Control of Hepatitis B and C virus infection in chronic haemodialysis patients

By

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Declaration

I, Maarten Willem Taal, hereby declare that the research described herein was performed by me with assistance as indicated in the ackowledgements. The dissertation was written by me and reviewed by my supervisor. Neither the whole thesis nor any part of it has been, is being or will be submitted for any other degree at this or any other University.

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Abstract

Chronic haemodialysis patients have a high prevalence of Hepatitis B and C virus infections both of which are associated with chronic liver disease and hepatocellular carcinoma.

Hepatitis B virus (HBV) was identified as a frequent cause of hepatitis during the early years of chronic haemodialysis therapy and strict adherence to infection control measures alone proved inadequate to control the transmission of infection between patients. A policy of regular screening of all patients and blood donations for hepatitis B surface antigen together with isolation of positive patients to separate dialysis units resulted in a significant decline in the incidence of new infections. Hepatitis B vaccination provided an important new means of protection. Despite the finding that haemodialysis patients did not respond to the vaccine as well as normal adults, randomized controlled trials showed significant protection in units with a previously high incidence of infection. Studies have identified both monocyte dysfunction and B cell inhibition by high levels of parathyroid hormone (PTH) as possible mechanisms for the reduced response in dialysis patients. Other factors which have been associated with this poor response include increased age, male gender, specific human leukocyte antigens, shorter time on a dialysis programme and poor nutritional status. One study has shown an increased response in patients receiving recombinant human erythropoietin and there is in vitro evidence that nifedipine improves B cell proliferation in dialysis patients with hyperparathyroidism.

Hepatitis C virus (HCV) infection in haemodialysis patients has been associated with blood transfusions in many studies. However, evidence exists that transmission between patients also occurs. There is disagreement as to what measures are necessary to prevent possible nosocomial spread. Some authors recommend isolation of HCV-infected patients to separate dialysis machines or units. There is also concern over the potential of dialyzer reuse to transmit the virus.

A protocol for surveillance of hepatitis B and C infections was established in the dialysis unit at Groote Schuur Hospital while HCV positive patients were not isolated and reuse of dialyzers was continued for all patients. HBV-infected patients are dialyzed in a separate unit and their dialyzers are not reused. A trial of hepatitis B vaccination of all antibody negative patients was undertaken using four doses of a plasma-derived vaccine given intramuscularly at month 0,1,2 and 4.

There were no new HBV infections during a period of 40 months prior to the initiation of vaccination. Of patients screened, 33% were positive for anti-hepatitis B core IgG, indicating that they had previously been infected. Of the 36 patients who received the full course of vaccination, 26 (72%) responded and 25 (69%) achieved levels above the minimum protective level of 10IU/L. The peak mean antibody titre occurred at 1 month after the 4th dose of vaccine and was 372 IU/L. Stepwise multiple regression analysis identified time on renal replacement therapy and serum PTH as factors affecting antibody response although together they accounted for only 19.2% of the variance. Patients responding to the vaccine with PTH levels of less than the mean of 502pg/ml had a significantly higher mean antibody titre than those with PTH levels above 502 pg/ml. The study was unable to demostrate a positive

effect of either recombinant erythropoietin or calcium channel blocker therapy on antibody response. Cost analysis revealed that due to reduced need for HBsAg testing, the vaccination programme would result in a nett saving of R7 223.90 (±R90/patient) at the end of the first year and R30 092.00 (±R380/patient) per year thereafter.

There was a marked decline in the prevalence of anti-HCV antibodies from 16.4% in December 1992 to 5.3% in December 1995 ($p = 0.04$). At both times, the anti-HCV positive patients, when compared to negative patients, had a longer time on haemodialysis (mean 101.6 ± SD 57.4 months vs. 30.3 ± 32.4 in 1992 and 105.5 ± 23.9 vs. 30.2 ± 32.8 in 1995) and a greater number of blood transfusions (mean 22.6 ± SD 17 units vs 6.8 ± 9.4 in 1992 and 14.8 ± 3.6 vs. 4.5 ± 7.1 in 1995). There was no difference in the number of renal transplants (mean 1 vs. 0.9 in 1992 and 1.25 vs. 0.8 in 1995). When the 1992 and 1995 groups were compared, there was no difference in time on haemodialysis (mean 42 months vs. 34.2 months). However, there was a significant reduction in the number of blood transfusions between 1992 and 1995 (mean 9.4 ± SD 12.5 vs. 5.1 ± 7.3; patients = 0.02). Of note was the introduction of screening of 1 in 5 units of transfused blood for anti-HCV in December 1992, and all units in December 1993. Review of all serology between 1992 and 1995, revealed 4 patients who became anti-HCV positive while on haemodialysis. Two of these were negative on later tests and both were negative for HCV RNA by polymerase chain reaction. The two remaining patients were both positive on repeated testing. One patient had received a total of 52 units of transfused blood, but the other had had only one unit, suggesting possible transmission in the dialysis unit.

We conclude that the existing strategy to control the spread of HBV is effective and that hepatitis B vaccination is a cost-effective additional measure. This study provides new evidence that hyperparathyroidism may be partially responsible for the impaired response of haemodialysis patients to the vaccine. Our results confirm the high prevalence of anti-HCV positivity in haemodialysis patients and the association with time on dialysis and blood transfusions. There was a marked decline in the prevalence of anti-HCV positive patients over 3 years which we attribute mainly to the introduction of anti-HCV testing of blood donations. The decline in prevalence occurred despite the routine reuse of dialyzers and the lack of isolation of anti-HCV positive patients, suggesting that neither contributed to the transmission of HCV.

1. Literature Review

1.1 Introduction

Hepatitis B and Hepatitis C virus infections are considered significant complications of chronic haemodialysis therapy. Both are capable of causing chronic hepatitis which may progress to cirrhosis and hepatocellular carcinoma. Currently available therapy is effective only in the minority of cases. Thus efforts to control these infections must focus on the prevention of new cases.

This dissertation describes the nature and extent of the problem and examines the measures required for control of these viruses in a chronic haemodialysis unit. Particular attention will be paid to vaccination of patients against hepatitis B infection and identification of the measures required to prevent transmission of hepatitis C.

1.2 Hepatitis B Virus

1.2.1 Biology of hepatitis B virus

1.2.1.1 Structure

Hepatitis B virus (HBV) is the smallest known DNA virus and is classified as a member of the family Hepadnaviridae. It has a diameter of 42nm and it's genome contains only 3200 bases.

The outer surface is composed of a lipophilic material and three envelope proteins which together are called hepatitis B surface antigen (HBsAg). The inner core has a diameter of 27nm and is composed of hepatitis B core antigen (HBcAg). The virus also produces a soluble, non-structural protein, hepatitis B e antigen (HBeAg) which is present in the blood of patients with replicating HBV. A single viral gene encodes both HBcAg and HBeAg. The transcript for HBeAg contains an additional 29 codons (pre-core) which code for a secretory peptide which allows the translated protein entry into the hepatocyte endoplasmic reticulum, where it is cleaved and processed for secretion.

HBV DNA is a partially double-stranded circular molecule. One strand is termed "minus" and is almost a complete circle. It contains genes for structural (pre-S, surface, core) and replicative proteins (polymerase, X protein). The other, "plus" strand is much shorter, variable in length and contains a segment of RNA. The genome contains two enhancer elements. The one (ENH I) functions effectively only

in hepatocytes and also contains a retinoic-acid-responsive element. ENH II stimulates the transcriptional function of the surface gene promoters.

Two clinically important mutant forms of HBV have been identified. The most common one has a mutation in the pre-core region, which results in a failure to produce HBeAg. Thus patients infected with this virus may have active liver disease with viral replication but are HBeAg negative. The second mutation results in a single amino acid substitution (arginine replaced by glycine) in the "a" determinant of HBsAg. Vaccines stimulate the production of antibodies to this domain and thus vaccinated patients may remain susceptible to infection by mutated HBV with an abnormal "a" determinant (1, 2, 3).

1.2.1.2 Replication

Binding to the hepatocyte surface takes place via attachment sites in the pre-S region of the viral envelope. After entry into the cell, the viral core is transported to the nucleus. The relaxed circular virus DNA is then converted to a covalently closed circular DNA which functions as the template for transcription into mRNA. Four mRNA transcripts with different functions have been identified. The largest (3.5kb) acts as a template for both HBV DNA replication via reverse transcription, and the synthesis of pre-core/core and polymerase proteins. The other transcripts encode the remaining structural and non-structural proteins.

New viral particles are assembled and packaged into HBsAg in the endoplasmic reticulum from where they are exported from the cell. Some of the newly synthesized

viral DNA is transported back into the cell nucleus to maintain a stable pool of DNA for further transcription (1, 2).

1.2.1.3 Transmission

HBsAg has been identified in blood, saliva, vaginal discharges, seminal fluid and serous exudates from infected patients. HBV is an extremely resistant virus and can survive extremes of temperature and exposure to many chemical agents. At room temperature it may survive for up to six months. It may therefore be spread by blood transfusions, sexual contact and accidental innoculation with small amounts of blood as may occur during medical procedures, sharing of needles during drug abuse, tattooing, ear-piercing and sharing of razors or toothbrushes. The virus may also be transmitted perinatally from mother to child. In certain rural areas of Southern Africa, horizontal transmission to young children is common (1, 3).

1.2.1.4 Prevalence of HBV infection

It is estimated that there are 300 million chronic carriers of HBV worldwide. The prevalence of carriers varies from 0.1%-0.5% in western Europe, to 8%-15% in Africa and the Far East. The prevalence of previous infection, as evidenced by the presence of antibodies to HBsAg (anti-HBs), is much higher and varies from 4%-6% to 70%-95% (1). In South Africa, serolgical studies have shown increased risk of exposure and chronic infection in rural areas (4). Overall, the prevalence of previous exposure is about 70% and that of chronic carriers, 5%-10%. In one survey of 607 black patients attending outpatient clinics in the Western Cape and Eastern Cape

provinces of South Africa, 61.1% of patients were anti-HBs positive and 4.3% HBsAg positive (5). In 1992 the total number of chronic carriers in the country was estimated to be 1 475 223 (6). (1, 3)

1.2.1.5 Natural history of infection

The incubation period for HBV infection is 60-180 days. The disease is usually mild and subclinical infection is common, although fulminant disease does rarely occur. Jaundice occurs in 20%-50% of cases and is usually preceded by anorexia, nausea and vomiting. Arthralgia and skin rashes occur in some patients.

Most adult patients recover completely within six months, however 5-10% progress to chronic infection. The risk of chronic infection is greatly increased in neonates (90%), children (30%-40%) and immunosuppressed adults. Chronic carriers generally have a mild or asymptomatic initial infection and therefore often do not recall having had acute hepatitis.

Patients with HBsAg persisting for longer than six months or HBeAg persisting for longer than three months are defined as chronic carriers. Chronic HBV infection may result in an asymptomatic carrier state or in chronic hepatitis which results in cirrhosis in up to 55% of cases. The detection of HBV DNA or HBeAg in the serum denotes ongoing viral replication. Follow up studies show that HBeAg is lost spontaneously at a rate of 13%-14% per year (7). However, this is an unstable state and patients may later again develop markers of viral replication.

An important complication of chronic HBV infection is the development of hepatocellular carcinoma (HCC). Although it has been shown that HBV DNA becomes integrated into the genome of hepatoma cells, the exact mechanism of oncogenisis has not yet been determined (8). The risk of developing HCC in patients with chronic HBV infection 23-223 times greater than in the uninfected population (9, 10). In regions where HBV infection is endemic, HCC is the largest cause of cancer-related deaths.

Extrahepatic manifestations of chronic HBV infection occur in a small number of patients and include a polyarteritis nodosa like syndrome, glomerulonephritis (usually membranous), cryoglobulinaemia, peripheral neuropathy and marrow aplasia (1, 3).

Therapy with interferon-α appears to halt viral replication, but a response is obtained in less than half of patients. In a multicentre study, treatment of 41 patients with chronic HBV infection with 5 million units daily for 16 weeks resulted in clearance of HBeAg in 37%. No reactivation occurred during 2 to 6 months of follow-up. However, only 12% of patients became HBsAg negative (11). Long-term studies will be required to determine whether interferon therapy alters the natural history of the disease.

1.2.2 Testing for HBV infection

The diagnosis of hepatitis is confirmed by the demonstration of increased activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum.

In the case of HBV infection, this will coincide with the onset of symptoms. A number of serologic markers may be used to identify HBV infection at different stages.

In acute infection, HBsAg is detectable at the time of onset of symptoms and remains present for about three months. Once HBsAg has been cleared from the serum, anti-HBs antibodies appear after a lag phase of about three weeks. HBeAg is also detectable at the onset of symptoms and is cleared after about 1 month. Anti-HBe antibodies then develop but levels decline at about a year post infection. IgM anti-HBc appears early in the infection and disappears by six months. It is then replaced by IgG anti-HBc. Anti-HBs and IgG anti-HBc both persist for many years and are thus the most enduring evidence of previous HBV infection. Patients vaccinated with HBsAg vaccines will become anti-HBs positive but will remain IgG anti-HBc negative.

By definition HBsAg remains detectable in the serum of patients with chronic HBV infection. Ongoing active viral replication is indicated by the detection of viral DNA in the serum by polymerase chain reaction (PCR) and by persistent HBeAg positivity. If replication stops, HBeAg is cleared and anti-HBe appears (1, 2).

1.2.3 HBV infection in haemodialysis patients

1.2.3.1 Prevalence

Soon after long-term haemodialysis had become established as standard therapy for chronic renal failure during the 1960's, numerous reports of outbreaks of hepatitis in haemodialysis units were received from the UK, USA and Europe. In one well documented case, all nine patients and six of fifteen staff in a unit developed hepatitis between September 1967 and October 1968 (12). "Australia antigen" tests became widely available in 1969 and studies showed that most of these outbreaks were due to hepatitis B. In the UK, a survey of 21 units revealed that 19% of units had outbreaks in 1969, all of which were associated with "Australia antigen" positivity. The incidence of hepatitis in 20 units in 1970 was 5.6% in patients and 0.4% in staff (13). In France the annual incidence of clinical HBV infection was reported to range between 25% and 40% from 1969 to 1976 (14). A Centre for Disease Control (CDC) study in the USA revealed that between 1967 and 1970, 53 of 65 (82%) of haemodialysis units reported cases of viral hepatitis in patients, staff or both. The incidence of viral hepatitis in these units in 1970 was 4.4% in patients and 3.2% in staff (15). A further study of 15 haemodialysis units in the USA from 1972 to 1973, reported a point prevalence of HBsAg of 16.8% in patients and 2.4% in staff. Antibodies to HBV were detected in 34.0% of patients and 31.3% of staff (16).

