituximab is as efficacious as cyclophosphamide even for severe disease as illustrated in patients in the RITUXVAS (rituximab versus cyclophosphamide in ANCA-associated vasculitis) and RAVE (rituximab in ANCA-associated vasculitis) trials who all had newly diagnosed severe disease(58, 64). This comment does not apply to patients on mechanical ventilation or those with very severe renal disease. They do concede though that there is not as much long term experience with rituximab as compared to cyclophosphamide.

The KDOQI commentary endorses the use of methotrexate and corticosteroids for induction of remission in non-severe extra renal disease but contends that the evidence for the use of MMF for induction is not strong enough. Furthermore they failed to find evidence for the use of azathioprine for induction therapy.

With regards to the use of plasmapheresis in Pauci-immune crescentic glomerulonephritis, KDOQI casts some doubt on the presence of strong evidence on the long term benefit based on new data from the MEPEX (methyl prednisolone or plasma exchange for renal vasculitis) trial. In addition they raise concerns about the need for clinicians to be aware of the risk of plasmapheresis removing rituximab

and therefore the need for appropriate dose timing. Other risks associated with plasmapheresis such as infection, hemodynamic shifts are also highlighted. In the context of pulmonary haemorrhage though the evidence is not strong since it was acquired from retrospective case series, the impact i.e. reduced mortality is recognised as high.

Both work groups advocated for the use of maintenance therapy after induction of remission for the duration of at least 18 months. Patients with renal limited disease and are still on dialysis 3 months after initiation of induction therapy should have immunosuppression withdrawn due to the higher risk of infection compared to the benefit of therapy. Choices of drugs include azathioprine, MMF and methotrexate. Both contend that there is no actual trial evidence for the use of maintenance therapy after rituximab though both groups advice for maintenance in this context due to evidence showing presence of relapse five months to years after use of rituximab.

2.5.2. Treatment of anti-GBM crescentic glomerulonephritis

The KDIGO 2012 glomerulonephritis clinical guidelines recognise that the anti -GBM antibodies in this condition are pathogenic and support the use of a treatment strategy that removes these antibodies, reduces further elaboration of antibodies by immune cells as well as mitigates the inflammatory damage that may be on going in the kidneys and other tissues(31).

In brief the guidance for induction of remission is that all patients should receive corticosteroids, cyclophosphamide as well as 14 days of plasmapheresis or until anti-GBM antibodies become undetectable. The exception is in patients with renal disease in which there is involvement of 80-100% of glomeruli by crescents. All patients with major pulmonary haemorrhage are meant to receive plasmapheresis regardless of the percentage of glomeruli involved in crescent formation. They do point out though the fact that the evidence for plasmapheresis is not very robust(31).

In commenting on the above guidance KDOQI essentially concurs with the exception of reiterating that there may be a case for trial of cyclophosphamide in some selected patients even in the presence of 80-100% of glomeruli that are involved with crescents(54)

With regards to maintenance therapy both groups advise against the use of this in the context of anti-glomerular basement disease. This is because of the fleeting nature of the anti-glomerular basement membrane antibodies. KDOQI, however offer the suggestion that azathioprine can possibly be used as maintenance therapy in the very rare event that a relapse doe as occur. Both guidelines counsel that transplantation be deferred until antibodies become undetectable as they are known to be pathogenic.

2.5.3. Treatment immune-complex crescentic glomerulonephritis

The specific therapy directed at immune- complex crescentic glomerulonephritis depends on what the underlying diagnosis is(3). The treatment for IgA nephropathy, Henoch Schonlein purpura or systemic lupus erythematosus associated crescentic glomerulonephritis is based on the respective protocols for these particular disease conditions. The situation is rather unclear for post infectious severe glomerulonephritis and idiopathic immune-complex crescentic glomerulonephritis but generally children are given an initial course of pulse corticosteroids followed by a tapering of prednisone over a three month period by most nephrologist with only a few preferring to add on cyclophosphamide for a select few patients.

2.6 Outcome

The long term renal outcome of childhood crescentic glomerulonephritis is described as poor in at least half of the patients in many of the case series (3, 44-46, 65). Table 4 below shows the renal outcome as reported in a few series from across the world.

Table 5: Renal outcome of crescentic glomerulonephritis from various series

	Thailand (66)	United kingdom(51)	Dilima(13)	New Delhi, India(44)	Natal, South Africa(14)
ESRD	50%	50%	75%	45%	45%
Death	-	10%	-	-	16.6 %

Some of the determinants that have been described as being associated with adverse renal outcome include the percentage of fibro-cellular crescents, the extent of global sclerosis, the time to treatment, the need for dialysis at presentation, the presence of oliguria as well as the presence of hypertension and nephrotic range proteinuria during the follow up period (25, 44, 46, 65, 66). One study noted that the prognosis of renal survival after post infectious rapidly progressive glomerulonephritis was particularly poor(67)

Thus, though crescentic glomerulonephritis is uncommon in the paediatric age group it is an important and preventable cause of end stage renal disease. The prevalence of the three immune-pathologic sub

types is influenced by factors such as age, ethnicity and geography. And as with many other disease conditions there is very little data describing crescentic glomerulonephritis with regards aetiology and outcome in children in an African setting. This study was an attempt to help bridge that knowledge gap and it is hoped that the data collected will both help improve patient care as well as act to guide future research efforts.

CHAPTER 3: MATERIALS AND METHODS

This study was conducted in an attempt to determine the aetiology, clinical features and outcomes of crescentic glomerulonephritis in a quaternary care hospital in Cape Town. This chapter outlines the study design, the data collection tools, the ethical considerations and the statistical methods used to analyse the collected data.

3.1 The study site

(68)

The study site is located in South Africa's fourth largest province, the Western Cape. The province has an estimated population of 5,223,908 with people of mixed race accounting for 50.2%, Black people accounting for 30.1%, White people accounting for 18.4% and Indian/Asian accounting for 1.3%. Two million of the population are aged below 20 years (68).

The study site was the Red Cross War Memorial Children's Hospital which was established in 1956 and is the only paediatric hospital in the sub-region. The hospital provides quaternary level care in all major specialties, to children referred from across the breadth of South Africa and even beyond the country's borders.

Children with renal problems are cared for in a consultant lead 12 bed nephrology unit which has the capacity to provide all forms of renal replacement therapy and has ready access to critical care, laboratory, radiology and pathology support services.

3.2 Study design and population

The study was a retrospective folder review and the population consisted of native kidney biopsies of children below 18 years that had been performed at the Red Cross War Memorial Children's Hospital between 2004 and July 2015.

3.3 Selection of Subjects

The results of renal biopsies conducted in children at the Red Cross War Memorial Children's Hospital between 2004 and July 2015 were reviewed and all those biopsy results meeting the case definition were included in the study.

3.4 Case definition

In this study a child was considered to have crescentic glomerulonephritis if they had crescents in at least 50% of glomeruli as determined in their original biopsy report.

3.5 Inclusion criteria

To be included in the study the following criteria had to be met:

- Child aged 18 years and below
- Child meeting the criteria for case definition
- Available case records(notes and/or laboratory reports and/or treatment charts)

3.6 Clinical Assessment

The record of all renal biopsies conducted in the relevant study period were reviewed. Once the children who were diagnosed as having crescentic glomerulonephritis were identified, their clinical records were assessed. The demographic data, clinical features, laboratory and pathological characteristics were then recorded in the data collection tool (Appendix A).

3.7 Data Management and Analysis

3.7.1. Dependent (outcome) variables:

The study dependant variables included the following:

- biopsy results
- aetiology
- outcome of renal function

3.7.2 Independent (predictor) variables

The study independent variables included the following:

- Age
- Sex

- Ethnicity – as self-identified by patient
- Region of origin i.e. province
- Duration of illness before diagnosis i.e. time elapsed between onset of symptoms and presentation.
- Number of rooms in home
- Estimated monthly family income
- Season
- Serologic markers(anti-nuclear antibody (ANA), anti- neutrophil cytoplasmic antibody (ANCA), anti-double stranded DNA (dsDNA) and serum complement levels , anti-streptolysin O titres (ASOT) , Anti DNase B),RPR.
- Admission blood pressure- A patient was considered to be hypertensive if their arterial blood pressure was greater than the 95 percentile for age, sex and height according to the paediatric hypertension percentile charts (69).
- Biochemical parameters (serum creatinine and estimated glomerular filtration rate (eGFR) at diagnosis and at last follow-up, highest creatinine, urine examination, urine protein level). The estimated GFR was calculated using the Schwartz formula(70).
- Human Immune Virus (HIV) serological status
- Duration of follow-up
- Therapeutic regime given(the number of doses of methyl-prednisone or cyclophosphamide)
- The clinical outcome (patient death, chronic kidney disease (CKD) stage, renal replacement therapy at one year and at last follow up).The Kidney Disease Outcome Quality Initiative (K/DOQI) classification of chronic kidney disease was used as shown in table 5 below(71).

Table 6: K/DOQI stages of chronic kidney disease(71)

Chronic Kidney Disease Stage	Glomerular filtration rate (ml/min/1.73m ²)
1	≥90
2	60-89
3	30-59
4	15-29
5	< 15

- The pathological characteristics of the patient biopsy as reported in the original report (total number of glomeruli, proportion of glomeruli with fibro-cellular/cellular/fibrous crescents, the degree of interstitial fibrosis and tubular atrophy).

3.7.3 Data management and analysis

A secondary data base was created in SPSS version 22. The baseline information on the patients was obtained from the data collection tool. We analysed the data using SPSS. Simple proportions were used to calculate the incidence of crescentic glomerulonephritis in the Red Cross renal biopsy series. Simple proportions were also used to analyse categorical data .Continuous variables were analysed by calculating means. The association of the categorical variables to renal outcome were assessed by using the Fischer’s exact test because in almost all the analyses there were some cells that contained less than 5 items due to a small sample size. A p value < 0.05 was considered as significant.

3.8 Ethical Considerations

The study was conducted in compliance with the South African Guideline for Good Clinical Practice and the Medical Research Council Ethical guidelines. Approvals to conduct this study were obtained from the University of Cape Town Department of Paediatrics and Child Health Research Committee, the University of Cape Town Ethics Review Board and the Red Cross Children’s Hospital management.

