SURGICAL RESECTION FOR HEPATOCELLULAR CARCINOMA IN CAPE TOWN — A CLINICAL AND HISTOPATHOLOGICAL STUDY

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Objective. Review of surgical resections performed for hepatocellular carcinoma (HCC) at our institution between 1990 and 1996, histology of resected specimens, and clinical outcome.

Design. Retrospective and prospective study of 14 patients who underwent resection for HCC.

Setting. The Hepatobiliary Unit and Liver Clinic, Groote Schuur Hospital, Cape Town.

Patients. Fourteen patients who underwent liver resections for HCC.

Interventions. Hepatic resections using prolonged vascular inflow occlusion.

Outcome measures. Clinical outcome and disease-free survival following resection.

Results. Fourteen patients (5.6% of the total number presenting with HCC) underwent liver resection for HCC at our institution between 1990 and 1996. There were 7 men, median age 40 years (range 18 - 74 years). Only 2 patients were black, and only 1 of these patients had evidence of hepatitis B virus (HBV) infection in the liver. Extensive liver resections were often required. The mean (SD) ischaemic time was 81 (26) minutes and mean estimated blood loss was 938 (649) ml. During hospital admission, 1 patient developed a minor bile leak that settled spontaneously, and 1 patient suffered a stroke and died. The mean hospital stay following operation was 12 days (range 7 - 21 days). Disease-free patient survival at 1, 2 and 3 years was 85%, 75%, and 62%, respectively. Histopathology of the resected specimens showed that 10 of 14 tumours had arisen in non-cirrhotic livers. Mean tumour size was 10.6 (4.6) cm. Only 1 specimen showed the fibrolamellar variant of HCC.

Conclusions. Only a small proportion of patients with HCC seen at Groote Schuur Hospital were eligible for resection, and only a minority of these had HBV-associated 'African' HCC. The results of hepatic resection at our institution compare favourably with literature reports, despite the relatively large size of the tumours. It is of interest that most tumours arose in non-cirrhotic livers. There was no evidence of proliferation of 'oval-like' cells in non-neoplastic liver tissue.

S Afr Med J 1998; **88:** 1575-1580.

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The treatment of patients with hepatocellular carcinoma (HCC) is influenced by the clinical stage of the disease, presence or absence of cirrhosis, and degree of liver impairment.[1,2] Surgery is the primary option for all patients without cirrhosis, and for many cirrhotics who have a single tumour less than 5 cm in diameter with well-preserved hepatic function.[1,2] Resection is the treatment of choice for patients with HCC arising in non-cirrhotic livers. In three studies the 5-year survival of 196 non-cirrhotic patients with HCC treated with hepatic resection was 43%.[3-5] Hepatic resection and liver transplantation offered the best chances of cure in cirrhotic patients with a single tumour, with a 50% reported 5-year survival.[3,4,6] The best long-term survivals were in patients with incidental tumours in the implanted liver. Their outcome was no different from that of patients transplanted for cirrhosis alone.[3,4]

Orthotopic liver transplantation (OLT) has the potential to cure both the tumour and cirrhosis, which is the most frequent site of occult satellite nodules, and is premalignant.[7,8] Patients with more advanced tumours are rarely eligible for surgery and have a dismal prognosis. For those with small tumours who are not eligible for surgery, percutaneous ethanol injection appears to be an effective and affordable treatment modality.[9-11] Randomised trials of targeted chemotherapy[12] and chemo-embolisation[13,14] have not shown any survival benefit, and have caused significant treatment-related morbidity.

In Western countries with a low incidence of HCC, the tumour usually complicates longstanding symptomatic cirrhosis in elderly patients, and the cause is usually alcoholic in origin.[15] However, in sub-Saharan Africa, where the incidence of HCC is very high, tumours frequently arise in young patients who do not have cirrhosis, and the commonest predisposing condition is chronic hepatitis B virus (HBV) infection.[16,17] Both forms of HCC are seen in South Africa because of the ethnic diversity of the population.

The aim of this study was to review the results of surgical resection for HCC at Groote Schuur Hospital and to examine the histopathology of the resected specimens.