1.2.3.2 Modes of transmission

The outbreaks of hepatitis provided strong evidence for nosocomial transmission of HBV between patients and staff. In some cases this was supported by the finding of the same subtype of HBsAg in all affected patients (17). In at least four units in one study, outbreaks could be linked to the introduction of a patient who was an asymptomatic chronic carrier of HBV (13). Furthemore, contamination of a haemodialysis unit was demonstrated by the detection of HBsAg on swabs taken from gloves, door handles, needle clippers, furniture and external ports of dialyzers (16). The subsequent demostration of the fact that HBV can remain viable and infectious after drying and storage for on week confirms that environmental contamination may be a source of infection (18).

Blood transfusions were also identified as an important potential source of HBV infection (13). In one study 87% of patients with hepatitis had received blood within six months of the onset of illness. These patients had also received twice as many units of blood as "average" dialysis patients (15). Another study was unable to demonstrate an association between blood transfusions and HBV infection independent of time on haemodialysis. However transfusion histories prior to admission to the dialysis centres were not taken into account (16).

In addition to transmission from other patients or staff and blood transfusions, patients remain susceptible to HBV aquired by the same routes as the general population. This is of particular relevance in areas with a high prevalence of HBV

infection because it means that patients on dialysis are continually at risk of aquiring the infection, which may then be spread to other patients in a haemodialysis unit.

1.2.3.3 Natural history of infection

From the earliest descriptions of outbreaks, it became apparent that HBV infection produced different clinical features in haemodialysis patients when compared to staff. Patients had less severe symptoms, less jaundice and lower transaminase levels, although these remained elevated for longer. In addition, more haemodialysis patients became chronic carriers (19). These findings were subsequently confirmed by other studies. In a prospective study conducted over five years, 32 of 61 (52%) of patients who developed HBV infection became chronic carriers and males became carriers more frequently than females (74%vs.26%) (19). Furthemore, a study involving fifteen haemodialysis centres revealed that of 495 patients with no history of clinical hepatitis, 11.5% were HBsAg positive and 37.2%, HBsAb positive. Seventy nine patients had a history of hepatitis and 50.6% remained HBsAg positive, while 16.5% were HBsAb positive. By contrast, the values for staff in these units were: 2.7% HBsAg positive, 28.2% HBsAb positive in those with no history of hepatitis, and 1.7% HBsAg positive, 52.5% HBsAb positive in those with previous hepatitis (16).

The high incidence of asymptomatic hepatitis and high prevalence of asymptomatic chronic carriers reflected in this data has important implications for the surveillance and prevention of HBV infection in haemodialysis patients. In addition, the finding of HBV DNA by PCR in serum from 6 of 43 HBsAg negative haemodialysis patients who were HBsAb and/or HBcAb positive, suggests that some patients who are

thought to have cleared the infection by serological criteria, may remain infectious (20).

Data on the long-term sequelae of HBV infection in this group of patients is limited. London et al. showed no difference in mortality between HBsAg positive and negative haemodialysis patients at 3 years follow-up (19). Similarly, Josselson et al. were unable to demonstrate an increased morbidity or mortality after 8 years follow-up, in 30 patients who were transiently or permanently HBsAg positive (21). By contrast, a retrospective study of 49 HBsAg positive patients with a mean follow-up of 52 months reported that 29% had chronically elevated liver enzymes and that one had died from liver disease (22). An early study of liver histology in 15 HBsAg positive patients with chronic renal failure (2 on supportive therapy only, 7 on haemodialysis, 6 renal transplant recipients) reported cirrhosis in 3, chronic active hepatitis in 2 and chronic persistent hepatitis in 4. All 3 patients with cirrhosis deteriorated rapidly and died (23). As part of a study examining the significance of HBeAg, 23 of 24 haemodialysis patients who had been HBsAg positive for longer than 4 months after acute hepatitis underwent liver biopsies. Twelve had chronic active hepatitis and of these, 4 had evidence of cirrhosis. The remaining 11 had unresolved acute hepatitis (24).

When chronic HBV carriers on haemodialysis were compared with those who had received renal transplants, the former had a lower prevalence of biochemically defined chronic hepatitis (29%vs.100%) and a lower mortality due to liver disease.(3%vs.37%) At least 2 of the 8 deaths in the transplant recipients were due to hepatoma (25). Similarly, Parfrey et al reported that 21 of 22 HBsAg positive

transplant recipients developed chronic liver disease (12 cirrhosis, 6 chronic active hepatitis, 3 chronic persistent hepatitis) and that after a mean follow-up of 83 months, 11 had died of liver disease (3 with hepatoma). By contrast, only 1 of 10 HBsAg positive patients on haemodialysis had evidence of chronic liver disease and none had died of liver disease over a similar period of time (26). Other studies have reported that HBsAg positive renal transplant recipients have good early graft and patient survival, although there is an increased mortality due to liver disease, especially after 10 years (27, 28). Some centres however, have reported a much lower prevalence of death due to liver disease in similar patients (29, 30).

Data on the use of interferon-α to treat dialysis patients or renal transplant recipients with chronic HBV infection is limited to isolated reports (31). The use of interferon-α to treat HCV infection in transplant recipients has been associated with graft rejection and it therefore seems inadvisable to consider this form of therapy in such patients (32).

It would appear that chronic hepatitis develops in about 50% of chronic HBV carriers on haemodialysis yet morbidity and mortality due to liver disease remain low. Following renal transplantation almost all HBV positive patients develop chronic hepatitis with a significant increase in mortality due to liver disease when compared to both HBsAg positive haemodialysis patients and HBsAg negative transplant recipients. This has led to debate as to whether HBsAg positive patients should be accepted for renal transplantation or not. Chronic HBV infection therefore has a significant negative effect on the long term outcome of patients with chronic renal failure.

1.2.3.4 Prevention

1.2.3.4.1 Infection control measures

Many reports of outbreaks of HBV infection identified inadequate infection control measures in the affected dialysis units. Yet measures such as improved hand washing, use of gloves, disposal of contaminated materials and avoidance of eating in units were unable to stop the spread of infection as evidenced by ongoing high infection rates (13, 33). Some centres began isolating HBsAg positive patients to separate units. Screening of blood donations for HBsAg also seemed justified although only relatively few infections could be directly attributed to blood transfusions (13).

In 1970, a policy of testing all patients for HBsAg before initiating dialysis and quaterly thereafter, combined with the transfer of positive patients to isolation units or home dialysis and the screening of blood donations for HBsAg where possible, resulted in 18 of 24 dialysis units in the UK remaining free of HBV infection during the first year. In 2 of the units where infections did occur, the above policy was not adequately applied and in another the infection could be attributed to an unscreened blood transfusion (13). Over the next two years, continued application of this policy, but with monthly screening of patients for HBsAg and testing of all blood for transfusion, led to a decline in the annual incidence of new HBV infections from 4.9% to 1.4% in patients and from 1.3% to 0.4% in staff (17).

In the USA, a CDC survey in 1974 revealed a lower annual incidence of HBV infection in dialysis units which separated HBsAg positive patients from negative patients within the same room (9.4% vs. 13.4%) and in those providing a separate room for positive patients (7.3% vs.11.7%) (33). Further evidence of the importance of isolation was provided by a trial conducted in 7 haemodialysis units in New Jersey from 1975 to 1978. The first phase was a retrospective study to document the annual incidence of HBV infection in patients and staff in 1975. In phase 2, attention was given to improving infection control measures for a one year period. The establishment of an isolation dialysis centre for all HBsAg positive patients constituted the third phase and five units participated in this phase. The remaining two did not transfer their positive patients and acted as controls. Results showed a progressive decline in the annual incidence of HBV infection in patients in intervention centres from 23.4% in phase 1 to 15.0% in phase 2 and 7.5% in phase 3. The decrease between phases 1 and 2 was not statistically significant, but that between phases 2 and 3 was highly significant. At the same time, the incidence in the control units increased from 14.0% to 17.6% and 27.7% in phases 1, 2 and 3 respectively (34). A subsequent CDC report which gathered data from 1135 dialysis centres in the USA, showed a decline in the incidence of HBsAg positivity in patients from 3.0% in 1976 to 0.5% in 1983. During this period the number of units screening patients monthly for HBsAg increased from 57% to 84%, and those isolating positive patients to separate rooms with separate machines increased from 75% to 86% (35).

Based on the above evidence, the following measures have been accepted by most centres as necessary for the effective prevention of HBV transmission in haemodialysis units:

1. Strict adherence to universal infection control precautions
2. Screening of all blood donations for HBsAg
3. Screening of all patients for HBsAg before starting haemodialysis and regularly thereafter.
4. Isolation of all HBsAg positive patients to separate dialysis units

1.2.3.4.2 Immunization

1.2.3.4.2.1 Hepatitis B immune globulin

Prior to the development of vaccines for active immunization, some protection was achieved using hepatitis B immune globulin in both staff and patients (14, 36, 37). However, the high cost and need for continued administration prevented this from becoming a widely used method of prophylaxis.

1.2.3.4.2.2 Hepatitis B surface antigen vaccines

1.2.3.4.2.2.1 Response and efficacy

The development of vaccines from HBsAg obtained from the serum of chronic carriers of HBV in the late 1970's, provided an important new method of protecting patients against infection. Although the vaccine induced anti-HBs antibodies at above the minimum protective level of 10 IU/l in 93%-100% of staff or normal controls (38-41), rates in haemodialysis patients were lower and varied from 55% to 83%. Peak geometric mean antibody titres (GMT) varied from 35 IU/L to 2551 IU/L (Table 1.2.1). In addition, all studies showed a decline in antibody levels with time. De Graeff et al. reported a decline in seroconversion rate from a peak of 74%, to 57% at 2 years post-vaccination (42). Similarly, in a study by Stevens et al., the rate dropped from 62.8%, to 49.1% at 2 years. Patients with low peak antibody levels were most likely to become seronegative with time (43).

Vaccine type	Dose	Regimen	Response rate (%)	Peak GMT (IU/L)	Reference
recombinant	40μg	0,1,6	55	91	Bruguera (44)
plasma	40μg	0,1,6	56.5	48.3 (males) 32.4 (females)	Kohler (45)
plasma	5μg	0,1,2	60	121	Crosnier (46)
plasma	40μg	0,1,6	62.8	139	Stevens (43)
recombinant	40μg	0,1,6	65.3	180.7	Jilg (40)
plasma	5μg	0,1,2,4	69.4	268	Benhamou (47)
plasma	3μg	0,1,2	77	35	Lelie (48)
recombinant	20μg	0,1,2,6	80	111	Bruguera (49)
recombinant	40μg	0,1,2,6	83	2551	Guan (50)

Table 1.2.1 Results of trials of hepatitis B vaccination in haemodialysis patients
"Regimen" lists the months after the start of the programme at which doses were administered

Nevertheless, two studies conducted in dialysis units with a high incidence of HBV infection showed significant protection after vaccination. Crosnier et al. randomized 138 patients from 31 units to placebo or vaccine. Protective antibody titres were achieved in 60% of patients after 3 monthly doses of vaccine. The annual incidence of HBV infection measured from the day that the first dose of vaccine was given, was 21% in the vaccinated patients and 45% in the placebo group. Of the 10 infections in vaccinated patients, 8 occurred before all 3 doses of vaccine had been administered (46). Similarly, Desmyter et al. randomized 435 patients from 18 units to placebo or vaccine. Four doses given at 0,1,2 and 5 months resulted in protective HBsAb levels in 75% of patients. At 435 days the infection rate was 4% in the vaccinated group and 18% in the placebo group. Five of the 7 infections in vaccinated patients occurred before the course had been completed and none of those developing infection had developed HBsAb (38). By contrast, a randomized trial involving 1311 patients from 41 units in the USA, failed to demonstrate a protective effect after vaccination. However, only 50.3% of patients responded to the vaccine and the

incidence in both groups was low (vaccine group 6.4%; placebo group 5.4%). HBV infection occurred in only 4 patients who had responded to the vaccine. Of these, 1 had had a renal transplant following vaccination which may be associated with loss of HBsAb. Another patient had had a weak response to the vaccine and was HBsAb negative prior to the infection. The remaining two had low levels (11 and 14 IU/L) of HBsAb (43).

Thus although haemodialysis patients do not respond to hepatitis B vaccines to the same degree as healthy adults, significant protection from infection is achieved in those who develop adequate antibody titres. The decline of antibody levels with time makes annual testing and the administration of booster doses where necessary, an important part of any vaccination programme.

1.2.3.4.2.2.2 Factors affecting response to vaccination

1.2.3.4.2.2.2.1 Patient factors

Numerous trials of vaccination have attempted to analyze patient-related factors which affect response to hepatitis B vaccines in chronic renal failure, although many of these were not specifically designed for this purpose.

The most obvious difference between dialysis patients and normal adults is the uraemia and several studies have examined the effect of the degree of renal failure on response to the vaccine. Dumann et al. reported a response in 60% of chronic renal failure patients with a serum creatinine of 1.5-3.5mg/dl (132-308μmol/l) and in 55.2%

of those with a creatinine of 3.6-16.2mg/dl (317-1426μmol/l) but this difference was not statistically significant (51). In a trial designed to look at different vaccination protocols, Seaworth et al. performed a block analysis of covariance which was unable to demonstrate any effect of serum creatinine levels on the seroconversion rate or on peak antibody levels (52). In a trial designed primarily to examine the effect of the degree of renal failure and patient age on the response to vaccination, Fraser et al. reported an 86% response in patients with a serum creatinine of ≤ 4mg/dl (352μmol/l) and a 37% response in those with a creatinine of >4mg/dl. ($p<0.002$) (53).

The precise nature of the immune defect responsible for the reduced response to hepatitis B vaccine in chronic renal failure patients remains to be determined. Kurz et al. demonstrated a reduced number of T lymphocytes (but with a normal T4 to T8 ratio) and a reduced T cell mitogenic response in 24 haemodialysis patients (54). Meuer et al. studied T cell function in 23 haemodialysis patients who received hepatitis B vaccine. T cells from those patients who were poor responders to the vaccine showed an impaired proliferative response after stimulation with anti-T3 monoclonal antibodies compared to T cells from patients showing a good response to the vaccine. When lymphocytes from non-responders were incubated with monocytes from normal adults, a proliferative response was obtained, suggesting that the defect was in the monocytes. Similarly, incubation of normal lymphocytes with monocytes from the uraemic patients with reduced T cell proliferation in the first experiment, produced an impaired T cell response. T cells of non-responders also demonstrated increased expression of interleukin-2 (IL-2) receptors and enhanced response to exogenous IL-2 . This increased IL-receptor expression was interpreted as being upregulation in response to defective IL-2 production (55). These findings are

supported by an in vivo study which demonstrated significantly higher levels of circulating IL-2 receptor in haemodialysis patients with a weak response to hepatitis B vaccine than in those with a strong response (56). Dumann et al. demonstrated increased IL-2 receptor expression in T cells from patients patients with varying degrees of renal failure not responding to hepatitis B vaccine, suggesting that the immune defect is secondary to renal failure itself, and is not caused by haemodialysis (51). Furthermore when exogenous IL-2 was given with a single dose of vaccine to 10 patients previously not responding to hepatitis B vaccine, 6 developed antibodies (57). It would therefore seem that in patients with chronic renal failure, an aquired defect in monocyte function causes impaired monocyte-T cell interaction which results in decreased IL-2 production, reduced T-cell proliferation and impaired antibody synthesis in response to hepatitis B surface antigen. This defect may also be the reason why a high percentage of dialysis patients are unable to clear the virus after HBV infection and consequently become chronic carriers.