1. Research procedure and data collection

As outlined above.

2. Outputs

The findings of the study will be presented as part of a thesis to the University of Cape Town and may be published in a peer reviewed journal.

3. Anticipated gain in scientific knowledge

There are only a handful of studies that have been carried out to study crescentic glomerulonephritis in Africa to try and characterise the aetiology and outcomes of this entity. Thus the results from this study will help to fill this knowledge gap and perhaps give clinicians information that may be useful when managing children with crescentic glomerulonephritis in this population.

4. Description of risks and benefits

As this was a retrospective study there were no additional risks to the patients.

There was no direct benefits to the patients included in the study. However, the study has provided information that may give new insights into the common aetiologies and usual outcomes of crescentic glomerulonephritis in children in this population. This knowledge may assist clinicians managing future patients who present with crescentic glomerulonephritis.

5. Privacy and Confidentiality

Once patient information had been entered into the study data base only a coded study number was used as an identifier. The identifying details were kept in a separate file with access restricted to only the investigators.

6. Reimbursement for participation

Not applicable as this was a review of patient records.

7. Emergency care and insurance for research-related injuries

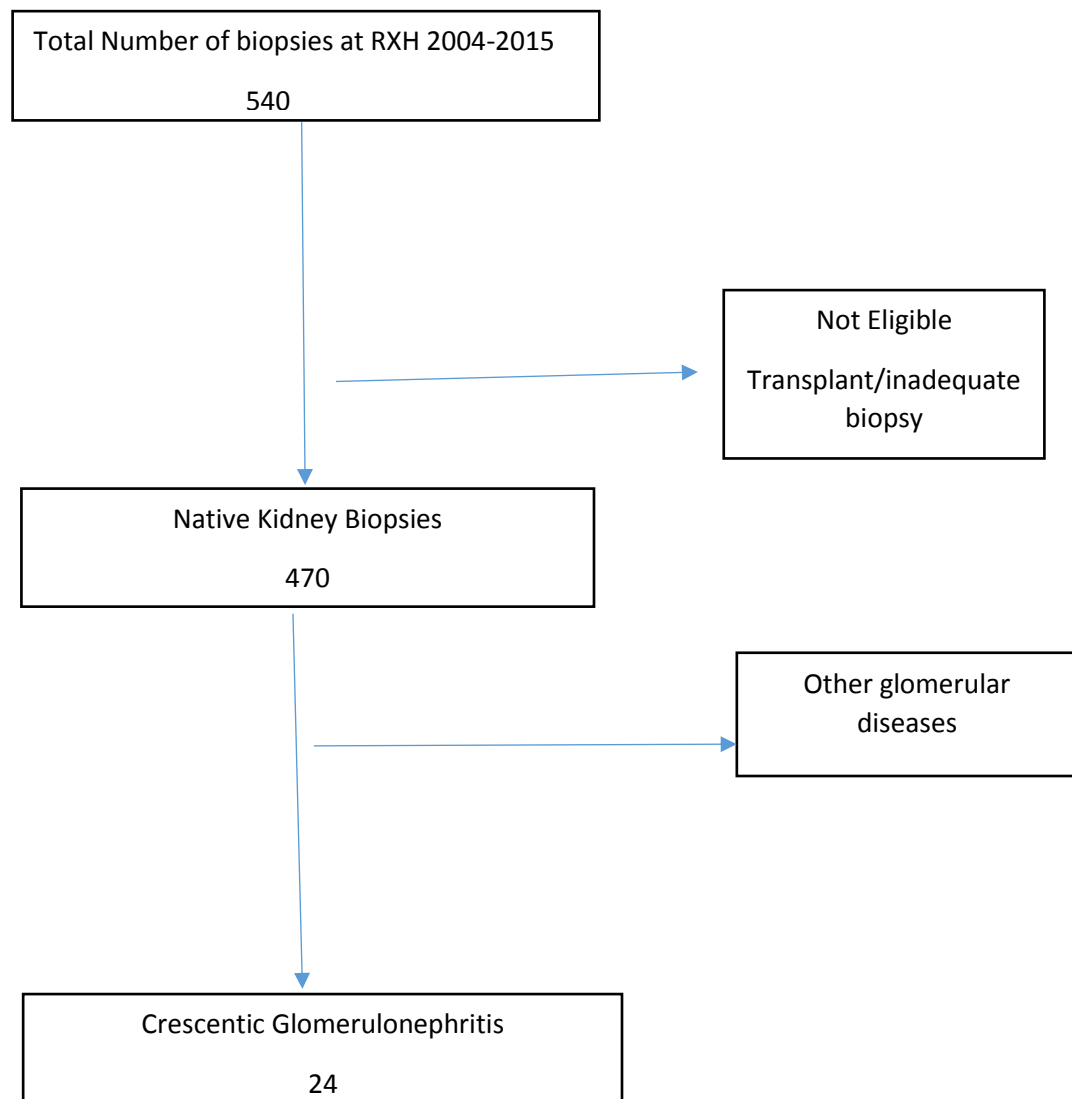
This did not apply as this was a review of patient records

CHAPTER 4: RESULTS

4.0 Recruitment of study subjects and incidence of crescentic glomerulonephritis

Figure 5. is a flow chart that illustrates how the selection of the subjects was conducted. A total of 540 renal biopsies were performed at the Red Cross children's hospital between 2004 and June 2015. Of these, transplant kidney biopsies and biopsies with insufficient material were excluded from the analysis. Thus a total of 470 native kidney biopsy results were assessed. Of these, 24 biopsies showed crescentic glomerulonephritis thus accounting for an incidence of 5.1% in this biopsy series.

Fig 5. Flow chart showing selection of study subjects



4.1 Demographic and Baseline Characteristics of the subjects

Table 7. Summarizes the demographic and baseline characteristics of the subjects. The children's mean age was 8.3+/- 3.4 year with an age range of 1 year to 14 years. Most of the children (14, 58.5%) were aged 5 to 10 years, while 7(29.2%) were aged greater than 10 years and 3(12.5%) were younger than five years old. The predominant sex of the children was male (14, 58.3%) thus giving a male to female ratio of 1.4.

The race of the subjects was defined as the race that the subject had identified themselves as belonging to as indicated in their hospital records. This information was unavailable for 17 (62%) of the children. The racial distribution of the remaining children was black 6 (25%), Asian 1 (4%) and mixed race 1 (4%).

We were unable to determine the living conditions of most of the children from the retrospective inspection of the records therefore this was left out of the analysis. Similarly the socio-economic status of families was difficult to establish in a meaningful way.

The Year was divided into four quarters roughly corresponding to the four seasons experienced in the Western Cape Province. As illustrated in Fig 6, most of the children (11, 45.8%) presented in the winter, followed by those that presented in the spring (7, 29.2%), summer (5, 20.8%) and autumn (1, 4.1%).

The majority of the subjects (13, 58.3%) had been referred from health facilities within the Western Cape Province. Of these children coming from within the Western Cape Province, 10 were referred from health centres within the city of Cape Town. In total 10 children came from outside the province. Of these 2 were foreign nationals (Namibia, Zimbabwe) while the remaining 8 were referred from the Eastern Cape Province. Due to missing record the referral health facility of one patient was undetermined.

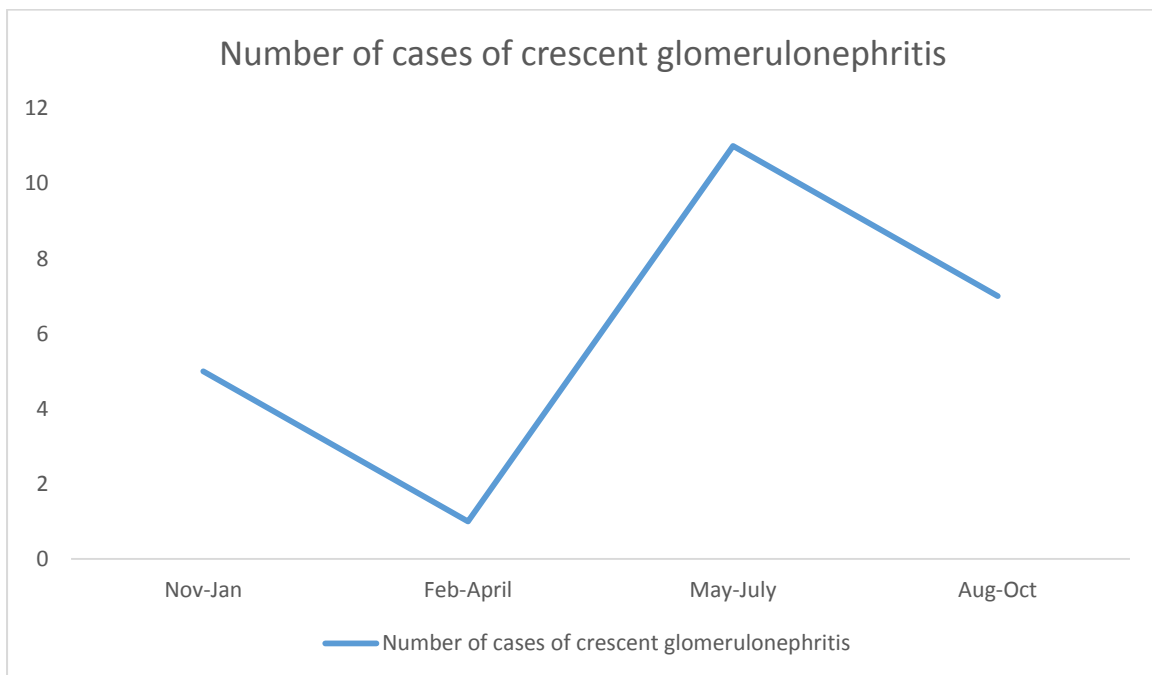
Table 7: Demographic characteristics of the participants