PATIENTS AND METHODS

Between January 1990 and February 1996 14 patients with HCC underwent surgical curative resection at Groote Schuur Hospital in Cape Town. During this period, a total of 248 HCC patients were seen at our institution. Selection criteria for hepatic resection included: tumour confined to either lobe of the liver, absence of vascular invasion or extrahepatic spread of tumour, good hepatic reserve, and absence of other significant medical illness. Pre-operative evaluation included full clinical, haematological, biochemical and radiographic assessment to confirm the diagnosis and to facilitate surgical planning. All potential candidates for resection had selective hepatic arteriography and CT portography in order to exclude vascular invasion and to obtain a precise knowledge of hepatic vascular anatomy.[18] Results of serum hepatitis B surface antigen (HBsAg) and serum α-fetoprotein (AFP) levels were available for 8 of 14 patients. Pre-operative biopsy to prove the nature of the liver mass or to assess the status of the surrounding liver was not performed. In 1 patient pre-operative selective hepatic artery embolisation of the tumour was performed to reduce tumour size and improve resectability by producing hypertrophy of the opposite lobe.

The individual liver resections were planned according to the site of the tumour and the lobar or segmental anatomy of the liver.[19,20] Intra-operative ultrasound was used to exclude additional tumours, and to determine the hepatic vascular anatomy in relation to the tumour. Hepatic resection was performed with the use of the Pringle manoeuvre with intermittent vascular inflow occlusion to reduce blood loss and blood transfusion requirements.[21] Complete haemostasis and control of bile leakage were important intra-operative considerations to prevent postoperative complications. The liver parenchyma was divided by a clamp fracture technique. Larger blood vessels and all bile ducts were sutured prior to ligation and division. Electrocautery was used for small vessels at the divided edge of the parenchyma.[19,20] Fibrin sealant (Tisseal Immuno, Österreichisches Institut für Haemoderivate, Vienna, Austria) was sprayed on the raw surface area of the resected liver, and abdominal drainage was not routinely performed.[21] All patients were ventilated in the intensive care unit for the first 24 hours. After discharge, patients were monitored for recurrent HCC by means of clinical examination, liver profile tests, ultrasound, AFP assay and chest radiography at 6-monthly intervals.

Pathology

On receipt of the resected liver tumours, the largest tumour diameter was measured and recorded. Histopathological blocks were selected from the tumours and surrounding non-neoplastic liver tissue. Formalin-fixed tissue was routinely processed and embedded in paraffin. Sections were cut at 5 μm for haematoxylin and eosin (H&E) and immunohistochemical staining. Immunohistochemical staining was performed using the following antibodies: rabbit polyclonal antibody to human AFP (DAKO, Copenhagen, Denmark); antibody against HBsAg (BioGenex, San Ramon, Calif.); monoclonal antibody to cytokeratin 19 (CK 19; Boehringer-Mannheim, Indianapolis, Ind.); monoclonal antibody to rat oval cells (OV-6; a generous gift from Dr S Sell, Albany, NY); antibody to endothelial cells (CD 34; Becton-Dickinson, Lincoln Park, NJ); and polyclonal carcino-embryonic antigen (CEA) stain to outline canaliculi (Poly CEA; DAKO, Copenhagen, Denmark).

Outcome measures

Operative mortality was defined as intra-operative death or death within 2 months of the operation. Operative mortality was not excluded from the survival curves. Total survival and disease-free survival were analysed according to the Kaplan-Meier survival method[22] using Statistica software.