Not all the patients studied by Meuer et al. had evidence of increased IL-2 receptor expression on their lymphocytes (55) and 3 of the 10 patients treated with vaccine plus IL-2 failed to respond (57), suggesting that other mechanisms may also determine antibody responsiveness. Alexiewicz et al. demonstrated that human B cell proliferation is inhibited by PTH in vitro (58). Subsequently, Gaciong et al. examined the effect of different concentrations of PTH on antibody production by B cells from 34 haemodialysis patients and 44 normal controls. Although B cells from control subjects were inhibited by all concentrations of PTH tested, those from dialysis patients were inhibited only at the highest concentration (10^{-6} M). The addition of forskolin or cholera toxin, agents which stimulate cAMP production or the cAMP

analogue 8-bromoadenosine, all inhibited antibody production in B cells from dialysis patients and controls to the same degree, suggesting that PTH may act via cAMP. Furthermore, intracytosolic calcium concentration was shown to be higher in B cells from dialysis patients than those from controls whilst the addition of a calcium ionophore (A23187) inhibited immunoglobulin production by all B cells (59). Further evidence of the role of intracellular calcium concentration was provided by a study of lymphocyte function in hyperparathyroid haemodialysis patients 11 of whom were treated with nifedipine and 12 not on a calcium channel blocking agent. B cell intracellular calcium was elevated to 50% above normal in untreated patients but less elevated in those on a calcium antagonist. The proliferative response of B cells stimulated with *Staphylococcus aureus* Cowan 1 (SAC) was reduced in untreated patients and intermediate in the treated group. B cells from all subjects were inhibited by PTH, but the degree of inhibition was greatest in normal controls, least in untreated patients and intermediate in patients receiving nifedipine. When the adenosine triphosphate (ATP) content of peripheral blood monocytes was measured, it was significantly reduced in untreated dialysis patients but less reduced in those on nifedipine. Finally serum IgG concentrations were significantly lower in patients not receiving nifedipine (60). It would therefore appear that secondary hyperparathyroidism is at least partially responsible for impaired antibody production in some haemodialysis patients and that this effect is mediated via increased cAMP production and raised intracellular calcium concentration. The raised calcium is associated with a reduction in ATP content of cells and an impaired proliferative response. B cells from dialysis patients have a degree of resistance to inhibition by PTH and this is thought to be the result of down-regulation of PTH receptors in response to raised intracellular calcium. These defects of B cell function may be

partially corrected by treatment with calcium channel blockers. PTH has previously been shown to stimulate T cell proliferation and IL-2 synthesis (61) and this mechanism therefore seems to be unrelated to the monocyte dysfunction described above.

Several studies have demonstrated that the seroconversion rate following hepatitis B vaccination decreases with increasing age and this has been attributed to a decline in T cell function (43-46, 53, 62). A similar relationship has been reported in healthy adults and dialysis staff (62, 63).

Genetic factors also affect the antibody response to vaccination in dialysis patients. Two studies have examined the effect of human leukocyte antigens (HLA) on the response to hepatitis B vaccine. Pol et al. found a higher frequency of HLA-A1, B8, and DR3 in dialysis patients who did not respond to the vaccine (64). Similarly, Stachowski et al. reported a higher frequency of HLA-A1, B8, DR3 and DQ2 in non-responders. Within the responder group, HLA-A1, B27, and Cw2 were associated with a strong response whereas patients with HLA-A2, B7 and DR4 had a weak response. Similar but less marked differences were found in major histocompatibility complex (MHC) class III alleles (65).

Stevens et al. reported seroconversion in 100% of female patients but in only 76.5% of male patients (66). Other studies have also reported higher seroconversion rates in females, but in none of these was the difference statistically significant (38, 40, 43, 45, 46, 48). One study found a higher seroconversion rate in males (67). Males are

more likely to become chronic carriers than females, also suggesting a gender-related difference in immune response to HBV (19).

One study has reported a positive association between time on dialysis prior to vaccination and antibody response. The explanation offered for this finding is that patients who survive longer on dialysis are healthier and thus better able to develop a response (62). Other studies however, have been unable to demonstrate such an association (45, 68).

Erythropoietin therapy was associated with a higher (though not statistically significant) seroconversion rate (80% vs. 54%) in a trial involving 37 haemodialysis patients. Peak antibody levels were significantly higher in the treated group and were associated with a higher T helper/suppressor cell ratio (68). However, this finding could not be confirmed by Navarro et al.(67). The latter study also reported a lower seroconversion rate in patients with chronic hepatitis C virus (HCV) infection.

Other patient-related factors where weak or conflicting effects on the response to hepatitis B vaccine have been reported include aetiology of primary renal disease (45, 62), nutritional status (69), zinc supplementation (70) and treatment with thymopentin (71, 72).

1.2.3.4.2.2.2.2 Vaccine factors

The low seroconversion rate in haemodialysis patients has led investigators to examine vaccine-related factors which may be manipulated in order to improve the response.

Studies in healthy adults have shown a better seroconversion rate with higher antibody levels in those subjects who recieved injections into the deltoid muscle than in those who received gluteal injections (73, 74). Different studies in dialysis patients have used either deltoid (44, 49, 68, 75), gluteal (40) or subcutaneous (46, 47) injections and have reported similar response rates.

Many of the earlier trials employed 3 doses of vaccine given at 0,1and 6 months, which is the recommendation in healthy adults. Several studies have examined the use of an increased number of doses or higher individual doses in dialysis patients. Benhamou et al. compared three 5μg doses given at 0,1 and 2 months with 4 doses given at 0,1,2 and 4 months and reported a significantly higher seroconversion rate and higher mean antibody levels with the 4-dose schedule (47). However, another study was unable to show an improved response in dialysis patients who received six 40μg doses at monthly intervals, when compared to a group who received only 3 doses (40). Bruguera et al. demonstrated that vaccine given at 0,1,2 and 6 months produced seroconversions at a similar rate to that given at 0,1,2 and 12 months (49). The first schedule is preferable because of it's shorter duration.

Attempts to improve the response by increasing the dose of vaccine have also been reported. Bruguera et al. compared the standard dose of 20μg recombinant vaccine with 40μg and found no difference in seroconversion rate or antibody levels after 4 doses of each (49). However, Jilg et al. reported a higher response rate (74% vs.42%) in patients receiving six 40μg doses than those receiving six 20μg doses (40). Similarly, another group using a plasma derived vaccine found an increased response in those patients receiving double the standard dose of vaccine. The increase in response was similar to that obtained if a fourth dose was added to the schedule of those receiving a standard dose (47). Lelie et al. demonstrated an improved seroconversion rate and higher antibody levels after using 27μg instead of the standard 3μg per dose in a schedule of 3 doses given at 1 month intervals. Similar results were obtained in another study using the same doseage schedule (42).

The development of vaccines employing HBsAg derived from yeasts through genetic engineering led to comparisons of immunogenicity with plasma-derived vaccines. Seaworth et al. found a significantly better seroconversion rate in chronic renal failure patients who received a plasma-derived vaccine (81% vs.58%) (52). However, Jungers et al. reported a higher serocoversion rate in similar patients who received recombinant vaccine (85% vs. 67%). In this trial antibody levels in responders from both groups were not significantly different.(75)

It would appear that plasma- and yeast-derived vaccines are of similar immunogenicity and that the response to vaccination may be improved by using higher individual doses of vaccine or by increasing the number of doses.

1.3 Hepatitis C Virus

1.3.1 Biology of Hepatitis C Virus

1.3.1.1 Structure and Replication

In 1988 the agent responsible for most cases of non-A non-B hepatitis was cloned and named hepatitis C virus (HCV). It is a small RNA virus with a diameter of 30-38nm and is classified as a separate genus in the family Flaviviridae.

HCV is composed of a single strand of RNA, a single putative nucleocapsid protein and two envelope glycoproteins. The genome consists of 9379-9481 nucleotides in a single large open reading frame. At the 5' end is a highly conserved region which is thought to be involved in translation of the viral genome. Downstream from this are regions coding for the core protein (p22) and two envelope glycoproteins (E1 and E2). The rest of the genome contains 4 non-structural regions which encode proteins responsible for viral replication. These include proteases (NS2, NS3), helicase (NS3), and an RNA-dependent RNA polymerase. The 3' terminal region is highly variable in length and sequence. Replication of viral RNA takes place via a minus-strand RNA intermediate template. The RNA is probably translated into a single protein of 3011 amino acids which is then split into different functional proteins.

Studies on different HCV isolates have revealed considerable sequence variation within the genome. A hypervariable region has been identified at the 3' end of the E2 region which may play a part in enabling the virus to escape host immune defences. Based on genomic variation, HCV has been classified into at least 6 distinct genotypes which differ in geographical distribution (Simmonds' type 1 and 2 - world-wide, type 3 - Europe, type 4 - Middle East and Egypt, type 5 - South Africa, type 6 - Hong Kong) and may also differ in virulence, transmissability, cell tropism and response to interferon therapy (76, 77).

1.3.1.2 Transmission

As HCV is present in the blood of infected patients at low levels, parenteral routes of transmission are most effective in spreading the infection. Prior to the screening of blood donations, transfusions were an important cause of transmission and HCV was recognized as the main cause of post-transfusion hepatitis. 50%-90% of haemophiliacs became infected through exposure to blood or blood products. Intravenous drug use with associated sharing of needles is another important means of transmission. The infection may also be spread by needle-stick accidents and the risk of infection after such an event is 3%-10%.

Up to 50% of patients with HCV infection have no history of parenteral exposure to the virus. Many of these cases are thought to be due to unnoticed parenteral transmission but other as yet unidentified routes may also be involved. Vertical transmission from mother to infant occurs in up to 10% of children born to mothers positive for HCV RNA. This figure is reported to be as high as 50% if the mother is

co-infected with HIV. Spread between members of the same family is uncommon, but has been described. Similarly, sexual transmission seems to occur at a low rate. (76, 77)

1.3.1.3 Prevalence of HCV infection

There is considerable geographical variation in the prevalence of HCV infection world-wide. Among blood donors in Northern Europe, Canada, and the northern part of the USA, the prevalence is reported to be 0.01%-0.05% while in Southern Europe, Japan, Africa, the Middle East and the southern part of the USA it is 0.5%-1.5%. The highest prevalence is in Egypt, where 22% of blood donors were found to be positive for HCV antibodies. Part of the explanation for this may be the use of paid blood donors.

In South Africa the prevalence of HCV infection varies in different ethnic groups and in different regions. One population-based study reported a prevalence of 3.84% in rural blacks and 1.2% in urban blacks. A similar study from KwaZulu-Natal showed HCV infection in 1.7% of urban blacks and 0.9% of rural blacks. In the Cape Town, a study of black adults presenting to Day Hospitals found a prevalence of 3.7% in those with formal housing and 2% in those from informal settlements (5, 76, 77).

1.3.1.4 Natural history of infection

Most of the data on acute HCV infection is derived from studies of post-transfusion hepatitis. In this setting the incubation period is usually 6 to 12 weeks but it may vary

from 2 to 26 weeks. Most patients are asymptomatic and jaundice occurs in only10%. Generally the disease is mild although rare cases of fulminant hepatitis have been described.

Most of the morbidity and mortality associated with HCV is related to chronic infection. Up to 90% of infections become chronic (78) and at 12 months 50-75% of patients will have abnormal liver transaminases and chronic hepatitis on histology (79). Chronic hepatitis may also be present in the absence of abnormal transaminases. Although most patients remain asymptomatic, the disease progresses and chronic hepatitis, cirrhosis and HCC are found at mean intervals of 10, 21 and 29 years post-infection respectively (80). Cirrhosis occurs in 20-50% of cases (81, 82). The incidence of HCC is thought to be low but has not yet been adequately quantified.

A high prevalence (18%-30%) of HCV infection has been reported in patients with alcoholic liver disease. The virus also seems to have a synergistic effect with alcohol in causing liver damage as patients develop liver disease at a younger age than those who are HCV negative.

HCV may induce an auto-immune process in the liver. Auto-antibodies to a variety of liver antigens have been identified in patients with HCV infection and some have responded to steroid therapy in the same way as uninfected patients with auto-immune hepatitis.

A strong association exists between sporadic cases of porphyria cutanea tarda (PCT) and HCV. 62%-82% of patients with PCT have HCV infection and it is thought that the virus either triggers or increases susceptibility to the disease (83, 84).

Several extrahepatic disorders are associated with HCV infection and these include type II mixed cryoglobulinaemia, glomerulonephritis (mainly mesangiocapillary), lymphocytic sialadenitis and lichen planus.

Therapy with interferon α results in normalisation of liver transaminases and clearance of HCV viraemia in about 50% of cases after 6 months of therapy. However, 50% of responders relapse within 6 months of stopping therapy and thus a sustained response is achieved in only 25%. Interferon therapy is expensive and is associated with significant adverse effects including flu-like symptoms, leucopaenia, thrombocytopaenia, depression and autoimmune phenomena. It is therefore not clear whether interferon therapy for these patients is cost effective (76, 77).

1.3.2 Testing for HCV infection

As HCV is present in very low concentrations in blood, infection is usually identified by the detection of antibodies to viral antigens.

First-generation assays employed a recombinant viral peptide (C100-3) derived from the non-structural NS4 region of the genome with an enzyme-linked immunosorbent assay (ELISA). As this peptide represents only 4% of the viral genome, the sensitivity of this test was low. Subsequently, sensitivity and specificity have been

improved by adding further recombinant antigens. Second generation tests use antigens derived from the core (C22-3), NS3 and NS4 regions (C200). In the third generation assays, an antigen from the NS5 region has been added.

The mean time from infection to the development of antibodies is 12 weeks but may be as long as 6 months. Antibodies to core antigen develop early in the course of infection and therefore second and third generation assays are able to detect infection earlier than first generation tests. Anti-core antibodies decline with resolution of the hepatitis but rise with active disease and may therefore be useful is assessing activity.

Measurement of IgM antibodies has shown that anti-core IgM appears early in the infection and persists for about 8 weeks. However the response is variable and IgM may appear after IgG in some cases or remain positive in chronic HCV infection. Thus the presence of IgM does not necessarily denote acute infection.

Although third generation ELISA assays have a high specificity (99.7%), the low prevalence of HCV particularly in blood donors means that false positives will occur according to Baye's theorem. Thus positive tests should be confirmed with supplementary tests. Recombinant immunoblot assays (RIBA) employ nitrocellulose strips with HCV antigens applied separately to them and are thus able to detect antibodies to individual antigens. Third generation RIBA tests employ recombinant and synthetic peptides derived from the core, NS3, NS4 and NS5 regions. Detection of antibodies to antigens from 2 or more regions is regarded as a positive result. There is a strong correlation between a positive RIBA and viraemia and it may therefore be useful in distinguishing between infectious and non-infectious patients.

