

Incidence, Etiologic Aspects and Clinicopathologic Features in Intrahepatic Cholangiocellular Carcinoma

A Study of 51 Cases From a Low-Endemicity Area

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A total of 51 cases (19 males and 32 females) of intrahepatic cholangiocellular carcinoma (CCC) from a low-endemicity area of primary liver cancer was analyzed during the periods from 1958 to 1979 and from 1984 to 1991. The mean annual age-adjusted incidence rate was 0.44 for males and 0.56 for females per 100 000 inhabitants. CCC was diagnosed before death in only 31%. There was a female predominance in patients over 70 years of age ($p < 0.05$). At presentation, malaise (85%), weight loss (73%), abdominal pain (50%) and hepatomegaly (80%) were common. The median survival time from diagnosis was 2 months. The mean age at the time of death was 72 years (range 41–92). At autopsy, cholelithiasis was found in 61% (81% in patients older than 70 years) and cirrhosis in 30% of patients. Cholelithiasis was more common in CCC ($p < 0.01$) than in hepatocellular carcinoma cases with the same mean age. Not one case of inflammatory bowel disease was found. The gross appearance of the tumor was predominantly massive (49%) or multinodular (35%). The most common histological features were tubular pattern of growth (82%) and abundant fibrous stroma. Metastases were particularly associated with the lymph nodes (41%), skeleton (26%) and lungs (16%).

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Primary liver cancer (PLC) is one of the most common malignancies in the world (1). The great variability of PLC incidence geographically (2, 3) appears to be due to variations in the predominant histological type, hepatocellular carcinoma (HCC) (4). Many studies have been conducted on HCC, and knowledge about its incidence, etiology and clinical characteristics has greatly increased over the past few decades (4–6).

Much less is known about the next most common histological type of PLC, intrahepatic cholangiocellular carcinoma (CCC). This tumor is believed to occur with generally the same frequency everywhere except in parts of South East Asia, where CCC is reported to be more frequent (4). The prognosis for CCC is usually very poor (7–10). CCC is refractory to chemotherapy (7, 10, 11) and surgical resection is the treatment of choice although the resectability is generally extremely limited (8). The results of liver transplantation for CCC have also been very disappointing (12). Consequently, it is of particular interest to define any possible etiologic factor and thus, if possible, prevent the development of the disease. Recognition of patients at risk of CCC and increased knowledge about clinical characteristics may lead to earlier diagnosis and a

better prognosis. It is also important to establish the role of different macro- and microscopic features, including the tendency of the tumor to metastasize, for the fatal outcome of this disease.

There are few reports on different aspects of CCC, including etiology, and those that are available usually only deal with a limited number of cases (7, 9, 10, 13). This is particularly true in low-incidence areas of PLC, where CCC is very rare (11, 14). Therefore, relatively little is known about whether there are differences in clinical characteristics, tumor growth and metastatic spread between patient cohorts from low-, compared with high-incidence areas of PLC.

Most of the reports on CCC derive from large referral centers without a defined population, and usually include highly selective samples of patients (7, 9–11, 13). Furthermore, since many cases with PLC may be found incidentally at autopsy (15, 16), a low autopsy frequency results in the exclusion of a significant number of patients with clinically unknown CCC.

In the present study, we report the data on 51 cases of intrahepatic CCC from Sweden, a low endemic area of PLC (3, 17). The aims of this study were to state the

incidence of CCC, to identify probable etiologic factors and to study various clinicopathologic features and their relation to metastatic spread in CCC. To minimize some of the disadvantages mentioned above, our report considers a well-defined population, and includes all known cases with CCC during a defined time period with high autopsy frequency.

MATERIAL AND METHODS

This CCC study was carried out in Göteborg, Sweden, as a retrospective survey during the period from 1958 to 1979, and as a prospective study over a period of 7 years (1984–1991). Göteborg is an industrial town, with a population varying between 395 000 and 465 000 during the study periods. The retrospective CCC survey was part of a comprehensive study of PLC (18). During these 22 years—according to a specially ordered material from the National Central Bureau of Statistics—as many as 67% of all deceased inhabitants in this area underwent either clinical or forensic autopsy. Since then the autopsy frequency has decreased to about 30%. All cases with liver cancer diagnosed after 1 January 1958, the date when the Swedish Cancer Registry started its activity, until 31 December 1979, were analyzed. The material of this Registry is based on compulsory reports from physicians working in hospital as well as pathologists and cytologists and will be appropriate for the study of PLC (19).