Patient No.	Aetiology	Age	Sex	Month of presentation	place of origin
1	Post infectious	5	Male	July	CTC
2	SLE	10	Female	May	OWCP
3	Post infectious	3	Female	October	CTC
4	p-ANCA renal limited vasculitis	11	Female	November	OWCP
5	HSP	11	Female	September	CTC
6	p-ANCA vasculitis	5	Female	December	WCP
7	Post streptococcal	6	Male	May	OWCP
8	IC-idiopathic	8	Male	January	OWCP
9	IC-idiopathic	14	Male	March	OWCP
10	IgA nephropathy	12	Male	November	WCP
11	Post-streptococcal	9	Male	August	OWCP
12	CGN-unspecified	12	Female	September	WCP
13	IC-idiopathic	13	Female	September	CTC
14	IC-idiopathic	4	Male	May	OWCP
15	Mesangiocapillary GN	5	Male	June	CTC
16	Post-streptococcal	10	Male	November	CTC
17	Post-streptococcal	6	Male	September	CTC
18	Post-streptococcal	9	Male	May	OWCP
19	Post-streptococcal	7	Female	June	OWCP
20	Post-streptococcal	10	Male	July	CTC
21	Post infectious	10	Male	August	CTC
22	Pauci-immune	13	Male	May	-
23	CGN- unspecified	6	Female	June	OWCP
24	Post-streptococcal	12	Female	May	CTC

CTC-Cape Town City, WCP- Western Cape Province, OWCP-Outside Western Cape Province, IC-immune-complex

CGN-crescentic glomerulonephritis

Fig.6 – Seasonal distribution of the crescentic glomerulonephritis cases



4.2 Clinical and Laboratory Characteristics of the subjects

Table 8. Shows the initial anthropometric measurements of the subjects. We were only able to find records of the initial anthropometric measurements for 20 out of all the 24 children who presented with crescentic glomerulonephritis during the study period. World Health Organisation (WHO) growth charts were used to evaluate the children's anthropometric measurements(72). The following categories are recognised based on these charts:

- Overweight: greater than +1 standard deviation
- Obesity: greater than + 2 standard deviations
- Thinness: less than -2 standard deviations
- Severe thinness: less than -3 standard deviations

None of the children was found to be stunted at presentation. Only 2 children were obese while only one child had severe thinness based on body mass index (BMI).

Table 8: Anthropometric characteristics of the study subjects

Patient No.	Age	Sex	Weight [Kg]	Height(z-score)	BMI (z-score)
1	5	Male	22.9	1.2 (+1)	17 (< +1)
2	10	Female	36	1.3 (< -1)	20 (< +2)
3	3	Female	12.9	0.93	15 (<median)
4	11	Female	41.1	1.4 (<med)	21 (< +2)
5	11	Female	34.4	1.5 (<+1)	16 (<median)
6	5	Female	24.7	1.0 (-1)	23 (> +3)
7	6	Male	23.4	1.2 (+1)	17 (< +2)
8	8	Male	24.4	1.3 (+1)	15 (median)
9	14	Male	50	1.5 (-2)	23 (< +2)
10	12	Male	28.4	1.5 (<med)	14 (< -3)
11	9	Male	25	1.2 (<-1)	17 (< +2)
12	12	Female	40	1.5 (med)	18 (median)
13	13	Female	47.3	1.5 (<med)	20 (< +1)
14	4	Male	21.9	1.0	22 (> +3)
15	5	Male	10	1.0 (-2)	10 (< -3)
16	10	Male	45.5	1.4 (<+1)	23 (< +3)
17	6	Male	18.6	1.2(<+1)	13 (-1)
18	9	Male	32.6	1.4(<+1)	18 (< +2)
19	7	Female	28.1	1.3(<+1)	17 (+1)
20	10	Male	32.8	1.3(<med)	19 (< +2)
21	10	Male	-	-	-
22	13	Male	-	-	-
23	6	Female	-	-	-
24	12	Female	-	-	-

Table 9. Tabulates selected clinical and laboratory characteristics of the study subjects. The mean number of days that symptoms had been present prior to presentation to Red Cross Hospital was 21.4 +/- 40.6 days. The range for the time it took for patients to present was 1 day to 180 days. The time that lapsed between onset of symptoms and presentation to Red Cross Hospital was categorised into three blocks: a) symptoms present for less than a week prior to presentation b) symptoms present between one and two weeks prior to presentation and c) symptoms present for more than two weeks prior to presentation to Red Cross Hospital. Symptoms had been present for less than a week in 6(30%), present between one to two weeks in 6(30%) and for greater than two weeks in another 6(30%). Records were only available for 18 children.

Table 9: Patient clinical characteristics at initial presentation

Patient [N=18]	Time between symptom onset and presentation to RXCH	Admission BP	Macroscopic Haematuria	Proteinuria g/mmol	Hb g/dl	Initial Creatinine micromoles/l	eGFR ml/min/1.73 m ²
1	3	130/100	Yes	-	10.6	534	8.7
2	28	118/60	No	1.1	5.4	1361	3.9
3	7	130/99	No	4.35	4.2	200	18.6
4	10	195/135	No	-	7.1	1381	4.0
5	180	135/60	Yes	1.0	10.6	95	62
6	28	115/80	No	0.73	7.3	273	15.2
7	2	140/75	Yes	0.20	5.9	1195	4.0
8	-	-	Yes	0.45	6.6	1108	4.6
9	14	160/100	Yes	0.56	10	218	27.2
10	21	133/73	Yes	0.69	11.1	155	37.4
11	7	126/89	Yes	1.36	8.1	1174	4.2
12	14	130/50	No	0.30	4.0	762	8.0
13	28	115/90	Yes	2.89	-	262	23.4
14	3	150/100	Yes	1.90	7.9	383	10.7
16	7	190/123	No	-	8.6	306	18.0
17	21	140/95	Yes	-	10	80	58.6
19	6	119/90	No	-	9.7	207	9.9
20	1	150/90	No	-	12		28.9

RXCH- Red Cross Memorial Childrens Hospital

The records of 19 children were available for assessment of blood pressure at presentation. Seventeen (90%) of the children were hypertensive while 2(10%) were not. Macroscopic haematuria was present in 10 (55.5%) of these children. Records on macroscopic haematuria were only available for 18 of the children

Records for the evaluation of proteinuria were available in 14 of the subjects. The mean urine protein to creatinine ratio was 1.1g/mmol +/- 1.2 g/mmol with a range of 0.1-4.3 g/mmol. Nephrotic range proteinuria [>0.2 g/mmol] was present in 11(80%) of the children.

Figure 7 below illustrates the frequency with which various clinical features occurred in the study subjects. Full records were only available for 18 of the children. Of note is the high prevalence of oedema (17, 94%), anaemia (15, 88%) and hyperphosphatemia (9, 50%). In contrast only 1 patient had haemoptysis, and only 5(30%) had hyponatraemia.

Figure 7. Frequency of various patient clinical characteristics

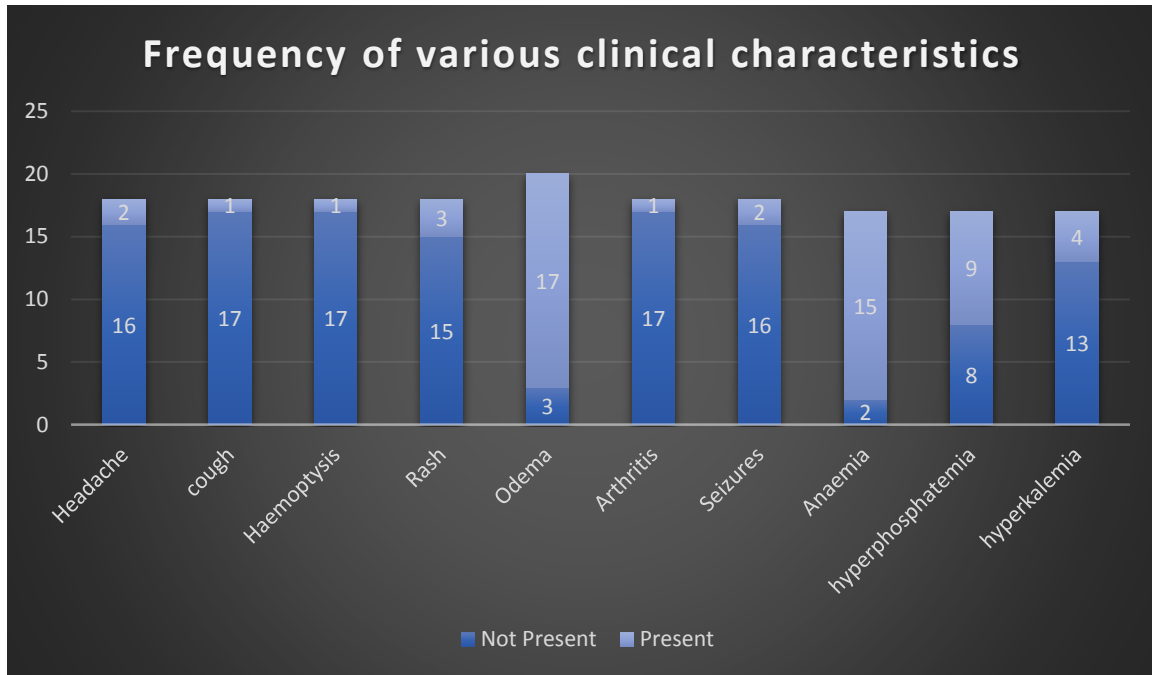


Table 10 below shows results of the initial renal function in the study subjects. A record was only available for 18 of the subjects. The mean urea was 33.3 mmol/l +/- 17.4 mmol/l while the mean serum creatinine at presentation was 566.8 +/- 465 micromoles/l. The range for serum creatinine was 80-1381 micromoles /l. The mean estimated GFR at initial presentation was 18 ml/min/1.73m² +/- 17.9 ml/min/1.73m² with a range of 3.9 to 62 ml/min/1.73 m² .Half (9) of the children had an initial estimated GFR below 10ml/min/1.73 m² .