Reactivity with only one antigen is indeterminate and may be the result of non-specific IgG binding, resolved or early infection.

Limitations of antibody testing make detection of viral RNA a useful diagnostic tool. Owing to the low levels of HCV RNA in blood, this can be achieved only by amplification of the RNA by PCR. Viral RNA is transcribed into complementary DNA by a reverse transcriptase enzyme before being amplified by the use of a DNA polymerase. Reverse transcriptase PCR (RT-PCR) and is able to detect very low levels of HCV RNA (100-1000 copies/ml). Primers which bind to the highly conserved 5' terminal region of the genome are used and thus RNA from all strains of HCV is detected. The test is useful for investigating patients with indeterminate RIBA results, early detection of infection, determination of infectivity in antibody positive patients, follow-up of anti-viral therapy and monitoring of perinatal transmission. However, the technique has been difficult to standardize and both false positive and false negatives are common. An HCV PCR kit has recently become available commercially and it is hoped that this will improve the problem. HCV RNA is rapidly degraded at room temperature and by freeze-thawing (85). Thus delays in getting specimens to the laboratory may account for some false negative tests. (76, 77, 86)

1.3.3 HCV infection in haemodialysis patients

1.3.3.1 Prevalence

Studies of hepatitis B drew attention to the fact that non-A non-B (NANB) hepatitis was also a significant problem in haemodialysis patients (35). It was not until after assays for anti-HCV antibodies became available in 1989, that the extent of the problem became apparent. Early surveys using first generation ELISA assays reported a prevalence of 0%-37% (87-90), but more sensitive second generation tests showed that that it was even higher than this. Centres from different countries found the prevalence to range from 4.7% to 68%. (Table 1.3.1)

Country	Number tested	% anti-HCV +	Reference
Denmark	340	4.7	Knudsen (91)
Belgium	399	13.5	Jadoul (92)
Germany	195	15	Da Silva Cardoso (93)
South Africa	103	21	Cassidy (94)
USA	208	21	Du Bois (95)
Hong Kong	51	21.6	Chan (96)
Italy	48	23	Cantu (97)
France	923	25	Dussol (98)
Venezuela	315	39	Muller (99)
France	217	39.6	Simon (100)
Japan	167	44	Oguchi (101)
France	115	54	Chaveau (102)
Italy	277	55	Mondelli (103)
Saudi Arabia	1147	68	Huraib (104)

Table 1.3.1. The prevalence of antibodies to HCV as measured by second generation assays at different centres.

Haemodialysis patients have been shown to have a higher prevalence than those on peritoneal dialysis (Table 1.3.2) and a much higher prevalence than blood donors.

Reference	ELISA	CAPD		HD	
		number tested	% positive	number tested	% positive
Chan (105)	1st generation	278	1.8	61	16.4
Brugnano (106)	1st generation	64	4.8	205	13.3
Cantu (97)	2nd generation	29	17	48	23
Dussol (98)	2nd generation	61	8	923	25

Table 1.3.2. Studies comparing the prevalence of anti-HCV in haemodialysis (HD) and peritoneal dialysis (CAPD) patients.

1.3.3.2 Modes of transmission

HCV seems to be transmitted by factors specifically associated with haemodialysis. Almost all studies have found an association between the risk of HCV infection and time on haemodialysis. Blood transfusions, renal transplantation from an HCV-infected donor and nosocomial spread have all been identified as modes of transmission.

Many studies have demonstrated an association with blood transfusions, which were the single largest source of HCV infections in the general population prior to the screening of blood donations (91, 92, 98, 99, 102, 103, 107). However, several centres have documented cases of HCV infection in haemodialysis patients with no previous blood transfusions (89, 98, 99, 108, 109), indicating that other modes of transmission must also exist.

Infection following transplantion of a kidney from an HCV-positive donor has been well documented (110-112). Another study reported an association between the number of previous renal transplants and anti-HCV positivity in haemodialysis patients (98). However, this route probably accounts for relatively few infections.

Of particular concern is the potential for the transmission of HCV between patients in haemodialysis units. Several studies have provided direct evidence that this does occur. Sampietro et al. employed single strand conformational polymorphism (SSCP) analysis of PCR product to investigate the viral strains in 28 patients positive for HCV RNA in one dialysis unit and compared these with strains from 16 patients with chronic liver disease but no renal failure. The study found a significantly higher prevalence of two SSCP patterns in the dialysis patients than in controls. Sequence analysis subsequently showed one of theses patterns to be associated with the type 4 HCV genotype, which is extremely rare in the general population of Italy (113). By sequencing the hypervariable region 1 of the E2-gene of HCV in isolates from 3 haemodialysis patients who seroconverted at the same time, Allander et al. were able to demonstrate that all three were infected by the same strain. The 3 seroconverted within 3 months of each other and were dialyzed on the same shift. A fourth patient from the shift was found to be infected with a similar strain and a fifth patient who had been on the shift later acquired a similar strain (114). Similarly, Stuyver et al. found that 23 anti-HCV positive patients from one dialysis unit, were infected with the same genotype, 1b. Sequencing of the core region of the genome revealed a rare but specific variation in the nucleotide sequence in 9 of 16 sera. Subsequent use of a

probe to detect this sequence showed that it was present in 20 of the 23 patients, providing strong evidence of transmission between patients (115).

The relative importance of different modes of transmission between patients requires further elucidation. Some authors have suggested that contamination of dialysis machines may cause "vertical" transmission between patients using the same machine. One outbreak of HCV in a dialysis unit may have been linked to the sporadic reflux of patient blood across transducers in the machines, although inadequate infection control measures were also identified as a contributing factor (116, 117). Chiaramonte et al. reported HCV infections in 12 patients who were using machines which either did not have fully disposable circuits or had ultrafiltration control devices which allow recirculation of a small amount of dialysate (118). In addition, Simon et al. reported seroconversion in 4 patients who were all dialyzed on the same machine in a two month period (100). The fact that the diameter of HCV (30-38nm) is at least 10 times the pore size of dialysis membranes (1-3nm) suggests that intact membrane should prevent the passage of HCV from the blood into the dialysate. However, it is possible that microfractures in the membrane may allow this to occur. Although some investigators have identified HCV RNA in dialysate from infected patients (119) others did not find this (120-123).

Evidence from different centres suggests that "horizontal" transmission of HCV between patients does occur. One study reported seroconversion in 3 patients who were all dialyzed next to an HCV positive patient (92) while another reported HCV infection with a similar strain of the virus in 5 patients who were all dialyzed on the same shift (114). Similarly, Okuda et al. noted 3 patients who developed acute

hepatitis C within 2 weeks of each other, who were dialyzed on adjacent machines (124). In a multicentre study in Italy, one unit reported 8 cases in patients on the same shift over a 9 month period (109). Thus, horizontal spread between patients due to defective infection control measures appears to be the most important mode of nosocomial transmission.

The widespread introduction of dialyzer reuse as a cost-saving measure has led to concern that this may contribute to the nosocomial transmission of HCV. Contamination of one dialyzer could lead to cross-contamination of other dialyzers during cleaning procedures if the virus is not inactivated by the sterilizing process. However, a multicentre study involving 401 patients which reported strong evidence of nosocomial transmission, was unable to demonstrate an association between seroconversion and dialyzer reuse (92). Furthermore, Chiapelli et al. were unable to detect HCV RNA in ultrafiltrate from the dialyzers of HCV RNA positive patients with either first use or reuse (120). Finally, in a study in which 103 patients were randomized to reuse or single use of dialyzers, there was no statistically significant difference in the incidence of HCV infection between the two groups (17.6% in the reuse group vs. 34.6% in the single use group) (125).

Haemodialysis patients remain at risk of acquiring HCV infection by the same routes as the general population. Two studies from the USA have identified intravenous drug use as an important risk factor for hepatitis C in these patients (90, 95).

1.3.3.3 Natural history of infection

In the majority of haemodialysis patients, acute HCV infection is a subclinical or mild illness and 0%-17% of patients become jaundiced (100, 124, 126, 127). However, liver transaminase levels are elevated in 65%-100% (92, 100, 126, 127). In one study the liver enzyme abnormality coincided with seroconversion in 50%, preceded it by 1 week to 5 months in 36% and occurred only after seroconversion in 14% (100). Another study however, found that elevated transaminase levels preceded seroconversion in all cases by 1 to 6 months (92). Enzyme elevation may consist of a single peak or multiple peaks (92, 100, 127).

The infection becomes chronic in the majority of cases. Studies have shown that 44%-85% of anti-HCV positive dialysis patients have HCV RNA detectable in their blood, implying that there is ongoing viral replication and that the patients remain infectious (95, 100, 128, 129). Some studies have identified patients who are anti-HCV negative and HCV RNA positive (93, 96, 130) although in other studies all PCR positive patients were also antibody positive (95, 129). Thus studies based on antibody testing alone may underestimate prevalence by up to 20% (130).

Only limited data is currently available on the long-term sequelae of HCV infection in haemodialysis patients. Three clinical patterns were identified in one study in which 78 anti-HCV positive patients were followed up for at least a year (median 5 years). The first was found in 69.2% and was characterized by persistently elevated gamma glutamyltransferase (γGT) levels, suggesting ongoing liver disease. ALT was elevated or fluctuating in 30.8%. 14 of 14 patients tested were positive for HCV RNA and all

8 patients who underwent liver biopsies had chronic active hepatitis. One patient also had HCC. Patients with the second pattern comprised 20.5% and had normal ALT and γGT levels although they remained persistently anti-HCV possitive. 5 of 7 patients tested were PCR positive and none of 8 liver biopsies showed chronic hepatitis. These features were interpreted as representing a healthy chronic carrier state. A third pattern suggesting clearance of the infection was seen in 10.3%. Liver enzymes remained normal and there was either complete loss of anti-HCV or gradual loss of different antibodies on the RIBA test. Two patients tested were negative for HCV RNA. In this series progression of the liver disease with time was suggested by the finding that only 2 of 8 liver biopsies performed within 2 years of the onset of infection showed chronic active hepatitis, while 6 of 11 biopsies performed between 3 and 13 years showed these changes. In two patients who had repeat biopsies, there was progession from discrete portal fibrosis to chronic active hepatitis in 6 and 7 years respectively (100). In another study, liver biopsies were performed in 17 anti-HCV positive dialysis patients. Chronic active hepatitis was present in 16 and cirrhosis in 2. Elevated liver enzymes were found in only 31.3% of those with chronic hepatitis (128). In a multicentre study in Spain, liver biopsies in 119 anti-HCV positive patients revealed normal histology in 7.5%, chronic active hepatitis in 35.3%, chronic persistent hepatitis in 26.8%, cirrhosis in 13.4%, haemosiderosis in 7.5% and fatty liver in 4.2% (131).

The course of HCV infection following renal transplantation has not yet been adequately defined. Berthoux et al. reported follow-up in 117 anti-HCV positive renal transplant recipients after a mean of 62 months (range 24-111 months). Persistently abnormal or fluctuating ALT levels were present in only 19.7%.

However liver biopsies performed in 59 patients showed normal histology in 13.6%, portal lesions in 23.7%, acute hepatitis in 8.5%, chronic active hepatitis in 44.1% and cirrhosis in 1.7%. Of the 26 patients with chronic active hepatitis, 12 (46%) had abnormal,liver enzymes. There was no difference in short term patient or graft survival between anti-HCV positive and negative patients. However, positive patients had a higher incidence of mesangiocapillary glomerulonephritis in the graft kidney (6% vs. 0.7%) (32). Ponz et al. followed up 67 transplant recipients for a mean of 32 months. At time of transplantation, 32 (48%) were anti-HCV positive, but 9 of these became seronegative at a mean of 27 months post transplant. Ten of 15 (66%) anti-HCV positive patients who had no clinical or biochemical evidence of liver disease developed raised transaminase levels after transplantation, although all remained asymptomatic. There was no difference in the incidence of acute rejection episodes, degree of renal impairment or mortality between anti-HCV positive and negative patients (132). By contrast, Roth et al. reported a higher incidence of serious infections and acute rejection episodes in anti-HCV positive recipients (133). Patients coinfected with HBV and HCV may be at risk of developing more aggressive liver disease. Huang et al. found a higher prevalence of biochemically defined chronic hepatitis (50% vs. 25%) and radiologically defined cirrhosis (21.4% vs. 0%) in coinfected patients than in those with only HCV infection (134).

Preliminary results of treatment of HCV infection in dialysis patients with interferon α-2b have been disappointing. In one study, patients received 5 million units thrice weekly for 4 months. Fifteen of 23 patients who completed the treatment course (65%) no longer had detectable HCV RNA at the end of the treatment, but within 5 months, 5 of the 15 responders had relapsed. Thus only 10 of 37 patients who

commenced treatment (27%) had a prolonged response (135). Following renal transplantation, interferon therapy has been complicated by severe graft rejection and is therefore contraindicated (32).

Available data thus suggests that HCV infection causes chronic liver disease in 40%-70% of both dialysis patients and renal transplant recipients. In the majority this is clinically and biochemically silent. To date studies have not found an increase in mortality associated with HCV infection but longer follow-up is required to define the natural history of the liver disease in these patients.

1.3.3.4 Prevention

Screening of all blood donations for anti-HCV and raised ALT is the logical way to prevent post-transfusion hepatitis. One haemodialysis centre reported a marked decline in the incidence of new infections after introduction of these measures (100). However, there is disagreement as to which measures are necessary to prevent the transmission of HCV between patients in haemodialysis units.

Several studies have demonstrated a decrease in the incidence of HCV infections after anti-HCV positive patients were isolated to separate units (136, 137). Chiaramonte et al. reported that transfer of anti-HCV positive patients to machines with fully disposable circuits stopped further transmission in an outbreak of NANB hepatitis (118). Furthermore, a multicentre study in Spain found a higher seroconversion rate in units not isolating anti-HCV positive patients (138).

However, although isolation is effective in preventing nosocomial transmission, other studies suggest that it may not be necessary. Gilli et al. compared one unit where anti-HCV positive patients were dialyzed on separate machines with a second unit where all patients shared machines. Although the prevalence of anti-HCV positivity at the start of the study was 29.8% and 53% respectively, no seroconversions occurred in either unit during 42 months of follow-up (139). Similarly, another study reported that confinement of positive patients to separate machines in two dialysis units and increased attention to infection control measures in a third, both resulted in a significant decline in the incidence of HCV infection (140). After seroconversion had occurred in 49 of 730 patients treated in 2 dialysis units in Japan during a 6 month period, inadequate glove-changing practices by nursing staff were identified as a potential factor contributing to transmission. Following re-education of nursing staff and the introduction of a new impermeable dressing for puncture sites after dialysis, no further seroconversions occurred during the following year (124). Thus a significant reduction in incidence was achieved without isolating positive patients.

1.4 Study Aims

This study was conducted to determine optimum strategies for the control of hepatitis B and C virus infections in chronic haemodialysis patients at Groote Schuur Hospital with the following specific aims:

1. To establish a protocol for routine surveillance HBV and HCV infections.
2. To assess response to hepatitis B vaccination and the cost-effectiveness of this intervention.
3. To identify factors responsible for the high prevalence of HCV infection.
4. To determine whether current infection control measures are effective in containing spread of the viruses without isolating HCV positive patients or confining them to separate machines.
5. To investigate whether routine reuse of dialyzers from HCV-infected patients can be practised without viral spread.