During the studied period, 663 cases were registered with a histopathological diagnosis of PLC. Tissue specimens were available for examination in 98% of these cases ($n = 649$). Re-evaluation of the preserved tissue revealed 48 cases with CCC and 532 cases with HCC (18). Mixed hepatocellular and cholangiocellular carcinoma ($n = 5$) were excluded from the survey. CCC was the next most common histological type and constituted 8% of all PLC cases. Autopsy was performed on all but one of these patients.

The prospective CCC study was conducted in the two main hospitals in Göteborg and was also part of a comprehensive PLC study (20). All patients who were admitted to the Departments of Medicine or Surgery because of strongly suspected PLC, and who came to the authors' attention, were included. PLC was the final diagnosis in 76 cases. There were three cases of CCC (4%) and two of these patients were presented for autopsy. The remaining cases were all diagnosed as HCC.

In both the retro- and prospective part of the study, the specimens for histopathological examination were obtained by biopsy ante mortem (needle, wedge biopsy or tissue blocks of resected tumors) or at autopsy. The formalin-fixed, paraffin-embedded tissue specimens were collected and slides for light microscopy were stained with hematoxylin and eosin. All the microscopic slides were reviewed by two of us (GH, JK) and studied also with

regard to the diagnosis and the histology of available, non-neoplastic liver tissue.

PLC was classified according to Anthony (4) and Wight (21). Only bile-duct carcinomas arising within the liver were considered as CCC (10, 21).

Histologically, the pattern of tumor growth was divided into tubular and papillary types (13, 21). The degree of differentiation of the tumor was classified into three grades: highly, moderately and poorly differentiated tumor (13).

In the retrospective part of the study, patient records were tracked down and scrutinized. In the prospective study all the patients were interviewed (JK) and followed up by personal examination or by examination of their clinical records. The CCC patients were all Swedish-born Caucasians and thus constituted a racially homogeneous population. The scrutiny of the case files, autopsy records and interviews gave information about clinical history and possible etiologic factors.

Autopsy records also contained information about cause of death, weight of the liver and the spleen, gross appearance and localization of the tumor, and possible involvement of blood vessels and metastases. For the macroscopic classification of CCC we used the system introduced by Eggel at the turn of the century and subsequently adopted by most investigators (13). This system distinguishes three forms:

- *The multinodular*: more or less rounded nodules of tumor scattered throughout the liver.
- *The massive*: a large mass of tumor occupying part of the liver
- *The diffuse*: a diffuse tumor infiltrate which replaces the liver parenchyma.

On the basis of this classification we have divided the gross appearance of the tumor into five subtypes in our study (Table 4).

Age-standardized incidence rate was calculated using the Swedish standard population (the census of 1970) as a standard (17).

Standard statistical methods were employed using the group comparison t-test for comparison of two mean values and the z or χ^2 test for comparison of two proportions.

RESULTS

Of the 51 biopsy-verified cases of CCC, 19 were males and 32 females. Eleven of the 20 patients aged 70 years or under were men. Of the 31 patients over 70 years of age, 8 were men. The uneven sex distribution regarding age was significant ($p < 0.05$). The mean age of all patients at the time of death was 72 years (range 41–92) with no significant sex differences.

The mean annual age-adjusted incidence rate during the period from 1958 to 1979 was 0.44 for males and 0.56 for

Table 1

The importance of autopsy for the definitive diagnosis in 48 cases of cholangiocellular carcinoma (CCC)

Diagnosis of CCC	n	(%)
CCC known/suspected ante mortem		
Histological or cytological diagnosis	13	27
Clinical and/or radiological suspicion	2	4
Other malignancy suspected ante mortem		
Histological or cytological diagnosis	3	6
Clinical and/or radiological suspicion	24	50
No malignancy suspected ante mortem	6	13

females per 100 000 inhabitants. In the retrospective study, CCC was known or at least suspected in only a minority of the cases before autopsy (Table 1).