Table 10: Summary of patient renal function at initial presentation

Parameter N=18	Minimum value	Maximum value	Mean Value	Standard Deviation
Serum Urea mmol/L	9	65	33.3	17.4
Serum Creatinine micromole/l	80	1381	566.8	465
Estimated GFR ml/min/1.73m ²	3.9	62	18	17.9

Table 11: Result of various serological immune markers in the subjects

<i>Patient No.</i>	<i>Aetiology</i>	<i>C3</i>	<i>C4</i>	<i>ASOT</i>	<i>Anti-DNase B</i>	<i>ANCA</i>	<i>Anti-GBM</i>	<i>Anti-dsDNA</i>
1	Post infectious	Low	Normal	High	High	Not done	Not done	Not Done
2	SLE	Low	Low	Negative	Not Done	High (p)	Negative	High
3	Post infectious	Low	Normal	Not done	Negative	Negative	Negative	Negative
4	p-ANCA renal limited vasculitis	Low	Low	Not done	Not Done	High (p)	Negative	Not Done
5	HSP	Normal	Normal	Negative	Negative	Negative	Not done	Negative
6	p-ANCA vasculitis	Normal	Normal	Normal	Negative	Negative	Negative	Negative
7	Post streptococcal	Low	Normal	High	High	Not Done	Not Done	Negative
8	IC-idiopathic	Normal	Normal	Negative	High	Not done	Not Done	Negative
9	IC-idiopathic	Normal	Normal	Negative	Negative	Negative	Not Done	Negative
10	IgA nephropathy	Normal	Normal	Negative	Negative	Negative	Not Done	Negative
11	Post-streptococcal	Low	Normal	High	High	Not done	Not Done	Not done
12	CGN-unspecified	Normal	Normal	Negative	Negative	Negative	Negative	Not done
13	IC-idiopathic	Normal	Low	Negative	Negative	Not done	Not Done	Negative
14	IC-idiopathic	Normal	Normal	?	?	Negative	Not done	Negative
16	Post-streptococcal	Normal	Normal	Negative	High	Negative	Negative	Not done
17	Post-streptococcal	Low	Normal	Negative	High	Negative	Negative	Negative
19	post-streptococcal	Normal	Normal	High	High	Not done	Not done	Negative
20	Post-streptococcal	normal	Normal	normal	High	Not done	Not done	Negative

4.3 Histopathology findings

During the study period 2004-2015, most (80%) of the patients identified as having crescentic glomerulonephritis had the immune-complex mediated sub-type. Over half (11, 60%) of these children had a suspected post-infectious aetiology.

Table 12: Aetiology of crescentic glomerulonephritis- RXH biopsy series

Classification (N=24)	Aetiology	Number (%)
Immune-complex CGN	SLE	1 (4.1)
	Post-infectious	11 (46)
	HSP/IgA	2 (8.3)
	Mesangiocapillary	1 (4.1)
	Idiopathic	4 (16.6)
Pauci-immune CGN	ANCA vasculitis	2 (8.3)
Anti-GBM CGN	-	0
CGN-unspecified	-	3 (12.5)

Figure 8 shows below the number of cases being diagnosed with CGN has remained constant over the years. The average number of cases per year is two cases per year.

Figure 8. Number of CGN cases by year 2004-2014

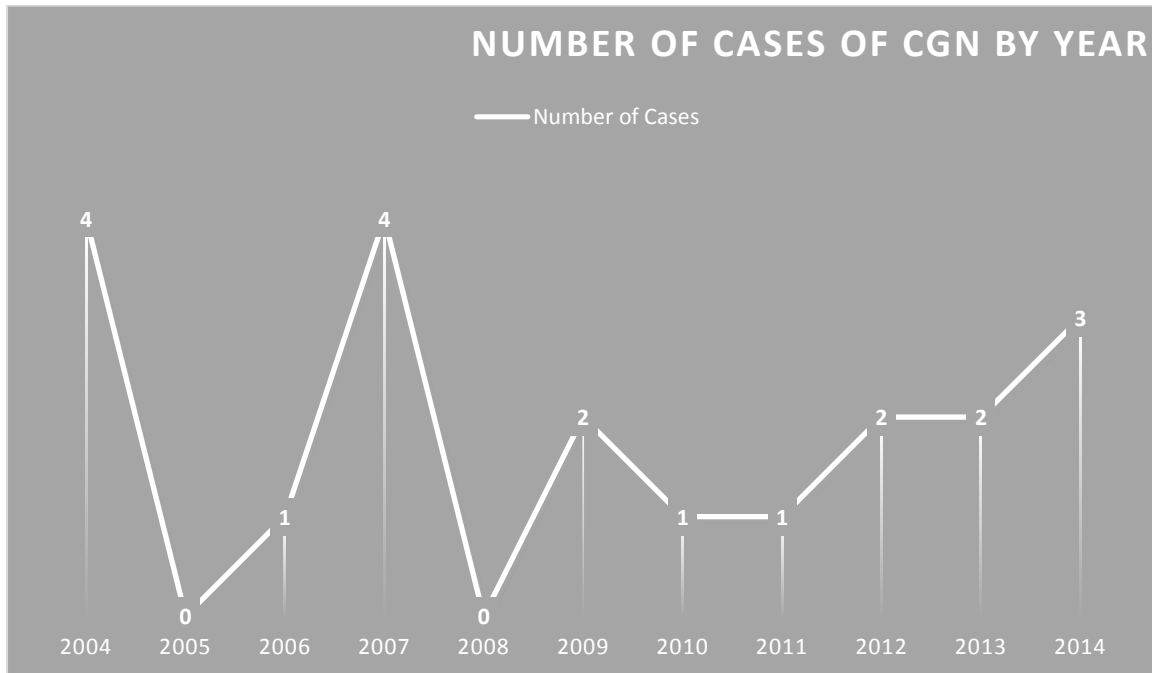


Table 13: Histopathology findings

Patient No.	Aetiology	Light Microscope				Immunofluorescence			
		Number of glomeruli	Percentage of Crescents	Type of crescents	Sclerosis?	IgA	IgG	IgM	C3
[N=24]									
1	Post infection								
2	SLE	12	100%	FC	No	1+	0	<1+	3+
3	Post infection	7	50%	C	No	0	1+	0	1+
4	ANCA-vasculitis	14	50%	F/FC	Yes	-	-	-	-
5	HSP	12	80%	C	No	3+	0	1- 2+	1+
6	ANCA-vasculitis	-	80%	-	No	0	2+	1+	2+
7	Post-strep	27	100%	C	No	-	<1+	-	3+
8	IC-idiopathic	21	100%	F	Yes	+/-	0	3+	3+
9	IC-idiopathic	20	Most	FC	Yes	2+	0	2+	2+
10	IgA N	12	Most	C/FC	No	2+	0	1+	1/2+
11	Post-strep	21	100%	F/FC	No	0	0	0	2+
12	CGN-unspecified	-	100%	FC	Yes	0	1+	2+	1+
13	IC-idiopathic	20	>50%	FC	Yes	3+	2+	1- 2+	1+
14	IC-idiopathic	14	Most	C,FC,F	No	0	0	2+	2+
15	Mesangiocapillary GN	5	80%	C	No	3+	3+	3+	3+
16	Post-strep	6	70%	FC	No	0	1+	2+	1+
17	Post-strep	8	50%	C	Yes	0	0	0	2-3+
18	Post-strep	23	Most	C	Yes	-	-	-	-
19	Post-strep	18	80%	C	Yes	+/-	+/-	+/-	4+
20	Post-strep	10	50%	FC	Yes	3+	0	0	2+
21	Post-strep	23	>50 %	C	Yes	0	0	0	0
22	Pauci-immune	29	>50%	C,FC,F	No	0	0	2+	1-2+
23	CGN-unspecified	14	80%	C	Yes	0	0	2+	1-2+
24	Post-strep	22	90%	C	Yes	1+	0	3+	3+

ANCA- anti neutrophil cytoplasmic antibodies, CGN-crescentic glomerulonephritis IC-immune complex GN, GN-glomerulonephritis,

4.5 Treatment

Records of treatment with cyclophosphamide were available for only 18 children. The unit uses a dose of 500mg per meter square of body surface area. Of the 18 children whose records were available,

9(50%) received a single dose, 1(6%) received two doses, 5(28%) received three doses while 1 child received more than three doses of cyclophosphamide. Two of the children did not receive cyclophosphamide.

Records of treatment with methyl-prednisone 10 mg per kilogram body weight pulses was available for 19 patients. Only one child did not receive any methyl-prednisone. Of the patients who received methyl-prednisone the vast majority (15, 79%) were given three doses, only 2(10.5%) got more than three doses and 1(5%) got one dose only.

Records regarding initial treatment with dialysis were available for 17 children. Of these 9 (52%) required dialysis at initial presentation while 8 did not. Only three (15%) of the children received plasmapheresis and all of these had more than five sessions. Of the three children who received plasmapheresis, one had systemic lupus erythematosus while two had ANCA associated vasculitis.

4.6 Outcome

Table 14. gives the renal outcome at one year. The mean follow up period was 29.9 +/- 27.3 months with a range of 0.5 to 72 months. A total of 24 children with CGN were seen during the period under review. Records were only available for 18 of these children. Of the 18 patients whose outcome records were available, 8(44.4%) were followed up less than 3 months, 7(38.9%) were followed up between 1 to 5 years and only 3(16.7%) were followed up more than 5 years.

Table 14: Renal outcome at one year

		Frequency [N=24]	Percentage [%]
Normal Renal Function		4	16.7
CKD stage II-IV		2	8.3
Dialysis dependant		4	16.7
Renal transplant		2	8.3
Followed < 1 year because:	not suitable for transplant	2	33.3
	Transfer out	1	
	Abscond	3	
	Death	1	
	Diagnosed 2015	1	
Missing data/unknown fate		4	16.7

Of all the 24 children, the fate of 4 is unknown as we could not locate their records. Four (16.7%) of the subjects had normal renal function at one year after first presenting while 2(8.3%) still had chronic kidney disease after a year of follow up. Furthermore 4 (16.7%) of the patients were dialysis dependant while 2(16.7%) had been transplanted at one year post initial presentation. Sadly one child (4.1%) had died. Most of the patients on dialysis were on peritoneal dialysis. Of the 2 patients with chronic kidney disease one was CKD stage 5 while the other was CKD stage 2.