RESULTS

Fourteen of 248 patients with HCC seen at Groote Schuur Hospital between January 1990 and February 1996 underwent liver resection for cure. Patient characteristics are shown in Table I. There were 7 male patients, median age 40 years (range 18 - 74 years). The proportion of patients undergoing resection according to ethnic group was as follows: 7 of 34 were white (20.6%), 5 of 95 were coloured (5.3%), and 2 of 119 were black (1.7%). Only 1 of the 2 black patients showed evidence of HBV infection. Serum HBsAg was present in 3 of 8 patients tested, and serum AFP was raised in 5 of 8 patients. Resections performed were left hepatectomy (6 patients), right hepatectomy (4 patients), extended right hepatectomy (3 patients), and segment IV resection (1 patient). The mean (SD) ischaemic time was 81 (26) minutes and mean estimated blood loss was 938 (649) ml. Only 5 patients required a blood transfusion, and the mean volume transfused was 1 050 (542) ml. During hospital admission 1 patient developed a minor bile leak that settled spontaneously, and 1 patient suffered a stroke and died. The mean hospital stay following

Table I. Surgical resections for HCC — patient details

Patients (N = 14)	5.6% of total
Male/female	7:7
Median age, yrs (range)	40 (18 - 74)
Ethnic group (%)	
White : coloured : black	7 (20.6) : 5 (5.3) : 2 (1.7)
Serum HBsAg positive	3/8
Serum AFP positive	5/8

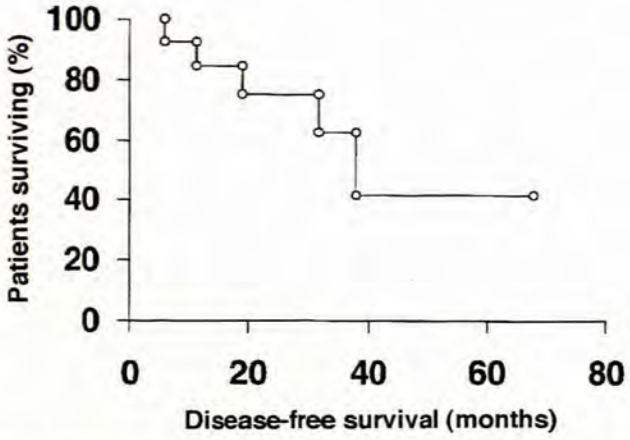

Fig. 1. Kaplan-Meier survival analysis of disease-free survival of patients following resection for HCC.

operation was 12 days (range 7 - 21 days). After a median follow-up period of 32 months (range 6 - 68 months) 8 of 13 patients were alive and disease-free, 1 patient was alive with recurrent tumour, and 4 patients had died from recurrent HCC. Tumour recurrence was intrahepatic in all 5 patients, and in addition 1 patient developed pulmonary metastases. The disease-free patient survival at 1, 2 and 3 years was 85%, 75%, and 62% respectively (Fig. 1).

Gross pathological examination of the resected specimens revealed a mean tumour diameter of 10.6 (4.6) cm. Histopathology showed that 10 tumours had arisen in non-cirrhotic livers (Fig. 2, a), and only 4 tumours complicated cirrhosis. H&E staining revealed that 13 tumours had a trabecular pattern, and only one fibrolamellar variant of HCC was found. Immunohistochemical staining showed tumour AFP positivity in 5 cases (Fig. 2, b) and HBsAg positivity of surrounding liver in 1 case. Staining with CD34 demonstrated the highly vascular nature of the tumours (Fig. 2, c), but did not stain sinusoids in the adjacent liver. Polyclonal CEA staining of the tumours outlined the canalicular network (Fig. 2, d).

The surrounding non-neoplastic liver tissue was examined for the presence of bile ductular and/or stem ('oval-like') cell proliferation. Immunostaining with CK19 and OV-6 showed 'reactive' bile ductular proliferation in the fibrous tissue at the edge of the tumours in cirrhotic livers and some non-cirrhotic livers (Fig. 2, e, f), with no evidence of proliferation of 'oval-like' stem cells in any of the livers examined.