2. Methods

2.1.1 Patients

Patients who had been on haemodialysis for 1 month or longer and who had renal failure which was not expected to recover were regarded as being on chronic dialysis. These patients receive 4 hours of dialysis three times per week on a variety of dialysis machines. A few patients receive "high flux" dialysis but the majority are on standard polysulphone dialyzers. Dialysis with bicarbonate as the buffer is used in about 30% of patients. HBsAg positive patients are dialyzed in an isolation unit. Anti-HCV positive patients are not isolated to separate units or separate machines.

2.1.2 Viral surveillance

Blood is taken monthly as part of an ongoing viral surveillance programme. HBsAg screening is performed monthly on all HBs antibody negative patients. Anti-HCV antibodies are measured every three months. In patients who are found to be anti-HCV positive, PCR is performed to detect viral RNA. Serum ALT and AST levels are measured monthly and any elevations are thoroughly investigated. Systematic recording of all these results was commenced in December 1992. Patient records were used to obtain the following details: age, gender, race, total time on haemodialysis including time prior to previous transplants, number of previous blood transfusions, number of previous renal transplants.

2.1.3 Reuse of dialyzers

After each use dialyzers are rinsed with water and then sterilized by means of a fully automated system (Renatron) which utilizes Hydrogen Peroxide and Peracetic Acid as sterilizing agents. Dialyzers from all patients including anti-HCV positive patients are reused, but those from HBsAg positive patients are not. Capillary patency is checked after each reuse and a dialyzer is discarded when fibre volume patency is reduced by more than 20%. Standard dialyzers are used approximately 15 times and high-flux dialyzers, 20 to 25 times.

2.1.4 Hepatitis B vaccination

In March 1996 all HBsAg negative haemodialysis patients were screened for anti-HBs and anti-HBc IgG antibodies. All patients who were negative for both antibodies were included in the vaccination programme. A plasma-derived vaccine containing heat inactivated HBsAg and aluminium phosphate was used (Hepaccine-B Vaccine, Cheil Foods and Chemicals Inc.). The standard dose of 3μg was administered intramuscularly into the deltoid region at 0,1,2 and 4 months starting in April 1996. Anti-HBs antibodies were measured at 1 and 2 months after the third dose and at 1 and 2 months after the final dose (ie. at 3, 4, 5 and 6 months after the first dose).

For purposes of the hepatitis B vaccination study, patient height was measured and post-dialysis weights were recorded on three consecutive dialysis days. Body mass index (BMI) was calculated using the formula: average post-dialysis weight (kg) ÷

height squared (m^2). Serum urea concentration before and after dialysis were measured at month 1 and the urea reduction ratio (URR) calculated using the formula: (pre-dialysis urea – post-dialysis urea) ÷ pre-dialysis urea. Serum albumin concentration was measured monthly and the average of the values at months 0, 1 and 2 as well as the lowest value were recorded. Parathyroid hormone levels were measured at month 4. Patient folders were scrutinized and the following details recorded: age, race, gender, total time on the renal replacement programme (including time during which patients had functioning renal transplants), number of previous renal transplants, erythropoietin therapy and use of calcium channel blocking agents.

2.1.5 Assay techniques

HBsAg was detected using a radioimmunoassy (AUSRIA, Abbott Laboratories). Antibodies to HBsAg and antiHBc IgG were both measured with microparticle enzyme immunoassays (IMx AUSAB and IMx CORE, Abbott Laboratories). Anti-HCV antibodies were detected using a second-generation ELISA test (Ortho HCV 2.0 ELISA, Ortho Diagnostic Systems) which detects antibodies to both structural C22-3 and non-structural C200 recombinant HCV antigens. In late 1993 this was changed to a third-generation ELISA test (Ortho HCV 3.0 ELISA, Ortho Diagnostic Systems) which detects antibodies to the NS5 region of the HCV genome in addition to the above two. PCR was performed using a reverse transcriptase PCR assay with primers from the highly conserved non-coding region 5 of the HCV genome. Serum urea, albumin, AST and ALT were measured using standard autoanalyser techniques. Parathyroid hormone was measured with a coated bead immunofluorometric immunoassay performed on an automated Immulite system (Diagnostic Products).

All tests were performed by the Virology and Chemical Pathology laboratories of Groote Schuur Hospital.

2.1.6 Statistics

Data was recorded on a personal computer on spreadsheets generated with "Quattro Pro" (Borland Office). Analysis was performed using statistical tests provided in the software. The students t-test was used for testing the difference between the means of independent samples, and a chi-squared (χ^2) test for 2 x 2 tables of frequency. Stepwise multiple regression analysis was performed using "Statgraphics".

3. Results

3.1 Hepatitis B

3.1.1 HBV infections

There were no new cases of HBV infection detected in chronic dialysis patients from the time that data collection was commenced in December 1992 until the start of the vaccination programme in April 1996 (40 months). All specimens tested were negative for HBsAg.

3.1.2 HBV antibodies prior to vaccination

In March 1996 there were 79 HBsAg-negative patients on chronic haemodialysis. Results of anti-HBs and anti-HBc testing are shown in Figure 3.1.1. 26 patients (33%) were positive for both antibodies, indicating that they have previously been infected with HBV but have cleared the virus. 5 patients (6%) were positive for anti-HBs but negative for anti-HBc suggesting previous vaccination. An analysis of data to test for relationships between previous HBV infection and demographic or treatment-related factors is shown in Table 3.1.1. The prevalence of previous infection was 40% in black patients, 35% in those of mixed race origin, and 0% in white patients. There was no difference in the mean age of previously infected and uninfected patients. Males and females had a similar prevalence of previous

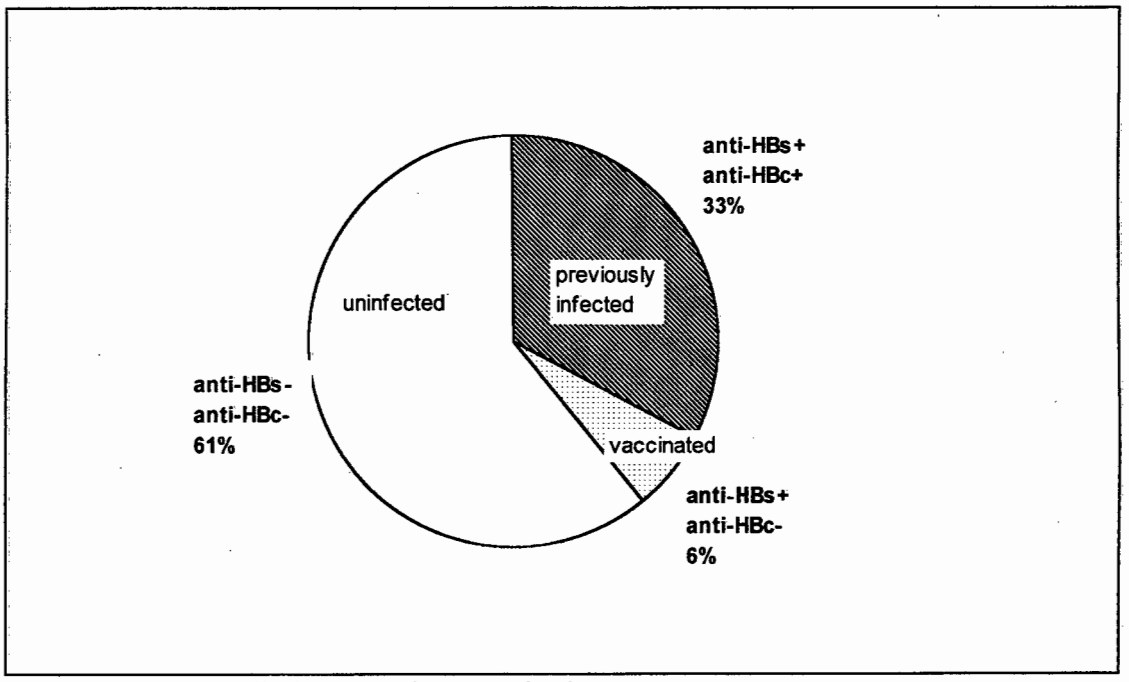

Figure 3.1.1 HBV antibody status prior to vaccination

infection. Means for time on haemodialysis, number of previous blood transfusions and number of previous renal transplants were also not significantly different.

	Total	anti-HBc +	anti-HBc -	p value
Number	79	26	53	
Black	20	8	12	0.046*
Mixed Race	52	18	34	0.064*
White	7	0	7	
Female	38	11	27	0.47**
Male	41	15	26	
Mean age (years)	43.2 ± 11.8	44 ± 9.3	42.8 ± 12.9	0.68***
Months on HD	35 ± 37.9	38 ± 45	33.5 ± 34.4	0.62***
Transfusions (units)	4.6 ± 7.9	3.9 ± 9.3	5 ± 7.3	0.56***
Renal transplants	0.81 ± 0.92	0.65 ± 0.8	0.89 ± 0.97	0.29***

Table 3.1.1 Demographic data and possible risk factors for previous HBV infection in chronic haemodialysis patients. Values for months on haemodialysis (HD), transfusion units and renal transplants are expressed as mean ± standard daviation.
* p value for comparison with white patients
** p value for comparison with males
*** p value for comparison between anti-HBc positive and negative patients

3.1.3 Response to vaccination

48 patients were negative for both antibodies and therefore eligible for vaccination. Three patients received renal transplants before the vaccination was commenced and thus 45 received the first dose. One patient died and a further 8 recieved renal transplants during the vaccination and follow-up period. Thus 36 patients received all 4 doses of vaccine and had their antibody response measured. No patients reported adverse effects related to vaccination. Figure 3.1.2 illustrates the mean antibody titres in those responding to the vaccine at different time intervals. After the initial three

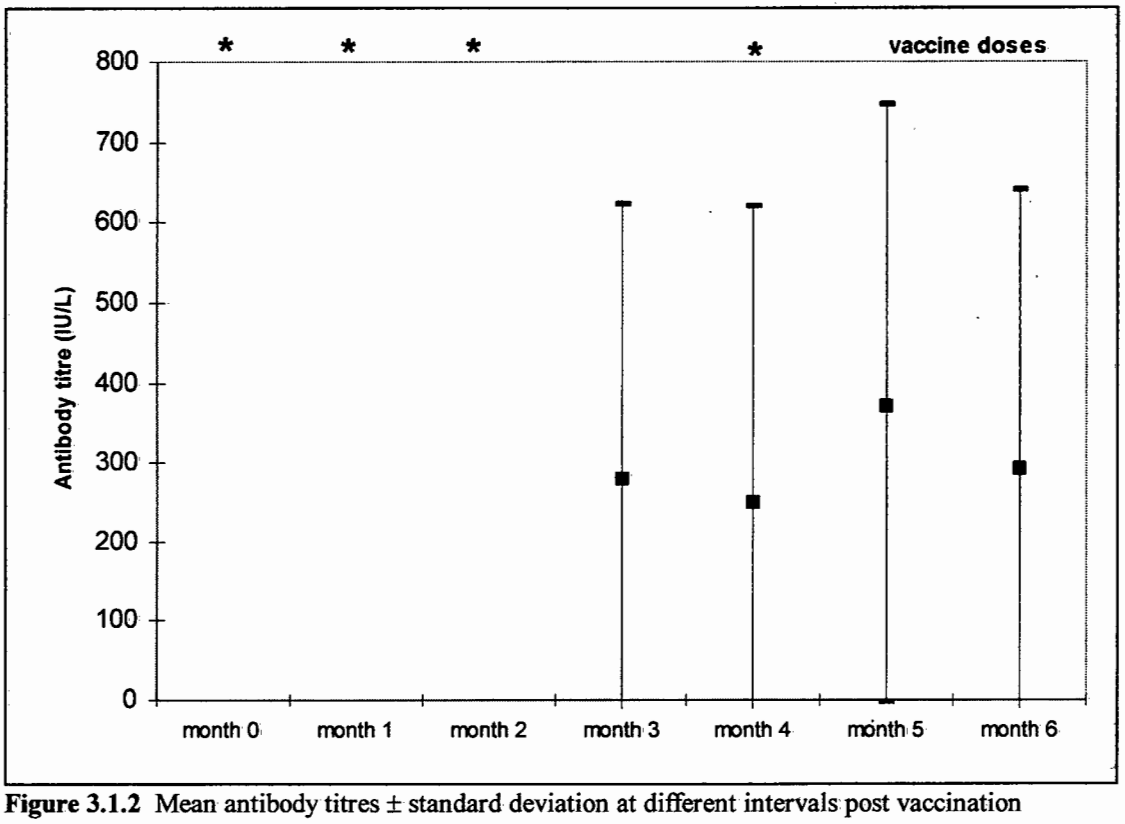

Figure 3.1.2 Mean antibody titres ± standard deviation at different intervals post vaccination

doses, the peak GMT (279 IU/L) was recorded at one month after the third dose (month 3), although there was a further rise in antibody titre from month 3 to month 4 in 15 patients. Following the booster dose, the peak GMT (372 IU/L) was again

noted at one month after the dose (month 5). Most patients had a decline in antibody level from month 5 to month 6 and only 4 had a further increase. Figure 3.1.3 illustrates the number of patients who had responded and the number who had achieved the minimum protective antibody level of 10 IU/L at different times post vaccination. There was a progressive increase in the number responding and the number achieving protective levels from month 3 to month 6.