There were 8 (33.3%) patients who were followed up for less than a year. Of these 2 [patient 11, patient 12] were recognised to have ESRD at initial admission but upon assessment by the transplant committee were found to be unsuitable for renal replacement therapy as per hospital protocol. They were sent home for palliative care and a search of the health system database showed that they have had no further contact with the Western Cape Province health care system. They are presumed deceased. Of the remaining 6 patients that were followed up for less than a year three were lost to follow up while one was referred back to another hospital and one died before one year had elapsed from the time of first presentation. The patient who was diagnosed in 2015 was already dialysis dependant from the start.

Thus of the 13 patients who had been diagnosed with rapidly progressive glomerulonephritis and whose records were available, 10 (77%) had a poor renal or patient outcome. Based on records of last contact with the patients at least five (20%) of the children are known to have received a renal transplant.

4.7 Association of various patient characteristics to renal outcome

The sample size was small therefore an exact Fischer test was used to measure the strength of association of various patient characteristics to the renal outcome. A p value of 0.05 was considered as significant. The results showed two sided p values: anaemia 1.0, macroscopic haematuria 1.0, hypertension 0.25, sex 0.51 and nephrotic range proteinuria 1.0. Thus none had a statistically significant association. This is illustrated in table 14 below.

Table 15: Association of various patient characteristics to renal outcome

Factor	Present or not	Good outcome	Poor Outcome	p-value*
Sex	Male	1	7	0.51
	Female	2	3	
Age	< 5 years	0	1	0.63
	5-10 years	1	6	
	>10 years	2	3	
Referring Hospital	CTC	0	2	0.07
	WCP	3	2	
	OWCP	0	6	
Time between start of symptoms and presentation	< 1 week	0	4	0.07
	1-2 weeks	0	3	
	>2 weeks	3	1	
Hypertension	Yes	2	9	0.25
	No	1	0	
Oedema	Yes	1	9	0.11
	No	2	1	
Proteinuria	Non-nephrotic Range	0	2	1.00
	Nephrotic Range	3	5	
Macroscopic Haematuria	Yes	2	5	1.00
	No	1	4	

*The Fischer exact test was used to measure strength of association

CHAPTER 5: DISCUSSION

5.0 Incidence of crescentic glomerulonephritis

This 10 year retrospective study was carried out to characterise the aetiology, clinical features and outcomes of crescentic glomerulonephritis in children presenting to a quaternary health care facility in Cape Town. A total of 24 cases were identified out of 470 native kidney biopsies performed during the study period therefore giving an incidence of 5.1 % which is similar to what has been reported elsewhere(14, 44). Gupta *et al* however reported a much lower incidence of 2.3 % of crescentic glomerulonephritis in their series of native kidney biopsies. Of note though is the fact that their study had included both children and adults and only covered a period of two years. The influence that age might have on the frequency of crescentic glomerulonephritis is further illustrated by an adult study performed in Cape Town but which showed a higher incidence of 11.3 percent over a ten year period(73). The number of cases of crescentic glomerulonephritis presenting per year in our series has not changed significantly with about two children with this entity being seen annually.

5.1 Demographic and baseline characteristics of the subjects

The mean age of the patients with crescentic glomerulonephritis was 8.3 +/- 3.4 years. The majority of these 11(45%) were aged 10 years and above. These findings are consistent with what has been reported by other investigators (25, 45, 46, 67). This age group is also the most commonly affected by classic post-streptococcal glomerulonephritis which accounts for quite a sizeable proportion [8 , 33%] of the children in our series(21). The mean age of the children described by Dewan *et al* however was higher at 12.2 years even though like us they had a majority of their patients presenting with immune-complex glomerulonephritis which was post-infectious in aetiology.

The majority(58%) of the patients were boys and most presented in winter or spring (45) .We hypothesize that perhaps this could be because a sizeable number of them had a post-infectious aetiology and it is known that post-streptococcal glomerulonephritis is more common in boys. Other series however have reported a predominance of girls (25, 67).

We were unable to collect data on the ethnicity of the subjects because this information was not recorded in the patient notes. Since a significant number of the children had post-infectious glomerulonephritis it would have been interesting to determine whether what has been observed by other investigators concerning the higher frequency of post-streptococcal glomerulonephritis in black patients is true in this population also (13, 14, 52) . Similarly we were unable to collect data on patient living conditions because this information was not recorded in the notes for the majority of the children.

5.2 Clinical and laboratory characteristics of the patients

The mean time that symptoms had been present before presenting to the Red Cross children's Hospital was 21.4 days. In fact 60 % of the children were referred within two weeks of falling ill. This is a much shorter period than what was reported by Dewan *et al* in India who had a mean of 2.4 months of symptoms having been present before the patients were referred. We attribute this relatively shorter time period in our cohort to the fact that the majority of the patients were residents of the Western Cape Province which has a good referral and patient transport system. It could also imply that primary health care providers in the catchment area of the hospital have been made aware of this entity.

None of the children in the cohort were stunted based on height for age while two of the children were obese based on body mass index. As these were admission weights it is possible that the presence of fluid overload may have distorted the results. On the other hand the absence of stunting seems to support the fact that in this cohort crescentic glomerulonephritis will often present as an acute illness. The most common presenting clinical features were oedema (94%), hypertension (90%), nephrotic range proteinuria (80%), anaemia (88%), gross haematuria (60%) and reduced renal function [GFR<15 ml/1.73m² /min in 50 % of those assessed]. This high prevalence of hypertension in this patient population has also been reported in other series (21, 44). In contrast the prevalence of hypertension seems to be lower in adults as illustrated by the Natal series that had included both children and adults(14). In fact a much earlier study by Cunningham *et al* also alluded to this (21). Could a possible explanation for this be the higher frequency of oliguria in children? The South-west Paediatric Nephrology study however, had a lower prevalence of hypertension (50%) and oedema in their cohort of 50 children(65).

Interestingly many of these patients had anaemia. This could reflect a dilutional anaemia secondary to fluid overload. Cunningham *et al* also described similar findings in their cohort of patients. They found that 9 out of 13 of their patients had a haemoglobin less than 9 g/dl. They concluded that the anaemia was out of proportion to the degree of fluid overload and that the anaemia was more severe in children with a post-infectious aetiology. They postulated that anaemia in a patient with crescentic glomerulonephritis made a post infectious aetiology more likely.

Only 3 of the 13 children for whom records were available presented with hyperkalaemia despite many of the children having severe renal failure. We attribute this to the fact that many of these children had already begun receiving symptomatic management of renal failure prior to being referred.

Of the 20 patients who had complement studies recorded in their notes, 7 had a low complement. Of these 1 had SLE, 1 had ANCA vasculitis and the rest were thought to have post-infectious crescentic glomerulonephritis. Of note is the fact that three of the children with post-streptococcal glomerulonephritis had a normal C3 at presentation. The diagnosis in this case was based on suggestive biopsy findings in the presence of anti-DNase B titres in the case of patient 16, 17 and 20 while it was

based on both raised ASOT and anti-DNase B in patient 19. Patient number 2 was diagnosed with SLE but surprisingly had raised ANCA titres. This has been described in the literature with suggestions that in cases of immune complex glomerulonephritis, the presence of ANCA antibodies contributes to the development of severe glomerular injury(2). Patient number 2 was dialysis dependant at the last contact.

Similar to reports in the literature about the severity of renal dysfunction at initial presentation, the mean estimated glomerular filtration rate was 18 ml/1.73 m² /min. Fifty percent of the patients had an estimated glomerular filtration rate less than 10 ml/1.73m²/min at initial presentation.

5.3. Histopathology

Of the 24 patients with crescentic glomerulonephritis, 11(46%) had a suspected post-infectious aetiology mostly post-streptococcal, 8(33%). Of interest though, patient 19 had been followed up earlier and prior to presentation with recurrent pyelonephritis that had been associated with some renal dysfunction. These findings are similar to earlier reports from other parts of South Africa in both children and adults as well as reports from India and the United Kingdom (12-14, 44, 46). Interestingly other reports from India indicate that immune-complex crescentic glomerulonephritis is less prevalent than the Pauci-immune subtype (25, 45, 74) . This was also the case in a nationwide survey of rapidly progressive glomerulonephritis that was carried out in Japan(15). One must bear in mind though the fact that the Japanese study included both adults and children which clearly would then distort the findings due to the higher prevalence of Pauci-immune crescentic glomerulonephritis in adults. An intriguing fact emerged from a report from northern India, which indicated that over time there had been a reduction in the incidence of immune-complex crescentic glomerulonephritis(45). This change in trends over time was also reported in the northern Indian study cited above. In the case of the northern Indian study there was a concomitant reduction in the cases of post-infectious glomerulonephritis which could account for this reduction. In fact numerous review articles have reported that there has been a general reduction in post-streptococcal glomerulonephritis in various regions but especially in industrialized nations (48-50). Based on the foregoing discussion it appears that post-infectious glomerulonephritis remains an important cause of crescentic glomerulonephritis in our cohort of patients contrary to what is being reported in other parts of the world.

One question that begs an answer is that of what factors make certain individuals susceptible to severe morbidity from a usually benign disease. Like in most conditions investigators postulate that the genetics of the pathogen and the host may have a role to play in this. Investigators in empirical research have described genetic alterations in mice models in which the mice became more susceptible to developing crescentic glomerulonephritis. One group elucidated the central role of IL-12p40 in inducing the Th1 response that leads to murine crescentic glomerulonephritis. (75-77). Whether these identified genes would play a similarly important role in humans remains to be seen.

The Western Cape is known to have a high prevalence of systemic lupus erythematosus (SLE) yet during the ten year period under study only one patient was diagnosed with crescentic lupus nephritis. Feng *et al* conducted a study at Peking University in China that included some adolescents. They investigated the factors associated with developing crescentic glomerulonephritis in a population of 327 patients with lupus nephritis. They described the presence of ANCA, high activity and chronicity scores on biopsy and high relapse rates as being significantly associated with crescentic lupus nephritis(78). It is interesting to note that our patient (number 2) did have raised ANCA titres. Though the exact role of ANCA has not been completely elucidated in such situations, their presence has been associated with more severe glomerular injury (2). Patient 2 was dialysis dependant at the last contact. It is also known that genetic, hormonal or ethnic factors could influence the severity and involvement of the kidney in SLE (79). This raises the question then of whether the lupus nephritis patients in this population may lack some pro-crescentic glomerulonephritis factors or alternatively whether they express protective factors.