Discussion

Less than 5% of patients seen with HCC at our institution were treated by surgical resection. On average, only two resections per year were performed for HCC in this tertiary referral centre. Furthermore most resections were clearly not performed for 'African' HBV-related HCC. Of salient interest in this small series was the observation that the majority of tumours were in non-cirrhotic livers. This is almost certainly due to selection bias, younger patients with good liver function being selected for surgery. The underlying cause(s) of HCC in these patients is not clear. The relationship between HCC and cirrhosis in southern African blacks differs substantially from that in low-incidence regions of the tumour, while in some areas the incidence of HCC may exceed that of cirrhosis.[16] In a study of southern African blacks with HCC, Kew[17] found that 37% of patients did not have cirrhosis. He found no differences in the age (mean 35 years), clinical features, hepatic function, serum AFP concentrations, or HBV status between HCC patients with cirrhosis and those without. However, as mentioned before, this surgical series is not representative of HBV-associated 'African' HCC. Although most of these patients present late in the course of their disease when surgical cure is not possible, there is evidence that HCC in Africa follows a more aggressive course than HCC in the Orient, and even small tumours are unencapsulated.[16]

The technique of hepatic resection for malignant tumours has been greatly simplified by the use of prolonged intermittent vascular inflow occlusion[21,23] and intra-operative ultrasound. Resections, which were often extensive, were well tolerated, as evidenced by the low transfusion requirement and short hospital stay of the patients. There was 1 hospital death from an unrelated cause (stroke), and 1 patient developed a minor bile leak that settled spontaneously. The long-term outcome of our patients compares favourably with that reported from larger surgical series.[3,24,25] This is encouraging, particularly as many of the patients had large tumours: the mean tumour diameter was 10.6 cm and the largest tumour resected had a diameter of 19 cm.

The overall prognosis for patients resected for HCCs arising in non-cirrhotic livers is not clear because in most series there is a low percentage of non-cirrhotic patients, who are rarely individualised in the survival curves. Iwatsuki and Starzl[26] reported a 5-year survival of 25% in 55 patients who underwent resection for an HCC in non-cirrhotic liver. Adson reported a 35% 5-year survival among 45 non-cirrhotic patients.[27] Better results were reported by Ringe *et al.*,[3] namely 45% at 5 years, while Nagasue *et al.*[25] reported 81% survival at 4 years in a series of 17 patients. Some authors compared results for resection of HCCs in cirrhotic and non-cirrhotic livers: Nagasue *et al.*[25] reported 4-year survivals of 34.0% and 81.2%, and Ringe *et al.*[3] reported 18.1% and 45.0%, respectively.

More recently Bismuth *et al.*[5] reported on 68 resections for HCCs in non-cirrhotic livers. The livers appeared normal at laparotomy in all cases, but in 13 cases (19%) there were minor histological abnormalities, such as steatosis or portal fibrosis. The mean diameter of the tumours was 8.8 cm. Operative mortality and morbidity were 2.9% and 19.0%, respectively. The disease-free survival at 1, 3, 5 and 10 years was 69%, 43%, 33% and 19%, respectively. These authors concluded that