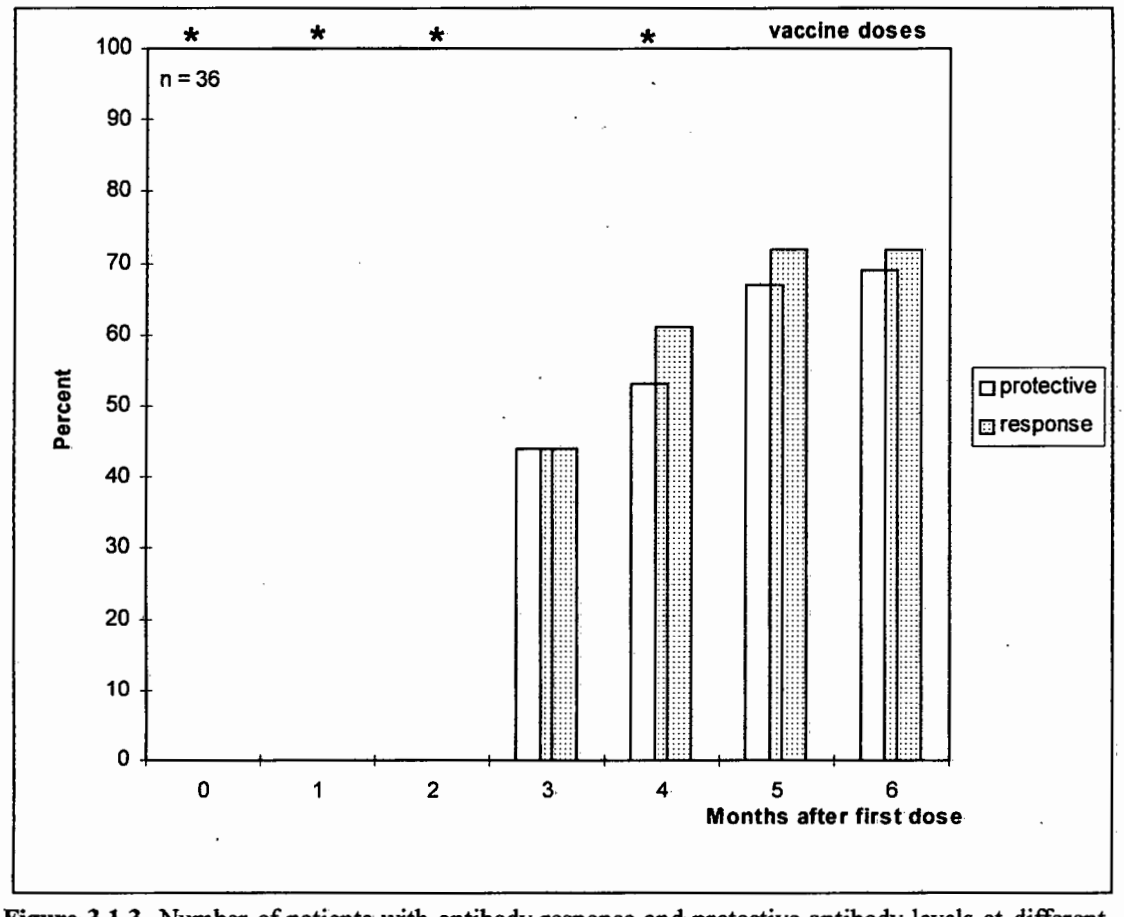

Figure 3.1.3 Number of patients with antibody response and protective antibody levels at different intervals post vaccination

The maximum response was achieved at 2 months after the 4th dose of vaccine, when 26 of 36 (72%) of patients were anti-HBs positive and 25 of 36 (69%) had achieved protective levels.

3.1.4 Factors affecting the response to vaccination

The response rate was 80% (16 of 20) in female patients and 63% (10 of 16) in male patients, but this difference was not statistically significant ($p = 0.24$). Although female patients had a higher mean antibody titre than males at 2 months after the 4th dose of vaccine (346 vs. 207 IU/L), this difference also was not significant ($p = 0.33$).

A stepwise multiple regression analysis was used to assess the effect of a number of different continuous variables on the response to vaccination. The variables included were: antibody titres at 3, 4, 5 and 6 months after the first dose of vaccine, patient age, total time on the renal replacement programme, lowest serum albumin during the initial course of 3 injections, average serum albumin, body mass index, urea reduction ratio, serum PTH concentration and number of previous renal transplants. Urea reduction ratio was not available in two patients and these were therefore excluded from the analysis. The mean and standard deviation for each of these factors is recorded in Table 3.1.2.

	All Patients		Non-Responders		Responders	
	n	mean ± SD	n	mean ± SD	n	mean ± SD
Antibody titre month 3 (IU/L)					16	279 ± 345
Antibody titre month 4 (IU/L)					22	249 ± 372
Antibody titre month 5 (IU/L)					26	372 ± 374
Antibody titre month 6 (IU/L)					26	293 ± 347
Age (years)	36	46.0 ± 12.6	10	43.3 ± 14.8	26	47.0 ± 11.7
Time on renal replacement (months)	36	78.9 ±74.4	10	66.9 ± 61.7	26	83.4 ± 79.3
Albumin (lowest) (g/L)	36	40.9 ± 4.6	10	42.3 ±4.1	26	40.4 ± 4.7
Albumin (average) (g/L)	36	42.7 ± 3.8	10	43.4 ± 4.0	26	42.5 ± 3.8
Body mass index (kg/m^2)	36	23.8 ± 4.5	10	22.8 ± 3.1	26	24.2 ± 4.9
Urea reduction ratio	34	65.1 ± 11.0	9	64.5 ± 10.8	25	65.4 ± 11.3
Parathyroid hormone (pg/ml)	36	438 ± 457	10	271 ± 242	26	502 ± 506
Previous renal transplants (number)	36	1.0 ± 1.0	10	1.1 ± 1.2	26	0.9 ± 0.9

Table 3.1.2 Antibody titres and patient factors which potentially affect response to vaccination

A table of simple correlations for the variables revealed significant correlations only between antibody titre at month 2 and average albumin ($r = 0.35$; $p = 0.04$), and antibody titres at month 5 and time on the renal replacement programme ($r = 0.37$; $p = 0.03$). A stepwise regression analysis using the mean antibody titre at 4 months as the dependent variable entered only average albumin as a significant factor and this accounted for only 6.7% of the variance. Using the mean antibody titre at 5 months (also the peak GMT) as the dependent variable produced the best results. Time on renal replacement ($t = 2.56$; $p = 0.015$) and serum PTH ($t = -2.08$; $p = 0.046$) were identified as significant factors affecting antibody response. Together these factors accounted for 19.2% of the variance.

Further analysis of the effect of PTH level revealed that in patients responding to the vaccine, those with a PTH level of ≤ 502 pg/ml (mean level for responders) had

significantly higher antibody titres at month 5 (526 IU/L vs. 161 IU/L; p = 0.007) and at month 6 (442 IU/L vs. 89 IU/L; p = 0.004) than those with PTH > 502 pg/ml. Patients who failed to respond to the vaccine had a lower mean PTH than those who did respond, although this was not statistically significant (271pg/ml vs. 502pg/ml; p = 0.07)

A separate analysis was performed to investigate the effect of treatment with human recombinant erythropoietin (rhEPO). 8 patients received rhEPO in a dose ranging from 2000 to 12000 u/week for at least 2 months during the vaccination period. The response rate was similar in treated and untreated patients (5 of 8 vs. 21 of 28; p = 0.49). There was also no difference in the peak GMT between the groups (398 IU/L vs. 365 IU/L; p = 0.86).

Treatment with nifedipine has been shown in vitro to improve the proliferative response of B cells from hyperparathyroid dialysis patients (60). Eight patients received calcium channel blockers for at least 2 months during the vaccination period. There was no difference in response rate (6 of 8 vs. 20 of 28; p = 0.84) or peak GMT (346 IU/L vs. 379 IU/L; p = 0.85) between treated and untreated patients.

3.1.5 Cost analysis of vaccination

A cost analysis of the vaccination programme was performed and results are shown in Table 3.1.3. Prices for laboratory investigations were based on South African Institute for Medical Research (SAIMR) rates for public sector institutions. The cost of the vaccine was based on the tender price at Groote Schuur Hospital. The total cost of the vaccination programme was R19 763.00 (±R250/patient entered). However, this could be reduced to R12 853.70 (±R160/patient) in future by measuring antibody response only on one occasion at two months after the 4th dose of vaccine. Savings result from the fact that patients identified to have immunity following a previous infection and those who develop protective levels of antibodies after vaccination, no longer require monthly screening for HBsAg. Thus the programme will have resulted in a nett saving of R7 223.90 (±R90/patient) at the end of the first year. After the first year the cost of maintaining protective antibody levels in previously vaccinated patients consists of the cost of an annual anti-HBs level and booster doses of vaccine which should be given to those whose levels have dropped below 100 IU/L. In the current analysis the figure of 16 is an estimate based on the number of patients with a titre of less than 200 IU/L after vaccination. Thus after the first year, antibody screening and vaccination of this cohort of patients can be expected to result in an annual nett saving of R30 092.00 (±R380/patient entered).

	cost/item (Rand)	number	total (Rand)
COST			
1. Pre-vaccination testing			
anti-HBs	59.20	79	4676.80
anti-HBc	59.20	79	4676.80
			9353.60
2. Post-vaccination testing			
anti-HBs 1	59.20	42	2486.40
anti-HBs 2	59.20	38	2249.60
anti-HBs 3	59.20	37	2190.40
anti-HBs 4	59.20	36	2131.20
			9057.60
3. Vaccine			
Initial course (3 doses)	8.10	126	1020.60
Booster dose	8.10	36	291.60
Incomplete courses	8.10	5	40.50
			1352.70
4. Total cost			**19763.90**
Total cost (2 post-vaccination anti-HBs levels)			15087.10
Total cost (1 post-vaccination anti-HBs level)			12853.70
5. Annual cost (after first year)			
Annual anti-HBs level	59.20	25	1480.00
Booster dose vaccine	8.10	16	129.60
			1609.60
SAVING			
1. First year			
HBsAg (vaccinated patients with protective levels)	51.80	209	10826.20
HBsAg (patients with immunity after infection)	51.80	312	16161.60
			26987.80
2. Annual after first year			
HBsAg (vaccinated patients with protective levels)	51.80	300	15540.00
HBsAg (patients with immunity after infection)	51.80	312	16161.60
			31701.60
NETT COST			
First year			**–7223.90**
First year (2 post-vaccination anti-HBs levels)			–11900.70
First year (1 post-vaccination anti-HBs level)			–14134.10
Annual after first year			**–30092.00**

Table 3.1.3 Cost analysis of hepatitis B vaccination programme

3.2 Hepatitis C

3.2.1 Prevalence and risk factors for infection

Table 3.2.1 shows the results of anti-HCV testing and analysis of risk factors for infection in patients who had been on dialysis for longer than 1 month in December 1992 and a similar group in December 1995. The groups were similar with respect to gender and age. In each group, the anti-HCV positive patients had a longer mean time on haemodialysis and a greater mean number of blood transfusions than anti-HCV negative patients. All anti-HCV positive patients had received blood transfusions. There was no significant difference in the mean number of previous renal transplants between anti-HCV positive and negative patients in either group.

December 1992	Total	anti-HCV +	anti-HCV -	p value
Number	55	9	46	-
Male	25	2	23	-
Female	30	7	23	-
Mean age (years)	42.7	48.1	41.6	NS
Months on HD	42.0 ± 45.5 (1-215)	101.6 ± 57.4 (14-215)	30.3 ± 32.4 (1-121)	0.006
Transfusion units	9.4 ± 12.5 (0-60)	22.6 ± 17.0 (3-60)	6.8 ± 9.4 (0-43)	0.03
Renal transplants	0.93 ± 0.88 (0-3)	1.00 ± 0.71 (0-2)	0.91 ± 0.91 (0-3)	NS
December 1995	Total	anti-HCV +	anti-HCV -	p value
Number	75	4	71	-
Male	33	1	32	-
Female	42	3	39	-
Mean age (years)	42.5	45.3	42.4	NS
Months on HD	34.2 ± 36.5 (1-134)	105.5 ± 23.9 (84-130)	30.2 ± 32.8 (1-134)	0.004
Transfusion units	5.1 ± 7.3 (0-31)	14.8 ± 3.6 (10-18)	4.5 ± 7.1 (0-31)	0.004
Renal transplants	0.87 ± 0.96 (0-4)	1.25 ± 0.50 (1-2)	0.85 ± 0.98 (0-4)	NS

Table 3.2.1 Demographic data and risk factors for anti-HCV positivity in haemodialysis (HD) patients in December 1992 and December 1995
Values for months on HD, transfusion units and renal transplants are expressed as mean ± SD and range in brackets
p values are for comparison of anti-HCV positive with negative patients

The prevalence of anti-HCV positive patients declined from 16.4% in December 1992, to 5.3% in December 1995 ($p = 0.04$). Of the 9 patients who were anti-HCV positive in 1992, 8 had PCR tests performed and 7 were positive for HCV RNA. In the 1995 group, all 4 anti-HCV positive patients had PCR tests and 2 were positive for HCV RNA.

When the 1992 and 1995 groups were compared, there was no significant difference in the mean time on haemodialysis. There was however, a significant reduction in the mean number of blood transfusions (Table 3.2.2)

	1992	1995	$\chi 2$	p value
Patient Number	55	75		
anti-HCV prevalence (number and percentage)	9 (16.4)	4 (5.3)	4.29	0.04
Months on HD (mean ± SD)	42 ± 45.5	34.2 ± 36.5		NS
Transfusion units (mean ± SD)	9.4 ± 12.5	5.1 ± 7.3		0.02

Table 3.2.2 Comparison of data showing a decline in prevalence of anti-HCV positivity and number of blood transfusions between December 1992 and December 1995.
p values are for comparison of patients from 1992 with those from 1995

The prevalence of anti-HCV positivity in peritoneal dialysis patients tested from April to June 1996 was 2.4% (1 of 41). This is not statistically different from the prevalence of 5.3% in haemodialysis patients in December 1995 ($\chi^2 = 0.54$; $p = 0.46$). The single positive patient however, had previously been on haemodialysis and was included in both the 1992 and 1995 groups of anti-HCV positive haemodialysis patients.

3.2.2 Seroconversions

Review of all serology obtained between 1992 and 1995, revealed 4 patients who appear to have become anti-HCV positive while on haemodialysis. Of these, one had a single positive result but all subsequent tests were negative. Her liver enzymes remained normal throughout the period of follow-up. Another patient had fluctuating positive and negative results, but from April 1995, he remained seronegative. His transaminases were abnormal on one occasion in 1991 and again in 1995 just prior to his death due to severe primary pulmonary hypertension. Both of these patients were PCR negative for HCV RNA and both were reported to be anti-HCV positive for the first time in 1993. The two remaining patients became anti-HCV positive in 1994.

Both had a rise in liver enzymes at the time of seroconversion and one had a mild clinical illness, although she did not become jaundiced. Both were positive for HCV RNA by PCR. One patient had received a total of 52 units of transfused blood (25 prior to the introduction of anti-HCV screening), but the other had received only one unit, in April 1994, which was screened for anti-HCV. There were no seroconversions during 1995. Three patients were found to be anti-HCV positive when tested for the first time on haemodialysis. One was already positive while he was on peritoneal dialysis, prior to commencing haemodialysis. The other two were tested for the first time at 3 and 10 months after commencing haemodialysis respectively. All three had previously been on haemodialysis and had received renal transplants and blood transfusions in the period before blood was screened for anti-HCV.

3.2.3 Liver transaminases

Analysis of the liver enzymes in the month of serological testing, revealed a significantly higher mean AST in anti-HCV positive patients in the December 1992 group. The mean ALT was also higher, but this was not statistically significant. Differences were less marked in the December 1995 group and not statistically significant. All values recorded in both groups were less than twice the upper limit of normal (i.e. less than 50IU/L). When all available data on monthly liver enzymes in each patient since the start of dialysis were reviewed, anti-HCV positive patients had a significantly higher number of AST or ALT values greater than 50IU/L than anti-HCV negative patients in both the 1992 and 1995 groups (Table 3.2.3). Review of

the patients' records revealed no documented episodes of clinical hepatitis in any patient with abnormal transaminases.