Four of the children were diagnosed as having idiopathic immune-complex crescentic glomerulonephritis. Thus the biopsy and serology results did not meet the criteria for any primary renal or secondary disease. This is similar to reports by other investigators(14).

Not a single patient presented with anti-glomerular basement disease during the period under study. It is well known that this entity is rare in the paediatric age group. In fact in one centre in the United States of America a 25 year retrospective review of biopsies only revealed four cases(16).

Surprisingly, only two children were diagnosed as having had ANCA associated glomerulonephritis. Both of these were girls. A female predominance of ANCA associated crescentic glomerulonephritis has been described elsewhere(80). One patient had renal limited microscopic polyangiitis while the other patient presented with a vasculitic skin rash in addition to the renal dysfunction. Both girls were p-ANCA positive. This low incidence is consistent with literature reports from many developing countries. Generally reports from more industrialised counties in more recent times tend to show a higher prevalence of ANCA-associated glomerulonephritis.

Also of note are the three children that appeared to have had a Pauci-immune crescentic glomerulonephritis but with negative serological markers. The clinical records of patient number 12 show that both ANCA and anti-GBM antibodies were negative at presentation. This state of affairs has been described before and is referred to as ANCA-negative Pauci-immune crescentic glomerulonephritis(22). This entity is characterised by a paucity of immune deposits on biopsy and a persistent absence of ANCA antibodies in the serum and is said to be present in between 10 to 30 percent of patients who have Pauci-immune crescentic glomerulonephritis(2, 22). Patients reportedly have fewer extra-renal manifestations of disease and investigators postulate that neutrophils are central to the pathogenesis of the condition(22). Earlier Jeanette had postulated that this state of persistent ANCA

being seronegative could be explained by the fact that other types of antibodies against neutrophil cytoplasmic antigens were responsible for the glomerular damage but that they were undetectable with the assays in use then(1). Indeed some reports suggest that lysosomal membrane protein 2 [LAMP-2], a novel ANCA subtype, is present in almost all patients with active untreated ANCA associated vasculitis (81). However other studies such as the one by Roth et al in 2012 found that LAMP-2 was not prevalent in patients with ANCA vasculitis and thus did not support a mechanistic relationship between it and ANCA vasculitis(82). A recent review by Kain et al addresses this controversy(83).

Most of the patients had histology that showed greater than 80% involvement of glomeruli with most of the crescents being either cellular or fibro-cellular. It was quite surprising that most of the children had such a high percentage of involved glomeruli despite reasonably quick referral and treatment. A similar phenomena was reported by Almera-Borja *et al* in the Philippines(67). Numerous studies have shown an association between poor renal outcome and a higher percentage of involved glomeruli and a high percentage of fibro-cellular crescents (18, 44). Jardim *et al* had a cohort of 30 patients but they did not demonstrate a correlation between percentage of involved glomeruli and renal outcome(51). Perhaps further strategies need to be put in place to sensitize primary level health workers about this renal syndrome and the absolute need for speedy nephrology referral in order to improve renal outcomes.

5.4 Treatment

The health referral system in South Africa is quite well developed with far flung medical workers being able to discuss patients with a renal consultant telephonically. This was reflected by the fact that many of the children in our series had in fact already received a dose of methyl-prednisone even before arrival. All the children received at least three doses of steroids intravenously.

Of the 18 children whose treatment charts available, 16 received at least one dose of cyclophosphamide. Among these were 11 with a suspected post-infectious aetiology. There is no consensus on the use of cyclophosphamide in the management of post-infectious crescentic glomerulonephritis (31, 84, 85). Nine (52%) of the children received acute dialysis.

Most of the patients on dialysis were on peritoneal dialysis. Of the 3 patients with chronic kidney disease two of the children were stage 5 while one child was stage 2.

5.5 Renal outcome

Only 13 patients were followed up for at least one year. Because the Red Cross Children's Hospital is a referral centre, eight patients were sent back to their home centres for continued follow up after initial stabilisation. Of the children followed up for at least a year 10 (77 %) had what could be described as a poor outcome (CKD-3, dialysis dependant-3, transplant-2, death-1). This bleak outcome is similar to earlier studies from South Africa and other countries (12-14, 44, 51, 66) . And like the earlier studies from South Africa, a post-infectious aetiology continues to play a major role in the disease morbidity. Of the patients sent to other hospitals for continued, care 2 were recognized to be in need of long term renal replacement therapy but this had been withheld due to the children's poor social circumstances. This highlights the fact that in resource poor regions many children do not have access to the expensive renal replacement therapies and that as far as possible preventable causes of end stage renal disease, like crescentic glomerulonephritis should be recognised early and prompt treatment should be instituted(5, 8).

Thus by one year post the diagnosis of rapidly progressive glomerulonephritis 10 (77%) of the 13 children whose records were available can be said to have had a poor renal or patient outcome while as at the last contact with the patients at least five (20%) of the all the children in the cohort are known to have received a renal transplant.

5.6. Correlation of various patient characteristics with renal outcome

The sample size was small therefore an exact Fischer test was used to measure the strength of association of various patient characteristics to the renal outcome. A p value of 0.05 was considered as significant. The results showed two sided p values: anaemia 1.0, macroscopic haematuria 1.0, hypertension 0.25, sex 0.51 and nephrotic range proteinuria 1.0. Thus none had a statistically significant association. In contrast other series have noted a correlation between presence of hypertension, nephrotic range proteinuria and the percentage of fibrous crescents with poor renal survival.(18, 44, 46, 86). Other

In summary then, crescentic glomerulonephritis in our biopsy series occurs primarily in children older than five years who are predominantly boys with a post-infectious aetiology in a large proportion. The level of poor renal outcome remains inordinately high despite quite reasonable speed of referral as well as administration of widely accepted therapies. There remains a need to better characterise some of the factors that may result in severe glomerular damage in children with post-infectious glomerulonephritis in this population.

CHAPTER 6

6.0 Conclusion

We conclude that the incidence of crescentic glomerulonephritis in the series of native kidney biopsies performed at the Red Cross Children's Hospital in the period 2004 to June 2015 was 5.1%. The most common sub-type is the immune-complex crescentic glomerulonephritis with a post-infectious cause being the leading aetiology. Renal outcome remains poor in about 77 percent of affected children, despite reasonably quick referral of patients and appropriate therapy. More needs to be done to reduce the time that it takes for patients to be seen by a nephrologist even further. In addition we were unable to prove any statistically significant correlation between the patient clinical features and outcomes perhaps due to the small sample size involved. Going forward, it may be prudent to begin a multi-centre prospective national registry to try and collect standardized patient data as well as possibly explore any genetic or immunological factors that may contribute to this severe presentation of post-infectious glomerulonephritis.

6.1 Recommendations

1. Paediatric nephrologists need to increase the awareness of primary health care providers concerning the presentation of rapidly progressive glomerulonephritis emphasising the need for prompt involvement of a nephrologist in patient care.
2. Crescentic glomerulonephritis is a rare entity. Thus it may be prudent for nephrologists to collaborate and establish a multicentre prospective registry that will have sufficient subjects so as to better describe the outcomes and their determinants and possibly explore how genetics may influence severity in the various conditions that can lead to crescentic glomerulonephritis in this population.

6.2 Study limitations

1. This was a retrospective folder review thus some data was not obtained because it had not been recorded in the notes. In addition the number of subjects was small thus no firm conclusions regarding associations can be drawn from the data.

LIST OF REFERENCES

1. Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney international*. 2003;63(3):1164-77.
2. Glassock RJ, Adler SG, al HJwe. primary glomerular diseases. 9th ed. Brenner BM, Rector FC, editors. Philadelphia: Saunders; 2012.
3. Jindal KK. Management of idiopathic crescentic and diffuse proliferative glomerulonephritis: evidence-based recommendations. *Kidney international*. 1999;55:S33-S40.
4. Warady BA, Chadha V. Chronic kidney disease in children: the global perspective. *Pediatric Nephrology*. 2007;22(12):1999-2009.
5. Wong CJ, Moxey-Mims M, Jerry-Fluker J, Warady BA, Furth SL. CKiD (CKD in children) prospective cohort study: a review of current findings. *American Journal of Kidney Diseases*. 2012;60(6):1002-11.
6. Andrassy K, Küster S, Waldherr R, Ritz E. Rapidly progressive glomerulonephritis: analysis of prevalence and clinical course. *Nephron*. 1991;59(2):206-12.
7. López-Gómez JM, Rivera F. Renal biopsy findings in acute renal failure in the cohort of patients in the Spanish Registry of Glomerulonephritis. *Clinical Journal of the American Society of Nephrology*. 2008;3(3):674-81.
8. Moroni G, Ponticelli C. Rapidly progressive crescentic glomerulonephritis: early treatment is a must. *Autoimmunity reviews*. 2014;13(7):723-9.
9. Jennette JC, Olson L, Schwartz MM, F.G.Silva. *Heptinstalls Pathology of the Kidney Vol1: 1; 2007*.
10. Couser WG. Basic and translational concepts of immune-mediated glomerular diseases. *Journal of the American Society of Nephrology*. 2012;23(3):381-99.
11. Gajjar P, Sinclair P, McCulloch M. A case of microscopic polyangiitis: case report. *Current Allergy & Clinical Immunology*. 2010;23(3):121-3.
12. Zent R, Van Zyl SR, Duffield M, Cassidy M. Crescentic nephritis at Groote Schuur Hospital, South Africa--not a benign disease. *Clinical nephrology*. 1994;42(1):22-9.
13. Dilima M, Adhikari M, Coovadia H. Rapidly p. rogressive glomerulonephritis in Black children. *South African Medical Journal* 60(21): 829-32, December 1981.
14. Parag K, Naran A, Seedat Y, Nathoo B, Naiker I, Naicker S. Profile of crescentic glomerulonephritis in Natal-A clinicopathological assessment. *QJM*. 1988;68(2):629-36.
15. Koyama A, Yamagata K, Makino H, Arimura Y, Wada T, Nitta K, et al. A nationwide survey of rapidly progressive glomerulonephritis in Japan: etiology, prognosis and treatment diversity. *Clinical and Experimental Nephrology*. 2009;13(6):633-50.
16. Williamson SR, Phillips CL, Andreoli SP, Nailescu C. A 25-year experience with pediatric anti-glomerular basement membrane disease. *Pediatric Nephrology*. 2011;26(1):85-91.
17. Waikhom R, Sarkar D, Bennikal M, Pandey R. Rapidly progressive glomerulonephritis in tuberculosis. *Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia*. 2014;25(4):872-5.
18. El-Husseini A, El-Agroudy A, Moustafa F, Fouda M, Sobh M. Impact of clinical and histopathological factors on outcome of egyptian patients with crescentic glomerulonephritis. *International urology and nephrology*. 2003;35(4):543-51.
19. Avner d HW, Niaudet P, Yoshikawa N. *Pediatric Nephrology*. sixth ed. Avner d HW, Niaudet P, Yoshikawa N, editor. Berlin: Springer; 2009 2009.