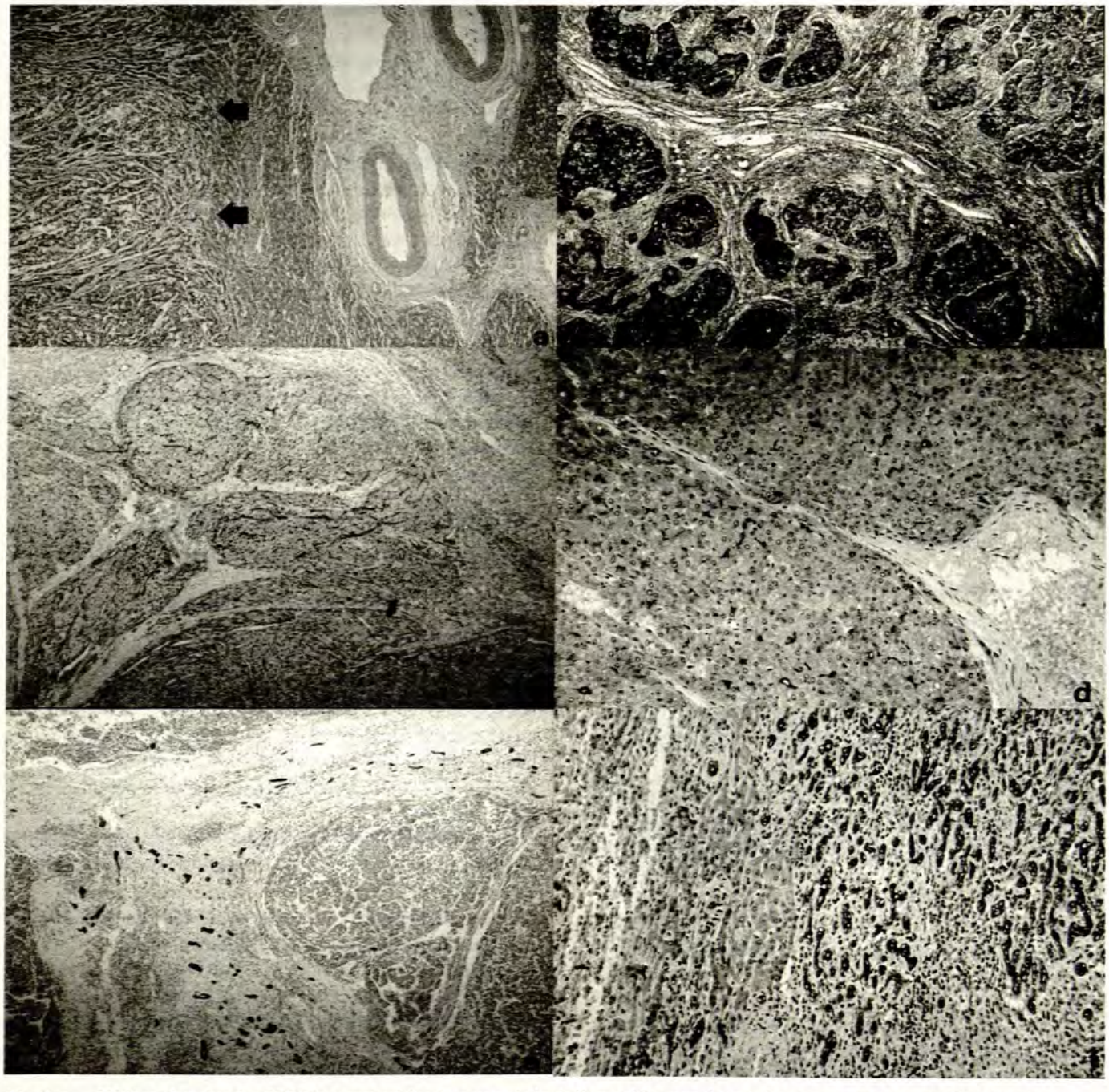

Fig. 2. a — H&E section showing HCC (arrows) arising in a non-cirrhotic liver, with a portal tract showing mild increase in fibrosis only (magnification ×2.5). b — Immunohistochemical stain demonstrating strong positivity of tumour for AFP (magnification ×2.5). c — Activated endothelial cells in tumour stain with anti-CD34, while sinusoidal cells in surrounding liver are CD34-negative (magnification ×2.5). d — Staining with polyclonal CEA outlines the tumour canalicular network (magnification ×10). e — Immunostaining with CK19 shows mild ductular proliferation in the fibrous tissue at the edge of a tumour (magnification ×2.5). f — Staining with OV-6 monoclonal antibody shows prominent bile ductular proliferation to the right of a tumour (magnification ×10).

survival rates were better after resection of HCCs in patients with non-cirrhotic livers than in those with cirrhotic livers.[6] The improved prognosis for patients operated on for HCCs in non-cirrhotic livers compared with those operated on for HCCs in cirrhotic livers may be accounted for by the fact that resection in patients with cirrhosis is associated with a higher mortality rate related to the surgical procedure.[6] The disease-free survival of our patients, most of whom had tumours in non-cirrhotic livers, was 85%, 75%, and 62% at 1, 2 and 3 years, respectively. The major long-term problem in our patients, as in all reported series,[3,6,24,28] was tumour recurrence, and this was the cause of all late deaths.