Clinical aspects and prognosis

Most patients presented with general malignant symptoms such as malaise, weight loss and anorexia (Table 2). Abdominal pain was common. Physical examination revealed hepatomegaly in 80% of the patients but jaundice was relatively uncommon (Table 2). Biochemical evaluation of the liver function showed an aspartate aminotransferase/alanine aminotransferase activity ratio above 1.0 in 84% of the patients and, on average, a threefold increased serum activity of alkaline phosphatase.

The prognosis for CCC in this unselected study was extremely poor. Despite chemotherapy in some cases, liver resection in one case and liver transplantation in another one, none of the patients was cured and the median survival rate from the time of the diagnosis was 2 months. The surgically treated patients survived 21 and 6 weeks, respectively. The longest survival time was one year. An autopsy had been performed in almost all (96%) cases. In general (92%), the cause of death was either directly or indirectly related to CCC and/or underlying liver disease such as hepatic failure and gastrointestinal bleeding. Three of the remaining four cases died of cardiovascular disease and one of an advanced gastric carcinoma.

Etiology

Not a single case of liver flukes was found on histological examination and none of these 51 patients with CCC had a history of inflammatory bowel disease. One patient had undergone a radiographic examination using the contrast dye Thorotrast 40 years before admission. Information about drinking habits was given in only 23 cases and among those there were 3 alcoholics (13%). In this population alcoholism was less common when compared with HCC cases from the same population and with the same mean age (corresponding figure 42%, $p < 0.01$) (18). Two patients had had other malignant tumors treated successfully before diagnosis of CCC and one had a gastric carcinoma at the same time. These three patients had a mean age of 80 years.

Cholelithiasis, defined as gallstones found in the gallbladder and/or in the bile ducts at autopsy ($n = 22$), or cholecystectomy before diagnosis of the tumor ($n = 8$), was found in 61% of CCC patients at autopsy ($n = 49$). A cholecystectomy had been performed because of gallstones in all cases, where this information was given ($n = 6$). Cholelithiasis was found in 81% of all the patients over the age of 70 years ($n = 31$). Cholelithiasis was more common in CCC patients compared with HCC cases from the same population and with the same mean age (36%, $p < 0.01$) (18).

Cirrhosis of the liver could be established in 14 out of 46 patients (30%), where non-neoplastic liver tissue was available for examination. There was no significant difference between the proportion of male CCC cases with cirrhosis (41%) and female cases (28%). Cirrhosis was known before diagnosis of CCC in 4 cases (29%). In 71% the etiology of the cirrhosis was unknown.

Microscopic and macroscopic appearance

The most common histological type of CCC was tubular pattern of growth (Table 3). Stromal connective tissue was usually abundant, particularly in the tubular type. The

Table 2

Clinical symptoms in 51 patients with cholangiocellular carcinoma

Symptoms	Patients with symptoms (n)	Evaluable cases (n)	Prevalence (%)
Malaise	35	41	85
Weight loss ¹	22	30	73
Anorexia	21	30	70
Abdominal pain	21	42	50
Ankle edema	13	41	32
Ascites	14	46	30
Jaundice	13	47	28
Fever	9	33	27
Gastrointestinal bleeding	6	45	13
Abdominal mass	3	36	8
Diarrhea	2	39	5

¹ mean value 12 (range 4–20) kg.

Table 3
Histological characteristics of choangiocellular carcinoma

Pattern of growth	Evaluable cases		Degree of differentiation					
			High		Moderate		Poor	
	n	(%)	n	(%)	n	(%)	n	(%)
Tubular	42	(82)	1	(2)	31	(74)	10	(24)
Papillary	9	(18)	1	(11)	6	(67)	2	(22)
Total	51	(100)	2	(4)	37	(73)	12	(24)

most frequent gross appearance of CCC in the 49 cases presented for autopsy was the massive and the multinodular form (49% and 35%, respectively) (Table 4). The tumor was usually grayish white and firm and was on average 11 cm in diameter (range 1 cm–25 cm). In 17% of the cases the tumor was hemorrhagic macroscopically. However, there was not a single case of ruptured CCC.