20. Corwin H, Schwartz M, Lewis E. The importance of sample size in the interpretation of the renal biopsy. *American journal of nephrology*. 1988;8(2):85-9.
21. Cunningham RJ, Gilfoil M, Cavallo T, Brouhard BH, Travis LB, Berger M, et al. Rapidly progressive glomerulonephritis in children: a report of thirteen cases and a review of the literature. *Pediatric research*. 1980;14(2):128-32.
22. Chen M, Kallenberg CG, Zhao M-H. ANCA-negative pauci-immune crescentic glomerulonephritis. *Nature Reviews Nephrology*. 2009;5(6):313-8.
23. Baldwin DS, Neugarten J, Feiner HD, Gluck M, Spinowitz B. The existence of a protracted course in crescentic glomerulonephritis. *Kidney Int*. 1987;31(3):790-4.
24. Choudhury TA, Singh RG, Singh S, Singh TB, Rathore SS, Usha, et al. Clinicopathologic spectrum of crescentic glomerulonephritis: a hospital-based study. *Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia*. 2014;25(3):689-96.
25. Srivastava RN, Moudgil A, Bagga A, Vasudev AS, Bhuyan UN, Sundraem KR. CRESCENTIC GLOMERULONEPHRITIS IN CHILDREN - A REVIEW OF 43 CASES. *American Journal of Nephrology*. 1992;12(3):155-61.
26. Couser WG. Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. *American Journal of Kidney Diseases*. 1988;11(6):449-64.
27. Hudson BG. The molecular basis of Goodpasture and Alport syndromes: beacons for the discovery of the collagen IV family. *Journal of the American Society of Nephrology*. 2004;15(10):2514-27.
28. Alsaad K, Oudah N, Al Ameer A, Fakeeh K, Al Jomaih A, Al Sayyari A. Glomerulonephritis with crescents in children: etiology and predictors of renal outcome. *ISRN pediatrics*. 2011;2011:507298-.
29. Chen Y, Bao H, Liu Z, Zhang H, Zeng C, Liu Z, et al. Clinico-pathological features and outcomes of patients with propylthiouracil-associated ANCA vasculitis with renal involvement. *Journal of Nephrology*. 2014;27(2):159-64.
30. Savage CO. ANCA-associated renal vasculitis. *Kidney international*. 2001;60(4):1614-27.
31. KDIGO G. Work Group. KDIGO clinical practice guideline for glomerulonephritis. *Kidney inter, Suppl*. 2012;2:139-274.
32. Harper L, Savage C. ANCA-associated renal vasculitis at the end of the twentieth century—a disease of older patients. *Rheumatology*. 2005;44(4):495-501.
33. Smeets B, Angelotti ML, Rizzo P, Dijkman H, Lazzeri E, Mooren F, et al. Renal progenitor cells contribute to hyperplastic lesions of podocytopathies and crescentic glomerulonephritis. *Journal of the American Society of Nephrology*. 2009;20(12):2593-603.
34. Tipping PG, Holdsworth SR. T cells in crescentic glomerulonephritis. *Journal of the American Society of Nephrology*. 2006;17(5):1253-63.
35. Holdsworth SR, Kitching AR, Tipping PG. Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. *Kidney international*. 1999;55(4):1198-216.
36. van Wijngaarden RAdL, van Rijn L, Hagen EC, Watts RA, Gregorini G, Tervaert JWC, et al. Hypotheses on the Etiology of Antineutrophil Cytoplasmic Autoantibody–Associated Vasculitis: The Cause Is Hidden, but the Result Is Known. *Clinical Journal of the American Society of Nephrology*. 2008;3(1):237-52.
37. Fujieda M, Hattori M, Kurayama H, Koitabashi Y. Clinical Features and Outcomes in Children with Antineutrophil Cytoplasmic Autoantibody–Positive Glomerulonephritis

- Associated with Propylthiouracil Treatment. *Journal of the American Society of Nephrology*. 2002;13(2):437-45.
38. Hellmark T, Segelmark M, Wieslander J. Anti-GBM antibodies in Goodpasture syndrome; anatomy of an epitope. *Nephrology Dialysis Transplantation*. 1997;12(4):646-8.
 39. Bombassei GJ, Kaplan AA. The association between hydrocarbon exposure and anti-glomerular basement membrane antibody-mediated disease (Goodpasture's syndrome). *American journal of industrial medicine*. 1992;21(2):141-53.
 40. Kalluri R, Cantley LG, Kerjaschki D, Neilson EG. Reactive oxygen species expose cryptic epitopes associated with autoimmune Goodpasture syndrome. *Journal of Biological Chemistry*. 2000;275(26):20027-32.
 41. Stevenson A, Yaqoob M, Mason H, Pai P, Bell G. Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. *Qjm*. 1995;88(1):23-8.
 42. Salama AD, Chaudhry AN, Holthaus KA, Mosley K, Kalluri R, Sayegh MH, et al. Regulation by CD25⁺ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. *Kidney international*. 2003;64(5):1685-94.
 43. Salama AD, Levy JB. Tolerance and autoimmunity in anti-GBM disease. *Journal of the American Society of Nephrology*. 2003;14(11):2988-9.
 44. Dewan D, Gulati S, Sharma RK, Prasad N, Jain M, Gupta A, et al. Clinical spectrum and outcome of crescentic glomerulonephritis in children in developing countries. *Pediatric Nephrology*. 2008;23(3):389-94.
 45. Sinha A, Puri K, Hari P, Dinda AK, Bagga A. Etiology and outcome of crescentic glomerulonephritis. *Indian pediatrics*. 2013;50(3):283-8.
 46. Jardim HM, Leake J, Risdon RA, Barratt TM, Dillon MJ. Crescentic glomerulonephritis in children. *Pediatric Nephrology*. 1992;6(3):231-5.
 47. Hedger N, Stevens J, Drey N, Walker S, Roderick P. Incidence and outcome of pauci-immune rapidly progressive glomerulonephritis in Wessex, UK: a 10-year retrospective study. *Nephrology Dialysis Transplantation*. 2000;15(10):1593-9.
 48. Rodriguez-Iturbe B, Musser JM. The current state of poststreptococcal glomerulonephritis. *Journal of the American Society of Nephrology*. 2008;19(10):1855-64.
 49. Kanjanabuch T, Kittikowit W, Eiam-Ong S. An update on acute postinfectious glomerulonephritis worldwide. *Nature Reviews Nephrology*. 2009;5(5):259-69.
 50. Stratta PS, Musetti C, Barreca A, Mazzucco G. New trends of an old disease: the acute post infectious glomerulonephritis at the beginning of the new millenium. *Journal of Nephrology*. 2014;27(3):229-39.
 51. Jardim H, Leake J, Risdon RA, Barratt TM, Dillon MJ. CRESCENTIC GLOMERULONEPHRITIS IN CHILDREN. *Pediatric Nephrology*. 1992;6(3):231-5.
 52. Wong W, Morris MC, Zwi J. Outcome of severe acute post-streptococcal glomerulonephritis in New Zealand children. *Pediatric Nephrology*. 2009;24(5):1021-6.
 53. Hattori M, Kurayama H, Koitabashi Y. Antineutrophil Cytoplasmic Autoantibody—Associated Glomerulonephritis in Children. *Journal of the American Society of Nephrology*. 2001;12(7):1493-500.
 54. Beck L, Bomback AS, Choi MJ, Holzman LB, Langford C, Mariani LH, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis. *American Journal of Kidney Diseases*. 2013;62(3):403-41.