Liver transplantation for HCC remains a controversial issue, particularly for HCC in a non-cirrhotic liver. We very rarely do transplants for HCC at our institution. Most patients seen by us have chronic HBV infection and extensive hepatic infiltration by HCC. There is a high risk of recurrence of both conditions in the transplanted liver, and the long-term survival is dismal. We feel that transplantation for malignant disease is not justified in the face of a shortage of donor organs, and where patients with non-malignant and non-recurring liver disease are dying while on the waiting list for transplantation. No liver transplants have been performed for known HCC in Cape Town since the establishment of a liver transplantation programme in 1988. However, a small incidental HCC was found in the liver explant of one patient transplanted at our institution for cryptogenic cirrhosis. He remains well with no evidence of tumour recurrence 7 years after transplantation.

Histopathological examination of the resected specimens confirmed the rarity of fibrolamellar carcinoma (FLC) in South Africa. In this study, we found only one case of FLC, in a 24-year-old white man. Diagnosis of FLC is nevertheless important as this histological subtype has been felt to have a better prognosis, with resectability rates of 47 - 75% and an overall survival at 5 years of 25 - 63%.[28] However, the belief that there is improved prognosis with FLC has recently been questioned.[30] Fibrolamellar tumours are generally advanced at the time of presentation;[30] indeed the tumour in our patient showed evidence of vascular invasion and breaching of the capsule on histology. Most HCCs examined showed a trabecular pattern of growth. The tumours were well to moderately differentiated; we did not use a grading system for tumours in this study because of lack of correlation with clinical behaviour and because of tumour heterogeneity, with several grades often found in the same tumour.[31]

Immunostains clearly demonstrated AFP in the cytoplasm, while canaliculi, often grossly distorted, could be shown with a polyclonal CEA stain, which cross-reacts with biliary glycoprotein.[32-34] Immunoreactivity of capillaries in HCC was clearly demonstrated using anti-CD34 for activated endothelial cells.[35,36]

Because staining of sinusoids in cirrhotic liver is virtually negative with anti-CD34, this antibody may be a good marker for distinguishing well-differentiated HCC from non-neoplastic regenerative nodules in a cirrhotic liver.[37,38] These immunocytochemical markers are particularly useful in the diagnosis of difficult cases of malignant hepatic tumours where the histogenesis was in doubt.[31,39]

When human liver cancer arises in cirrhotic livers, the increased incidence of HCC is probably related to increased liver cell turnover.[15] In the non-neoplastic areas of cirrhotic liver in patients with HCC, cells have been identified that resemble the 'oval cells' (stem cells) and 'transitional cells' seen in rats during experimental hepatocarcinogenesis. These cells are thought to be possible progenitors of mature hepatocytes.[40] In a study of 14 HCC resection specimens from China, Hsia *et al.*[41] observed the proliferation of a new population of epithelial cells in actively regenerating nodules and in liver tissue surrounding the cancers. These 'oval-like' cells stained strongly positive for CK19 and with OV-6 monoclonal antibody against rat oval cells. Oval cells and transitional types of cells appeared to be the principal producers of AFP in the regenerating liver.[41] In a subsequent study of 26 HCC resection specimens, oval cells and transitional cells were shown to contain HBsAg and/or hepatitis B core antigen by immunohistochemistry.[42] In this study of 14 surgical specimens, immunostaining with CK19 and OV-6 demonstrated mild nonspecific ductular proliferation in non-neoplastic livers of cirrhotic patients, but no AFP production. No proliferation of stem cells was observed in liver adjacent to HCC. Presumably the oval cell proliferation observed by others is related to the increased cell turnover in livers with end-stage cirrhosis.[43]

Conclusions

Surgical resection for cure is appropriate for a small group of carefully selected patients with HCC. Extensive resections for large tumours are well tolerated in young patients with non-cirrhotic livers. The main long-term problem in these patients remains recurrence of HCC. Liver transplantation for HCC is not a realistic option in South Africa. The vast majority of HCC patients present with advanced disease and the prognosis is dismal.

The authors wish to thank Dr Sedick Isaacs for calculating the survival curve, Mrs Heather McCleod and Mrs Rochelle Barnard for expert technical assistance with the immunohistochemistry, and Professor Peter Cruse and Dr Wendy Spearman for critical review of the manuscript.