	1992				1995			
	HCV+	HCV-	$\chi 2$	p value	HCV+	HCV-	$\chi 2$	p value
Number	9	39			4	69		
AST ± SD	16.2 ± 6.5	9.5 ± 4.4		0.01	9.5 ± 1.3	11.2 ± 7.3		NS
Number	9	38			4	69		
ALT ± SD	15.1 ± 9.8	8.2 ± 4.5		NS	14.5 ± 12.5	10.2 ± 6.5		NS
≥ 1 Peak	8/9	8/40	15.85	0.0001	4/4	13/71	14.42	0.0001
≥ 2 Peaks	8/9	3/40	27.95	<0.0001	4/4	2/71	48.59	<0.0001

Table 3.2.3 Mean AST and ALT values in IU/L and the prevalence of patients with previous abnormal transaminase peaks (>50IU/L) in December 1992 and December1995
p values are for comparison of anti-HCV positive with negative patients

3.2.4 Clinical outcome in anti-HCV positive patients

Of the 9 patients who were anti-HCV positive in December 1992, 4 had died, 2 had received renal transplants and 3 were still on haemodialysis by December 1995. None of the deaths could be attributed to HCV-related disease and none of the 5 living anti-HCV positive patients had clinical or biochemical evidence of chronic liver disease. There was no difference in survival between the anti-HCV positive and negative patients (5/9 alive vs. 37/46 alive; χ^2=2.58; p = 0.11).

4. Discussion

4.1 Hepatitis B

4.1.1 HBV infections

The zero incidence of HBV infections in our haemodialysis patients during 40 months of follow-up is remarkable when one considers the high prevalence of chronic carriers in the general population. It provides further proof that the policy of screening of new patients for HBsAg prior to the initiation of dialysis and regularly thereafter coupled with testing of blood donations and isolation of positive patients to separate dialysis units, is effective in preventing new infections in this high risk group.

The prevalence of previous infection in these patients (33%) seems lower than that in the general population although the prevalence in black dialysis patients (40%) was not statistically different to the 61.1% in 607 black South Africans in the study by Tucker et al. (5). The difference may be a result of the fact that patients from rural communities, which have the highest prevalence of HBV infection, often are not referred for dialysis or are not accepted onto dialysis programmes for social and logistic reasons.

4.1.2 Response to vaccination

Using a standard dose of a plasma-derived vaccine given intramuscularly at months 0,1,2 and 4, we achieved a response rate (72%) and peak GMT (372 IU/L) similar to that reported by other centres (see table 1.2.1). It is difficult to compare results directly because of the differences in vaccine type, dose, doseage schedule and patient population. However, our results are very similar to those of Benhamou et al. (47), who used the same schedule, but a different plasma-derived vaccine.

The administration of a booster dose after the initial 3 doses led to an increase in seroconversion rate from 61% to 72% and an increase in the number of patients with protective antibody levels from 53% to 69%. The peak GMT rose from 279 IU/L to 372 IU/L. Although these differences are not statistically significant, a randomized controlled trial has prevoiusly demonstrated a significantly improoved response when a 4-dose schedule was compared to a 3-dose schedule (47). Shortening the doseage schedule by giving the booster dose at 4 months instead of 6 months makes the vaccination programme slightly easier to manage, and does not appear to affect the response adversely. Determination of the optimum vaccine, dose and schedule in haemodialysis patients requires further randomized controlled trials.

4.1.3 Factors affecting immune response to vaccination

We observed a higher seroconversion rate and higher GMT in female patients than in males, although the difference was not statistically significant. This is similar to the findings of several other studies (38, 40, 43, 45, 46, 48). Taken together with the

observation that males are more likely to become chronic carriers of HBV (19), this suggests that there is a gender-related difference in immune response to HBV. Further studies are required to confirm this and to investigate the mechanisms involved.

Stepwise multiple regression analysis with the peak GMT as the dependent variable identified only time on the renal replacement programme and serum PTH concentration as significant factors affecting antibody response, although they accounted for a relatively small proportion of the variance (19.2%). The former finding is similar to that by Steketee et al. who found a positive association between time on dialysis prior to vaccination and antibody response (62). A possible explanation for this is that prolonged survival selects a group of patients with failed renal transplants and high cytotoxic antibody levels. These patients are therefore good immune responders and are more likely to respond to vaccination.

The negative association between serum PTH levels and peak GMT and the finding that amongst patients responding to the vaccine, those who had a PTH of >502 pg/ml developed significantly lower antibody titres, suggests that the high levels of PTH associated with the secondary hyperparathyroidism of chronic renal failure may inhibit antibody synthesis. As far as we are aware, there is no published data on the effect of hyperparathyroidism on the response to hepatitis B vaccine in haemodialysis patients. However, in vitro studies have shown that PTH inhibits B cells from both normal controls and haemodialysis patients. The hormone appears to act via cAMP and raised intracellular calcium to cause a reduction intracellular ATP and an impaired proliferative response (59, 60). In B cells from dialysis patients, inhibition took place

at only very high concentrations of PTH implying a degree of resistance which is thought to be due to down-regulation of receptors. This may explain why the reduced antibody response in our study was seen only in patients with very high levels of PTH. Our results require confirmation by other studies. We measured serum PTH on only one occassion, at 4 months after the first dose of vaccine was administered. A clearer relationship may emerge if PTH is measured at the start of vaccination and at monthly intervals thereafter. If confirmed, these findings will have far-reaching implications as they may provide a partial explanation not just for the impaired response to hepatitis B vaccine, but also for components of the immunocompramised state of haemodialysis patients.

The finding that treatment of haemodialysis patients with calcium channel blocking agents partially corrects the inhibition of B cells by PTH in vitro (60), confirms the importance of raised intracellular calcium in this process and suggests that treatment with calcium antagonists may improve the antibody response to vaccination in patients with hyperparathyroidism. In our study we were unable to demonstrate a better response in those on calcium antagonists, but there were only a very small number of patients on these agents and the study was not designed for this purpose. A randomized controlled trial is required to investigate this matter further. If calcium antagonists are shown to improve the response to vaccination, further studies would be warranted to test for a beneficial effect on the immune system as a whole.

In our study, patients who did not respond at all to the vaccine had a lower mean PTH level than those who did respond, although the difference was not significant. This suggests that factors other than hyperparathyroidism were responsible for their

failure to respond. In vitro studies have shown impaired proliferation in T cells from haemodialysis patients and in particular, in those not responding to hepatitis B vaccine (54, 55). This abnormality has been shown to be secondary to an acquired defect of monocyte function which is thought to result in impaired interaction with T cells and reduced IL-2 production. Thus there are at least 2 separate immune mechanisms responsible for the poor response of dialysis patients to hepatitis B vaccine.

Serum albumin concentration is a reliable measure of nutritional status in dialysis patients. The correlation between mean antibody titre at month 4 and average serum albumin on the table of simple correlations, together with the identification of average albumin as a significant factor in a stepwise regression analysis using this antibody titre as the dependent variable, suggests that nutritional status may affect the response to vaccination. However, no relationship could be shown with the mean lowest serum albumin or with another measure of nutritional status, body mass index. There is little published data on this subject. Lombardi et al. found that haemodialysis patients responding to hepatitis B vaccine had a better nutritional status than non-responders, but the difference was not significant (69). Our study may have failed to detect the effect of nutritional status because of the small number of patients and the fact that the majority are well nourished as evidenced by a mean average albumin of 42.7g/L and a mean body mass index of 23.8 kg/m^2 (Table 3.1.2).

We were unable to identify any relationship between patient age and the response to vaccination. Several other studies have found a decrease in seroconversion rate with increasing age (43-46, 53, 62). The small sample size and relatively low mean age (46 years) in our study may provide an explanation for this.

Urea reduction ratio is a measure of dialysis efficacy and correlates with patient survival. We found no association between this and the response to vaccination and are not aware of any published data on the subject. Larger studies may be necessary to detect such a relationship.

There was also no association with number of previous renal transplants, suggesting that previous exposure to immunosuppressant drugs does not have a residual effect on vaccine response. Larger studies are required to confirm this.

Only 8 of 36 patients received recombinant erythropoietin during the vaccination period and thus one should not draw any conclusions from the failure to detect a beneficial effect as reported by others (68). A randomized controlled trial would be necessary to investigate this further.

4.1.4 Cost effectiveness of vaccination

In this study, the initial cost of antibody screening and vaccination was R250/patient. The largest component of this cost is the antibody assays which were done repeatedly to determine the optimum time for testing in the future. Thus future vaccination programmes will require only pre-vaccination testing and a single post-vaccination assay at 2 months after the booster dose. This will reduce the cost to approximately R160/patient. The initial costs are offset by the large saving resulting from the reduced need for monthly HBsAg testing. Thus by the end of the first year, the current programme will have resulted in a nett saving of R90/patient. In future, with

the use of only 1 post-vaccination antibody level, the saving will be R180/patient. After the first year, the cost of maintaining protective levels of antibody is relatively small and thus an annual saving of R380/patient can be expected. These figures do not take into account the fact that patients are constantly leaving the dialysis programme due to transplantation. However, the mean time on dialysis at the start of the programme was 35 months (Table 3.1.1), indicating that the majority of patients will be on dialysis long enough for nett saving to occur.

The fact that we observed a zero incidence of new HBV infections during the 40 months prior to the vaccination programme implies that existing measures to prevent the infection were effective, and that it would be difficult to demonstrate an added benefit due to vaccination. Nevertheless, haemodialysis patients remain at risk of acquiring HBV infection in the community and secondary transmission to other patients could have serious consequences for the individuals affected and for the unit as a whole. In order to contain an outbreak, all infected individuals would have to be transferred to an isolation unit and no new patients could be accepted into the unit until there was reasonable certainty that no further seroconversions would occur (ie. for about 6 months). In addition, reuse of all dialyzers would have to be discontinued for the same period. These measures would severely disrupt the delivery of dialysis support to patients and would also lead to greatly increased costs in the vicinity of R200 000 - 300 000 due to the suspension of reuse. Thus any measure which could reduce the risk of infection further would be important to consider. The fact that hepatitis B vaccination has minimal adverse effects and results in cost saving means that it can be recommended even though the response rate is less than in healthy subjects and additional benefit is difficult to prove in our setting. We therefore

conclude that all patients should be screened for hepatitis B antibodies and vaccinated as required at or before the commencement of haemodialysis.

4.2 Hepatitis C

4.2.1 Prevalence and risk factors for infection

Our results concur with those from many other units which have reported a high prevalence of HCV infection in haemodialysis patients. By comparison, the prevalence of anti-HCV positivity in 66 314 blood donors in the Western Cape Province of South Africa has recently been reported to be only 0.41% (Tucker et al. personal communication). Like several other units we have demonstrated a positive association of HCV infection with time on dialysis and with number of previous blood transfusions. These findings imply that HCV infection is transmitted by factors specifically associated with haemodialysis and in particular by blood transfusions.

There was a marked decline in prevalence of anti-HCV positive patients in our unit over a three year period. Our local blood transfusion service commenced testing blood for anti-HCV in December 1992. Initially only 1 unit in 5 was tested but since December 1993, all units have been tested. During the same period, there has been a significant reduction in the mean number of blood transfusions per patient. This is probably attributable to an increase in the use of recombinant erythropoietin and parenteral iron supplementation. These facts together suggest that the main source of HCV infection in our unit has been blood transfusions and that the decline in prevalence has largely been due to screening of blood donations. A reduction in the use of blood products may also have had a minor effect. A similar trend was reported by Simon et al. (100).

4.2.2 Seroconversions

Four apparent seroconversions to anti-HCV positive were recorded between 1992 and 1995. Of these, one patient had only a single positive test and another had fluctuating positive results followed by two negative tests, suggesting that they were false positives. Both were PCR negative. The remaining two were unequivocal seroconversions who became PCR positive. Both of these patients had received blood transfusions. One had received 52 units, including 25 which were not screened for anti-HCV. The other however, had received only a single unit of blood which was screened, and is unlikely to have been the source of infection. This patient is therefore the only case in our study where nosocomial transmission could be implicated.

4.2.3 Measures to prevent HCV infection

We observed a significant decline in the prevalence of HCV during a three year period when anti-HCV positive patients were not isolated to separate machines or dialysis units. This finding is in agreement with other studies which have shown that although isolation of HCV-infected patients is effective in preventing nosocomial transmission of the virus, strict adherence to universal infection precautions (141) is equally effective. There are several reasons why isolation of anti-HCV positive patients to separate units may not be desireable or effective in preventing transmission of the virus. Firstly the isolation of both HBsAg and anti-HCV positive patients to separate units would require the creation of different units for each of 4 categories of patient: 1) negative for both viruses 2) anti-HCV positive, HBsAg negative 3) anti-HCV negative, HBsAg positive 4) positive for both viruses. This would be difficult

logistically and would lead to increased costs due to less efficient use of personel, dialysis equipment and hospital space (142). Secondly, antibodies which develop after HCV infection do not appear to protect against reinfection and the high variability of the viral genome enables it to escape immune defences. There is evidence that HCV-infected subjects can be reinfected by an homologous strain of the virus and that haemodialysis patients have become coinfected with different strains (86, 115, 143). Thus, placing all HCV-infected patients in one unit may increase their chances of becoming reinfected. Finally, seroconversion may occur only 6 months after infection with HCV. An infected patient could therefore transmit the virus to other patients before being identified by serological testing. We conclude that isolation of anti-HCV positive patients to separate units is not essential for the prevention of nosocomial transmission and that the added cost associated with this approach cannot be justified. Efforts to prevent transmission should be focussed on strict adherence to infection control measures.

Concern that the reuse of dialyzers may contribute to the nosocomial transmission of HCV has led some authors to recommend that this practice be discontinued in anti-HCV positive patients. Our study has demonstrated a decrease in the prevalence of HCV infection during a three year period when reuse of dialyzers was standard practice, suggesting that it does not increase the risk of transmission. This is supported by the fact that although a large multicentre study involving 401 patients reported strong evidence of nosocomial transmission, it was unable to demonstrate an association with the reuse of dialyzers (92). Furthermore, a randomized study of reuse verses single use of dialyzers showed no statistical difference in the incidence of HCV infection after a mean of 32 months follow-up (125). Although some authors

have detected HCV RNA in ultrafiltrate from infected patients (119), there is little evidence of transmission of the virus between patients as a result of reuse. The discontinuation of reuse in anti-HCV positive patients would lead to a significant increase in the cost of dialysis. In our unit, changing to single use in the 9 positive patients identified in 1992 would have resulted in an increase in cost of approximately R97 000.00 per annum. We have provided additional evidence that reuse is safe and can be continued in HCV-infected patients without increased risk of transmission to others.

4.2.4 Liver transaminases

Measurement of serum transaminase levels appears to have a low sensitivity for the detection of HCV infection (96, 107, 128). Several workers have however, reported an association between anti-HCV positivity and previous elevations of ALT (91, 92, 94, 103, 107). Simon et al. reported elevated ALT levels which persisted or fluctuated for at least 6 months in 41 of 42 patients who seroconverted on dialysis. Long term follow-up of anti-HCV patients revealed fluctuating ALT levels in 69.2% (100). In our study, retrospective analysis showed a much higher prevalence of previously elevated transaminases in anti-HCV positive patients. Whilst we agree that transaminases have too low a sensitivity to be used for surveillance of HCV infections, the finding of previous or current elevated transaminases in an haemodialysis patient should prompt investigation for HCV infection by serology and PCR.