55. Hogan SL, Falk RJ, Chin H, Cai J, Jennette CE, Jennette JC, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody–associated small-vessel vasculitis. *Annals of internal medicine*. 2005;143(9):621-31.
56. van Wijngaarden RAdL, Hauer HA, Wolterbeek R, Jayne DR, Gaskin G, Rasmussen N, et al. Chances of renal recovery for dialysis-dependent ANCA-associated glomerulonephritis. *Journal of the American Society of Nephrology*. 2007;18(7):2189-97.
57. de Groot K, Harper L, Jayne DR, Suarez LFF, Gregorini G, Gross WL, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody—associated vasculitis: a randomized trial. *Annals of internal medicine*. 2009;150(10):670-80.
58. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *New England Journal of Medicine*. 2010;363(3):221-32.
59. Jayne DR, Gaskin G, Rasmussen N, Abramowicz D, Ferrario F, Guillevin L, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *Journal of the American Society of Nephrology*. 2007;18(7):2180-8.
60. Cole E, Cattran D, Magil A, Greenwood C, Churchill D, Sutton D, et al. A prospective randomized trial of plasma exchange as additive therapy in idiopathic crescentic glomerulonephritis. *American journal of kidney diseases*. 1992;20(3):261-9.
61. Nachman PH, Hogan SL, Jennette JC, Falk RJ. Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *Journal of the American Society of Nephrology*. 1996;7(1):33-9.
62. Lauque D, Cadranet J, Lazor R, Pourrat J, Ronco P, Guillevin L, et al. Microscopic Polyangiitis with Alveolar Hemorrhage A Study of 29 Cases and Review of the Literature. *Medicine*. 2000;79(4):222-33.
63. Klemmer PJ, Chalermkulrat W, Reif MS, Hogan SL, Henke DC, Falk RJ. Plasmapheresis therapy for diffuse alveolar hemorrhage in patients with small-vessel vasculitis. *American journal of kidney diseases*. 2003;42(6):1149-53.
64. Jones RB, Cohen Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *New England Journal of Medicine*. 2010;363(3):211-20.
65. REISCH JS, Fox W, Centers S. A clinico-pathologic study of crescentic glomerulonephritis in 50 children. A report of the Southwest Pediatric Nephrology Study Group. *Kidney International*. 1985;27:450-8.
66. Tapaneya-Olarn W, Boonpucknavig V, Tapaneya-Olarn C, Boonpucknavig S. Rapidly progressive glomerulonephritis in Thai children. *Journal of the Medical Association of Thailand*. 1992;75(SUPPL. 1):32-7.
67. Almera-Borja A, Rosel MB, Antonio ZL, Tadoy SI, De León O, Marbella A. Clinico-pathologic profile and outcome of rapidly progressive glomerulonephritis in children: a 5 year experience. *Philippine Journal of Nephrology*. 2005;20(1):36-43.
68. . Available from: https://www.westerncape.gov.za/assets/departments/premier/2012.06_western_cape_overview1_0.pdf.
69. Falkner B, Daniels SR. Summary of the fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Hypertension*. 2004;44(4):387-8.

70. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *Journal of the American Society of Nephrology*. 2009;20(3):629-37.
71. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics*. 2003;111(6):1416-21.
72. Group WMGRS. WHO Child Growth Standards based on length/height, weight and age. *Acta paediatrica (Oslo, Norway: 1992) Supplement*. 2006;450:76.
73. Okpechi I, Swanepoel C, Duffield M, Mahala B, Wearne N, Alagbe S, et al. Patterns of renal disease in Cape Town South Africa: a 10-year review of a single-centre renal biopsy database. *Nephrology Dialysis Transplantation*. 2010:gfq655.
74. Gupta R, Singh L, Sharma A, Bagga A, Agarwal SK, Dinda AK. Crescentic glomerulonephritis: A clinical and histomorphological analysis of 46 cases. *Indian Journal of Pathology and Microbiology*. 2011;54(3):497.
75. Kinjoh K, Kyogoku M, Good RA. Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. *Proceedings of the National Academy of Sciences*. 1993;90(8):3413-7.
76. Kitching AR, Turner AL, Wilson GR, Semple T, Odobasic D, Timoshanko JR, et al. IL-12p40 and IL-18 in crescentic glomerulonephritis: IL-12p40 is the key Th1-defining cytokine chain, whereas IL-18 promotes local inflammation and leukocyte recruitment. *Journal of the American Society of Nephrology*. 2005;16(7):2023-33.
77. Deplano S, Cook HT, Russell R, Franchi L, Schneiter S, Bhargal G, et al. P2X7 receptor-mediated Nlrp3-inflammasome activation is a genetic determinant of macrophage-dependent crescentic glomerulonephritis. *Journal of leukocyte biology*. 2013;93(1):127-34.
78. Yu F, Tan Y, Liu G, Wang S-x, Zou W-z, Zhao M-h. Clinicopathological characteristics and outcomes of patients with crescentic lupus nephritis. *Kidney international*. 2009;76(3):307-17.
79. Ntatsaki E, Isenberg D. Risk factors for renal disease in systemic lupus erythematosus and their clinical implications. *Expert review of clinical immunology*. 2015(0):1-12.
80. Siomou E, Tramma D, Bowen C, Milford DV. ANCA-associated glomerulonephritis/systemic vasculitis in childhood: clinical features–outcome. *Pediatric Nephrology*. 2012;27(10):1911-20.
81. Schulte-Pelkum J, Radice A, Norman GL, López Hoyos M, Lakos G, Buchner C, et al. Novel clinical and diagnostic aspects of antineutrophil cytoplasmic antibodies. *Journal of immunology research*. 2014;2014.
82. Roth AJ, Brown MC, Smith RN, Badhwar AK, Parente O, Chul Chung H, et al. Anti-LAMP-2 Antibodies Are Not Prevalent in Patients With Antineutrophil Cytoplasmic Autoantibody Glomerulonephritis. *Journal of the American Society of Nephrology*. 2012;23(3):545-55.
83. Kain R, Rees AJ. What is the evidence for antibodies to LAMP-2 in the pathogenesis of ANCA associated small vessel vasculitis? *Current opinion in rheumatology*. 2013;25(1):26-34.
84. Roy S, Murphy WM, Arant BS. Poststreptococcal crescentic glomerulonephritis in children: comparison of quintuple therapy versus supportive care. *The Journal of pediatrics*. 1981;98(3):403-10.
85. Raff A, Hebert T, Pullman J, Coco M. Crescentic post-streptococcal glomerulonephritis with nephrotic syndrome in the adult: is aggressive therapy warranted? *Clinical nephrology*. 2005;63(5):375-80.

86. El-Husseini AA, Sheashaa HA, Sabry AA, Moustafa FE, Sobh MA. Acute postinfectious crescentic glomerulonephritis: Clinicopathologic presentation and risk factors. *International urology and nephrology*. 2005;37(3):603-9.

18. Haematuria

19. Plasmapheresis 1) Yes 2) No

If Yes, number of sessions

Date first session

20. Methyl prednisone 1) Yes 2) No

If Yes, number of doses

Date first dose

21. Cyclophosphamide 1) Yes 2) No

If Yes, number of doses

Date first dose

22. Maintenance drug

23. Duration of follow-up (months)

24. Outcome at one year a) eGFR

b) CKD stage

c) Haemodialysis dependant 1) Yes 2) No

d) Peritoneal Dialysis 1) Yes 2) No

e) Renal Transplant 1) Yes 2) No

f) Death 1) Yes 2) No

g) lost to followup 1) Yes 2) No

Part III: Laboratory data

25. FBC at presentation

- a. Hb
- b. WBC
- c. Plt
- d. ESR

26. Electrolytes/Urea/Creatinine at presentation

- a. Na
- b. K
- c. Cl
- d. Ca
- e. Mg
- f. iPO4
- g. Ur
- h. Cr

27. Peak Creatinine (date)

28. Creatinine at one Year

29. Creatinine at last follow-up

30. Autoimmune markers

	<u>Value</u>	<u>Interpretation</u>
a. CRP	_____	1) High 2) Low 3) Normal 4) Not done
b. ANA	_____	1)high 2)Low 3) Normal 4) not done
c. Anti Dnase B	_____	1) high 2) Low 3) Normal 4) not done
d. pANCA	_____	1) high 2) Low 3)normal 4) not done
e. cANCA	_____	1) high 2) low 3)normal 4) not done
f. Anti- GBM antibodies	_____	1)high 2)low 3)normal 4) not done
g. C3	_____	1)High 2) Low 3) Normal 4) Not done
h. C4	_____	1) High 2) Low 3) Normal 4) Not done
i. Anti-ds DNA	_____	1) High 2) Low 3) Normal 4) Not done

31. HIV status

- 1) Positive 2) Negative 3) Not done

Part IV: HISTOPATHOLOGY DATA SHEET

Subject study number

Subject hospital number

Date specimen collected

Light Microscopy

- 32. Crescentic glomerulonephritis 1) Yes 2) No
- 33. Number of glomeruli
- 34. Proportion of glomeruli with crescents
- 35. Proportion of fibrocellular crescents
- 36. Proportion of cellular crescents
- 37. Proportion of fibrinous crescents
- 38. Presence tuft necrosis 1) Yes 2) No
- 39. Presence of rupture of Bowman's capsule 1) Yes 2) No
- 40. Presence of fibrinoid necrosis
- 41. Degree of interstitial fibrosis
- 42. Degree of tubular atrophy
- 43. Degree of arteriolar fibrin deposition

Immunofluorescence

44. Immunoglobulins

- i. IgA 1) yes 2) No
- ii. Ig M 1) Yes 2) No
- iii. IgG 1) Yes 2) No

45. Complement

- i. C 1q 1) yes 2) No
- ii. C3 1) Yes 2) No
- iii. C4 1) Yes 2) No
- iv. Other.Specify.

Electron Microscopy

- 46. Podocyte effacement 1) Yes 2) No
- 47. Subendothelial deposits 1) Yes 2) No
- 48. Subepithelial deposits 1) Yes 2) No
- 49. Other, Specify

Conclusion

Signature

APPENDIX B: HOSPITAL APPROVAL OF RESEARCH



Dr R Mistry
Manager: Medical Services
Email: Roshni.Mistry@westerncape.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166

Dr C Mwaba
Red Cross War Memorial Children's Hospital

Dear Dr C Mwaba

APPROVAL OF RESEARCH

PROJECT TITLE: A STUDY OF THE AETIOLOGY AND OUTCOME OF CRESCENTIC GLOMERULONEPHRITIS IN CHILDREN PRESENTING TO THE RED CROSS CHILDREN'S HOSPITAL CAPE TOWN, SOUTH AFRICA.

We have the pleasure of informing you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

Signed

DR ROSHNI MISTRY
MANAGER: MEDICAL SERVICES
DATE: 03 AUGUST 2015

APPENDIX C: ETHICS APPROVAL LETTER



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 June 2015

HREC REF: 321/2015

Dr P Gajjar
Paediatric Nephrology
Red Cross Children's Hospital

Dear Dr Gajjar

PROJECT TITLE: AETIOLOGY AND OUTCOME OF CRESCENTIC GLOMERULONEPHRITIS IN CHILDREN PRESENTING TO THE RED CROSS CHILDREN'S HOSPITAL (MPhil candidate - Dr C Mwaba)

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee received on 08 June 2015.

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

Approval is granted for one year until the 30th June 2016.

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.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the MPhil student, Dr Chisambo Mwaba will also be involved in this study.

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

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

HREC 321/2015