Tumor distribution

At autopsy, the tumor distribution included both the right and left lobes of the liver in 74% of the cases, with an even distribution in both lobes in 46% of all patients. In cases with an uneven tumor involvement in the liver, the largest tumor mass was in either the right (11%) or the left lobe (11%), or in the central part of the liver (7%). The right lobe only was implicated in 15%, the left lobe in 2% of cases, and in 9% of the cases only the central part of the liver was implicated. In three cases there was insufficient information about the tumor involvement.

Involvement of veins and biliary tract

Invasion of portal and hepatic veins was observed in 4 out of 24 cases (17%), where the information in the autopsy records was sufficient to confirm or exclude a macroscopic venous involvement of CCC. A portal invasion was present in 3 out of these 4 cases. If microscopic tumor growth in

the vessels is regarded as vascular invasion, this feature was noted in 33% of the cases. Involvement of the biliary tract was unusual (10% of 48 evaluable cases). In 6% of the cases, CCC was growing in the ductus hepaticus, choledochus or the gallbladder and in 4% the choledochus and the gallbladder were incorporated in the tumor, which did not originate from the extrahepatic bile tree as judged from the macroscopic appearance.

Metastases

The most common sites of metastases were the lymph nodes, skeleton, lungs and peritoneum (Table 5). There was not a single case of metastases in the brain. CCC involved lymph nodes in the region of the liver in 30% (n = 15) and lymph nodes elsewhere in 20% of the cases (n = 10). Apart from regional lymph nodes, metastases occurred mainly in the para-aortal nodes (n = 6) and mediastinum (n = 3).

There was one case each of metastases in costa, os frontalis, humerus and spina iliaca anterior, but in most cases of skeleton metastases there was vertebrae involvement (70%). It must be pointed out, however, that the vertebrae are the only parts of the skeleton that are examined systematically during a clinical autopsy.

In 17 cases the metastatic growth could be excluded, although all sites (Table 5) were investigated and sufficient information was given in the autopsy records. This finding was more common when compared with HCC cases from the same population (22) (35% and 21%, respectively, $p < 0.05$).

The mean weight of the liver at autopsy was 2 446 g, (range 870 g–6 200 g) and it was higher in cases with metastases than in cases without metastases (2 725 g and 1 933 g respectively, $p < 0.05$). The average weight of the spleen was 218 g (range 66 g–650 g) with no significant difference between cases with and without metastases. Metastases in lymph nodes were found in 50% of all evaluable cases with a tubular pattern of growth (n = 40) but not in a single case with a papillary pattern of growth (n = 9). This difference in metastasis frequency was significant ($p < 0.01$), but there were no significant differences regarding other metastasis sites between cases with tubular and papillary patterns of growth. Sex, age, presence of cirrhosis, macroscopic appearance of the tumor, involvement of both liver lobes, vascular invasion and the grade of differentiation were not found to be significantly related to metastatic growth.

Table 4

Macroscopic appearance of cholangiocellular carcinoma

Macroscopic classification Subtypes	Evaluable cases	
	n	(%)
1. Multinodular	17	(35)
2. Massive		
With satellites	15	(31)
Without satellites	9	(18)
3. Diffuse		
Mixed (2 and 3)	3	(6)
Diffuse	5	(10)
Total	49 ¹	(100)

¹ One case evaluated at laparotomy, all others at autopsy.

Table 5

Sites of metastases at autopsy in 49 patients with cholangiocellular carcinoma. Direct invasion and overgrowth of the tumor is included as metastatic spread

Sites of metastases	Patients with metastases (n)	Evaluable cases (n)	Prevalence (%)
Lymph nodes ¹	20	49	41
Skeleton	10	39	26
Lung	8 ²	49	16
Peritoneum	7	49	14
Pleura	6	49	12
Adrenal gland	5	47	11
Pancreas	4	49	8
Omentum	3	49	6
Duodenum	3	49	6
Pericardium	2	49	4
Others ³	4	49	<3 ³
Brain	0	42	0

¹ Includes lymph nodes in the region of the liver and in other sites (see text).