4.2.5 Clinical outcome

The small number of anti-HCV positive patients and short follow-up period in our study makes it impossible to make any meaningful observations about the natural history of this disease. The absence of clinical or biochemical evidence of liver disease in our HCV-infected patients is similar to findings in other studies and does not exclude liver pathology which should be investigated by means of a liver biopsy (96, 107, 128).

4.3 Conclusions and Recommendations

Research over the past three decades has identified of HBV and HCV as the two most important causes of viral hepatitis in haemodialysis patients. Characterization of the viruses and elucidation of the modes of transmission have led to successful strategies for preventing this once common problem.

In the case of HBV it has been shown that serological screening of patients and blood donations together with isolation of infected patients to separate dialysis units is essential to prevent trasmission between patients. The zero incidence of HBV infections in our unit over 40 months is evidence of the effectiveness of these measures. Although dialysis patients have an impaired response to hepatitis B vaccine, vaccination has been shown to reduce the number of infections in units with a high annual incidence. In addition, it results in reduced management costs because patients with protective antibody levels no longer require monthly screening for HBsAg. We therefore recommend it as an additional preventative measure. New evidence has been provided that hyperparathyroidism may be partially responsible for the impaired antibody response in haemodialysis patients.

Our study has shown that the prevention of HCV infection in a haemodialysis unit can be achieved by screening blood donations for anti-HCV and the strict application of universal infection precautions. Although the isolation of anti-HCV positive patients to separate units is effective in preventing spread of the infection, it is not essential and the increased expense associated with the creation of such units does not seem

justified. Available data supports our finding that the reuse of dialyzers does not contribute to the spread of HCV and can be safely continued in positive patients.

The differences in measures required to prevent the spread of infection between HBV and HCV probably relate to differences in characteristics of the viruses. HBV may be present in high concentrations in peripheral blood whereas HCV is usually present at very low levels. Furthemore, HBV has been shown to persist in a viable form in the environment for a week (18), while HCV degenerates rapidly in stored blood samples (85).

Although the above measures have been effective in containing the transmission of HBV and HCV infection, haemodialysis patients remain at risk for acquiring blood-borne pathogens. This is illustrated by the finding of an increased prevalence of the recently discovered hepatitis GB virus C in haemodialysis patients in Japan (144). Thus ongoing surveillance, thorough investigation of new pathogens and the maintenance of infection control standards remain essential for the safe delivery of haemodialysis therapy.

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Appendix 1

Patient data for hepatitis B vaccination trial

Patient	T1(Jul)	T2(Aug)	T3(Sep)	T4(Oct)	R	S	Age	Time	AlbL	Alb	URR	Tx	BMI	PTH	Ca	EPO
1	0	0	0	0	2	2	45	40	40	42.7	66	0	21.9	218		
2	0	0	0	0	3	2	19	70.5	42	43.7	83	2	19.6	125		12
3	0	0	0	0	2	1	56	29	45	45.3	67	0	17.4	87		
4	0	0	0	0	2	1	55	142	48	48.7	63	2	21.9	838		4
5	0	0	0	0	3	1	44	15.6	33	33.7	47	0	26.4	278	N	12
6	0	0	0	0	1	1	59	52.8	43	45	64	2	23.9	221		
7	0	0	0	0	3	1	39	6.41	42	43	57	0	27	89		
8	0	0	0	0	2	2	24	121	41	41.7	76	2	22.1	369		
9	0	0	0	0	2	2	32	184	46	46.7		3	22.1	18		
10	0	0	0	0	2	1	61	8.38	43	43.3	57	0	26.1	463	A	
11	0	0	4	5	3	1	37	12.8	35	40	65	0	23.2	706	N	12
12	0	0	8	11	2	2	49	156	34	35	52	0	14.9	654		
13	0	7	36	18	2	2	49	151	44	45	74	2	22.9	1167		
14	0	0	87	43	1	1	74	3.85	39	39.3	67	0	21.2	61		12
15	44	58	80	47	3	1	34	12.6	45	46	61	1	19.8	522		
16	47	52	85	54	1	2	74	22.8	41	43.7	65	0	30.2	1790		6
17	0	6	73	57	2	2	51	76.3	45	45.7	72	1	30.6	1089		
18	0	0	98	58	2	2	54	160	40	41.3	69	1	28.7	1409		
19	0	15	58	62	3	2	42	70.3	30	39.3	68	1	17.6	340	A	
20	0	6	46	78	2	1	41	10.4	39	39.7	56	0	30.2	820	A	
21	23	16	138	78	2	1	52	31.9	46	47	57	1	26.6	53		
22	15	20	98	89	2	2	43	44.2	35	36.7	76	2	34.5	131		
23	24	57	96	92	2	2	44	11.7	42	43	78	0	27	27		
24	47	147	300	121	2	1	37	130	38	39	32	2	20	708	A	
25	760	1000	532	152	3	1	56	33.5	42	43.3		0	22.1	58		
26	0	24	231	153	3	2	45	4.11	33	37.7	77	1	22.2	1268		
27	0	13	195	166	2	1	60	184	39	42	69	1	23.6	434		
28	38	33	437	210	2	2	53	96.4	44	47	44	0	32.8	32		
29	105	100	814	376	1	1	35	165	47	48	60	2	23.1	836		2
30	415	1000	667	589	2	2	25	26.8	48	49.7	70	2	19.5	98	A	
31	1000	945	826	623	2	2	59	101	45	45.3	78	1	24.3	107		
32	1000	1000	752	692	2	2	39	141	44	45.7	64	2	21.8	350		
33	220	271	1000	833	2	2	46	290	39	39.7	73	3	18.7	211		
34	105	84	1000	1000	2	2	46	10.3	39	39.7	63	0	28.6	140	A	
35	241	155	1000	1000	2	2	28	217	35	41.7	80	1	21.7	10		6
36	373	476	1000	1000	2	1	51	6.51	42	44.7	65	0	24.5	41		

Key to headings:

T1-T4	antibody titre at months 3, 4, 5, and 6 post vaccination in IU/L
R	race: 1 = white; 2 = mixed race; 3 = black
S	sex: 1 = male; 2 = female
Age	patient age in years
Time	time on renal replacement programme in months
AlbL	lowest serum albumin during first 3 months of vaccination in g/L
Alb	average serum albumin during first 3 months of vaccination in g/L
URR	urea reduction ratio
Tx	number of previous renal transplants
BMI	body mass index in kg/m^2
PTH	parathyroid hormone level in pg/ml
Ca ant	calcium antagonist therapy: A = amlodipine; N = nifedipine
EPO	erythropoietin therapy: number = weekly dose (units) $\div 10^3$

Appendix 2

Patient data for hepatitis C surveillance: December 1992

PATIENT	AGE	RS	TF	TMH	TX	HCV	ALT	AST	Peak
1	24.3	4	18	94	2	P	19	11	2
2	53.1	3	60	120	2	P	19	22	2
3	42.6	3	3	14	1	P	12	26	0
4	50.6	4	17	50	1	P	6	8	2
5	57.5	2	41	144	1	P	8	9	2
6	80.7	2	29	215	0	P	10	16	2
7	37.8	8	10	86	1	P	5	22	2
8	43.7	4	15	114	0	P	36	20	2
9	42.1	8	10	77	1	P	21	12	2
10	40.6	3	0	13	1	N	20	11	0
11	19.4	4	1	3	0	N	4	6	0
12	47.8	3	2	22	0	N			
13	44.9	4	2	48	2	N	6	11	1
14	65.4	8	9	80	0	N	11	15	2
15	48.2	4	3	23	0	N	4	7	0
16	35.4	3	2	5	1	N	4	2	0
17	56.8	3	1	16	1	N		14	1
18	38.9	4	0	46	1	N	17	12	0
19	58.4	4	25	38	3	N	6	11	
20	37.8	4	10	12	0	N	10	6	0
21	36.6	4	4	4	0	N	2	7	0
22	55.3	4	4	44	1	N	6	8	0
23	31.6	2	13	12	2	N			0
24	26.1	7	26	48	1	N	11	13	0
25	36.2	3	0	3	0	N			
26	60.7	7	5	35	0	N	8	7	0
27	37.6	3	0	4	1	N	5	7	1
28	37.3	3	5	14	1	N	11	15	0
29	42.7	4	8	32	3	N	5	11	0
30	60.1	4	20	117	1	N	4	7	0
31	51.7	3	3	92	2	N	14	14	0
32	49.5	3	2	3	0	N			0
33	56.2	3	3	15	1	N	12	6	0
34	32.5	8	0	1	0	N	9	6	0
35	31.3	4	3	7	0	N	10	19	
36	55.7	1	0	8	0	N	10	10	0
37	35.8	4	17	98	2	N	6	9	0
38	35.5	8	6	31	2	N	5	6	0
39	52	7	6	57	2	N	15	21	2
40	45.7	8	0	15	1	N	7	12	0
41	38.7	4	0	59	2	N	9	7	2
42	43.1	7	4	5	1	N	4	6	1
43	28.4	8	2	25	1	N	1	3	0
44	45.9	3	19	35	3	N	9	12	0
45	40.7	1	0	1	0	N	11	7	0

PATIENT	AGE	RS	TF	TMH	TX	HCV	ALT	AST	Peak
46	22.4	7	0	2	0	N			
47	25	4	25	68	1	N	1	5	0
48	55.2	4	1	4	0	N	5	6	0
49	27.9	4	0	2	0	N			
50	31	3	43	121	1	N	12	17	0
51	32.1	1	13	45	2	N	12	14	1
52	44.8	7	4	3	1	N	8	7	0
53	31.7	3	0	4	0	N			0
54	37.5	3	0	1	0	N	15	9	0
55	47.8	4	22	74	1	N	3	4	0

Key to headings:

Age	patient age in years
RS	race and sex: 1 = white male; 2 = white female; 3 = mixed race male; 4 = mixed race female; 7 = black male; 8 = black female
TF	number of units of blood transfusion
TMH	time on haemodialysis in months
TX	number of previous renal transplants
HCV	anti-HCV antibody status: P = positive; N = negative
ALT	serum ALT level in IU/L
AST	serum AST level in IU/L
Peak	number of previous abnormal transaminase peaks

Appendix 3

Patient data for hepatitis C surveillance: December 1995

PATIENT	AGE	RS	TF	TMH	TX	HCV	ALT	AST	Peak
1	27.3	4	18	130	2	P	8	9	2
2	53.6	4	17	86	1	P	6	8	2
3	40.8	8	10	122	1	P	11	10	2
4	59.5	3	14	84	1	P	33	11	2
5	45.2	4	0	36	0	N	8	9	0
6	43.6	3	10	47	2	N	21	15	0
7	40.2	3	0	6	0	N	7	6	0
8	47.2	4	2	84	2	N	5	9	1
9	30.2	1	0	4	0	N	11	6	0
10	51.2	4	3	22	1	N	11	13	0
11	60.1	3	2	10	1	N	10	9	1
12	17.9	8	4	9	2	N	12	15	0
13	41.9	4	0	82	1	N	9	11	0
14	46.9	3	0	5	1	N	3	5	0
15	36.7	3	0	21	1	N	25	17	0
16	36.3	7	0	9	0	N	9	18	0
17	45.5	4	0	6	0	N	7	8	0
18	37.6	4	2	5	0	N	8	4	0
19	48.3	4	1	27	2	N			1
20	37.5	8	1	21	0	N	5	6	1
21	52.1	1	0	4	0	N	10	2	0
22	58.3	4	4	80	1	N	6	7	0
23	24.9	8	9	8	0	N	9	16	2
24	31.2	1			0	N	11	6	0
25	55.4	3	0	27	0	N	6	11	0
26	51.4	3	18	28	2	N	8	7	0
27	19.6	4	29	44	1	N	5	9	0
28	22.1	8	0	8	0	N	5	11	0
29	42.8	4	1	15	2	N	21	14	0
30	63.7	7	5	71	0	N	5	4	0
31	40.6	3	0	40	1	N	19	21	1
32	21.7	8	0	5	0	N	18	16	0
33	45.7	4	8	68	3	N	4	9	0
34	54.7	3	3	128	2	N	14	17	0
35	54.2	4	4	4	0	N	6	9	0
36	36	4	0	1	0	N	11	13	0
37	37	3	10	35	2	N	36	14	1
38	57.6	3	0	5	0	N	7	8	0
39	32.6	8	4	7	2	N	13	10	0
40	38.7	3	1	4	0	N	14	12	0
41	41.6	3	3	32	1	N	4	4	0
42	43.4	7	2	12	0	N	30	43	0
43	55	8	0	23	1	N	7	8	0
44	34.8	5	31	12	1	N	3	8	0
45	41.5	8	10	39	1	N	8	13	1

PATIENT	AGE	RS	TF	TMH	TX	HCV	ALT	AST	Peak
46	58.7	1	0	14	2	N	7	12	0
47	38.8	4	17	134	2	N	6	10	0
48	34.9	3	7	45	1	N	6	8	1
49	59	4	2	13	0	N	8	8	0
50	41.2	4	1	95	4	N	7	9	2
51	40.7	4	0	2	0	N	7	10	0
52	43.5	4	0	8	0	N	22	15	0
53	53	4	1	31	0	N			0
54	28	4	25	104	1	N	6	10	0
55	41.5	4	4	50	1	N	6	11	0
56	33.6	7	1	9	1	N	14	24	0
57	23.3	4	9	48	2	N	5	8	0
58	54	1	0	4	0	N	19	15	1
59	48.8	4	1	11	0	N	20	18	0
60	35.1	1	13	81	2	N	12	16	1
61	50.3	3	0	2	0	N	4	4	0
62	40.2	8	2	7	0	N	19	21	0
63	47.8	7	5	39	3	N	9	12	0
64	55.3	4	4	63	0	N	6	6	0
65	31.3	4	15	46	3	N	8	9	0
66	33.6	8	0	13	0	N	9	15	0
67	38.5	3	3	5	0	N	5	3	0
68	57.6	3	0	3	0	N	6	6	0
69	27.1	3	2	12	1	N	6	11	0
70	42.3	7	0	2	0	N	9	6	0
71	52.9	1	5	10	1	N	11	4	0
72	50.8	4	22	110	1	N	6	7	0
73	25.1	4	2	4	2	N	5	6	0
74	60.5	3	0	2	0	N	7	5	0
75	39.8	4	2	51	0	N	18	44	1

Key to headings:

Age	patient age in years
RS	race and sex: 1 = white male; 2 = white female; 3 = mixed race male; 4 = mixed race female; 7 = black male; 8 = black female
TF	number of units of blood transfusion
TMH	time on haemodialysis in months
TX	number of previous renal transplants
HCV	anti-HCV antibody status: P = positive; N = negative
ALT	serum ALT level in IU/L
AST	serum AST level in IU/L
Peak	number of previous abnormal transaminase peaks