References

1. Colombo M. Treatment of hepatocellular carcinoma. *J Viral Hepat* 1997; **4:** suppl, 125-130.
2. Bruix J. Treatment of hepatocellular carcinoma. *Hepatology* 1997; **25:** 259-262.
3. Ringe B, Pilchmayr R, Wittekind C, Tush G. Surgical treatment of hepatocellular carcinoma: experience with liver resection and transplantation in 198 patients. *World J Surg* 1991; **15:** 270-285.
4. Iwatsuki S, Starzl TE, Sheahan DG, *et al.* Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann Surg* 1991; **214:** 221-229.
5. Bismuth H, Chiche I, Castaing D. Surgical treatment of hepatocellular carcinomas in noncirrhotic liver: experience with 68 resections. *World J Surg* 1995; **19:** 35-41.
6. Bismuth H, Chiche L, Adam R, Castain D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993; **218:** 145-151.
7. Okuda K. Hepatocellular carcinoma: recent progress. *Hepatology* 1992; **15:** 948-963.
8. Tsukuma H, Hiyama T, Tanaka S, *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328:** 797-801.
9. Shiina S, Yasua H, Muto H, *et al.* Percutaneous ethanol injection in the treatment of liver neoplasms. *Am J Radiol* 1987; **52:** 949-952.
10. Ebara M, Otho M, Sugiura N, *et al.* Percutaneous ethanol injection for the treatment of small hepatocellular carcinoma. Study of 95 patients. *J Gastroenterol Hepatol* 1990; **5:** 616-626.
11. Livraghi T, Giorgio T, Marin G, *et al.* Hepatocellular carcinoma in cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. *Radiology* 1995; **197:** 101-108.
12. Madden MV, Krige JEJ, Bailey S, *et al.* Randomised trial of targeted chemotherapy with lipiodol and 5-epidoxorubicin compared with symptomatic treatment for hepatoma. *Gut* 1993; **34:** 1598-1600.
13. Pellettier G, Roche A, Ink O, *et al.* A randomised trial of hepatic arterial chemoembolization in patients with unresectable hepatocellular carcinoma. *J Hepatol* 1990; **11:** 181-184.
14. Groupe d'Étude et de Traitement du Carcinome Hépatocellulaire. A comparison of lipiodol

chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. *N Engl J Med* 1995; **332:** 1256-1261.

15. Kew MC, Popper H. Relationship between hepatocellular carcinoma and cirrhosis. *Semin Liver Dis* 1984; **4:** 136-146.
16. Kew MC. Hepatocellular carcinoma in Africa. In: Terblanche J, ed. *Hepatobiliary Malignancy: Its Multidisciplinary Management*. London: Edward Arnold, 1994: 189-202.
17. Kew MC. Hepatocellular carcinoma with and without cirrhosis: a comparison in southern African blacks. *Gastroenterology* 1989; **97:** 136-139.
18. Lai ECS, Wong J. Hepatocellular carcinoma: surgical management. In: Terblanche J, ed. *Hepatobiliary Malignancy: Its Multidisciplinary Management*. London: Edward Arnold, 1994: 111-129.
19. Couinaud C. *Le Foie: Études Anatomiques et Chirurgicales*. Paris: Masson, 1957.
20. Bismuth H. Surgical anatomy and anatomical surgery of the liver. *World J Surg* 1982; **6:** 3-9.
21. Terblanche J, Krige JEJ, Bornman PC. Simplified hepatic resection with the use of prolonged vascular inflow occlusion. *Arch Surg* 1991; **126:** 298-301.
22. Altman DA. *Practical Statistics for Medical Research*. 7th ed. London: Chapman & Hall, 1996: 365-395.
23. Rees M, Plant G, Wells J, Bygrave S. One hundred and fifty hepatic resections: evolution of technique towards bloodless surgery. *Br J Surg* 1996; **83:** 1526-1529.
24. Tobe T, Aril S. Improving survival after resection of hepatocellular carcinoma: characteristics and current status of surgical treatment of primary liver cancer in Japan. In: Tobe T, Kameda H, Okudaira M, *et al.*, eds. *Primary Liver Cancer in Japan*. Tokyo: Springer, 1992: 215-220.
25. Nagasue N, Yukaya H, Ogawa Y, Sasaki Y, Chang YC. Clinical experience with 118 hepatic resections for hepatocellular carcinoma. *Surgery* 1986; **99:** 694-699.
26. Iwatsuki S, Starzl TE. Personal experience with 411 hepatic resections. *Ann Surg* 1988; **208:** 421-426.
27. Adson MA. Primary hepatocellular cancers: Western experience. In: Blumgart LH, ed. *Surgery of the Liver and Biliary Tract*. Edinburgh: Churchill Livingstone, 1988: 1153-1165.
28. The Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. *Cancer* 1994; **74:** 2772-2780.
29. Rosen CB, Nagorney DM. Fibrolamellar and less aggressive hepatocellular carcinomas. In: Terblanche J, ed. *Hepatobiliary Malignancy: Its Multidisciplinary Management*. London: Edward Arnold, 1994: 204-214.
30. Stevens WR, Johnson CD, Stephens DH, Nagorney DM. Fibrolamellar hepatocellular carcinoma: stage at presentation and results of aggressive surgical management. *AJR* 1995; **164:** 1153-1158.
31. Ishak KG. Pathology of hepatic malignancy. In: Terblanche J, ed. *Hepatobiliary Malignancy: Its Multidisciplinary Management*. London: Edward Arnold, **1994:** 4-26.
32. Chedid A, Chejfec G, Eichorst M, *et al.* Antigenic markers of hepatocellular carcinoma. *Cancer* 1990; **65:** 84-87.
33. Brumm C, Schultz C, Charels K, Moroshi T, Klöppel G. The significance of alpha-fetoprotein and other tumour markers in differential immunocytochemistry of primary liver tumors. *Histopathology* 1989; **14:** 503-513.
34. Imoto M, Nshimura D, Fukuda Y, Sugiyama K, Kumada T, Nakamo S. Immunohistochemical detection of alpha-fetoprotein, carcinoembryonic antigen, and ferritin in formalin-paraffin sections from hepatocellular carcinoma. *Am J Gastroenterol* 1985; **80:** 902-906.
35. Ito A, Nomura S, Hirota S, Suda T, Kitamura Y. Enhanced expression of CD34 messenger RNA by developing endothelial cells of mice. *Lab Invest* 1995; **72:** 532-538.
36. Fina L, Molgaard HV, Robertson D, *et al.* Expression of the CD34 gene in vascular endothelial cells. *Blood* 1990; **75:** 2417-2426.
37. Ruck P, Xiao J-C, Kaiserling E. Immunoreactivity of sinusoids in hepatocellular carcinoma. *Arch Pathol Lab Med* 1995; **119:** 173-178.
38. Nakamura S, Muro H, Suzuki S, *et al.* Immunohistochemical studies on endothelial cell phenotype in hepatocellular carcinoma. *Hepatology* 1997; **26:** 407-415.
39. Van Eyken P, Sciot R, Paterson A, Callea F, Kew MC, Desmet VJ. Cytokeratin expression in hepatocellular carcinoma: an immunohistochemical study. *Hum Pathol* 1988; **19:** 562-568.
40. Evarts RP, Nagy P, Marsden E, Thorgeirsson SS. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. *Carcinogenesis* 1987; **8:** 1727-1740.
41. Hsia CC, Evarts RP, Nakatsukasa H, Marsden ER, Thorgeirsson SS. Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. *Hepatology* 1992; **16:** 1327-1333.
42. Hsia CC, Thorgeirsson SS, Tabor E. Expression of hepatitis B surface and core antigens and transforming growth factor-α in 'oval cells' of the liver in patients with hepatocellular carcinoma. *J Med Virol* 1994; **43:** 216-221.
43. Vandersteenhoven AM, Burchette J, Michalopoulos G. Characterization of ductular hepatocytes in end-stage cirrhosis. *Arch Pathol Lab Med* 1990; **114:** 403-406.

Accepted 14 Feb 1998.