² Includes 1 case with tumor emboli in the blood vessels but no macroscopic metastases in the parenchyma.

³ One of each: diaphragm, spleen, mesentery, vena cava.

DISCUSSION

Our study was designed to minimize many sources of error which afflict other reports on CCC. Our report concerns a specified region with a well-defined, racially homogeneous population, and during the period of the retrospective part of the study all known cases with PLC were taken into consideration (19). Owing to a high autopsy frequency during this period, histologically proved diagnosis in the vast majority of cases, and preservation of biopsy material for re-evaluation, we had a unique opportunity to present reliable figures about the incidence of CCC in a low endemic area. The size of our material and performance of autopsy in almost all cases should also render the results of the clinicopathological part of our study reliable.

The relative proportion of CCC in relation to PLC cases in our study from a low-incidence area is similar to the results from other surveys, where CCC accounts for between 5 and 30% of all cases of PLC (7, 8, 11, 23–25). The patients were mostly old, which is also in accordance with other reports (4, 7, 21). CCC is reported either to affect both sexes equally (4, 7, 11, 14, 21, 26) or to be more common in men (10, 13, 25). However, in our study, CCC cases were twice as numerous in women than in men, and CCC was more common in women even though the mean annual incidence was age-adjusted. Our study indicates that there is a higher risk of CCC in women than in men in a population older than 70 years. Thus, the female preponderance in our patient cohort with a higher mean age than in other reports should not be surprising.

Malaise, anorexia and weight loss were more common symptoms in our study than reported by others (7, 8, 11, 13). However, the frequency of other common symptoms and findings in CCC patients in our study, such as right upper abdominal pain, jaundice, fever and hepatomegaly,

is essentially in agreement with other reports (7, 8, 11, 13). Our study also confirms that the prognosis for CCC is extremely poor.

The increased incidence of intrahepatic CCC in South East Asia is clearly related to infestation with liver flukes (4, 21) but otherwise relatively little is known about the etiology of CCC. Ulcerative colitis and Crohn's disease, with or without sclerosing cholangitis, have been postulated as important etiologic factors in CCC (11, 27). However, there is some confusion about the definition of CCC in these studies and cancer of extrahepatic origin is often included (27), although it had been pointed out that only bile duct carcinomas arising peripherally within the liver should normally be called CCC (21). There is no doubt that there is a strong association between inflammatory bowel disease and cancer originating in the major hepatic ducts (11, 27–29), but this etiologic association with intrahepatic CCC seems to be much weaker and tends to vary between 0% and 7% (11, 13). None of the patients in the present study was found to be infested with liver flukes, nor had any of them a history of inflammatory bowel disease.

Congenital anomalies of the biliary tree (4, 21, 30) and exposure to Thorotrast (31–33) have been reported to increase the risk of CCC. CCC has also been reported to occur in women on oral contraceptive steroids (34, 35), in men on anabolic/androgenic agents (36) and in patients with alpha-1-antitrypsin deficiency (37), but the etiological significance is unclear. With the exception of one patient who was exposed to Thorotrast 40 years prior to diagnosis, none of these factors were identified in patients with CCC in the present study. Alcoholism as a prominent feature in CCC patients (45%) is reported in only one study (11). The frequency of alcoholism in our study was much lower.

Contrary to HCC, there seems to be no association between cirrhosis and CCC. Cirrhosis in cases with CCC is sometimes thought to be the consequence rather than the cause of the tumor (21) and is reported in between 0 and 38% of all cases with CCC (7, 11, 13, 14, 25). Almost one-third of the cases with CCC in our study had liver cirrhosis and though the frequency is lower than in patients with HCC (18), cirrhosis cannot be excluded as a contributory factor in the etiology of CCC.

There was a remarkable correlation between cholelithiasis and CCC in the present study. Cholecystectomy has been reported to decrease the risk of extrahepatic bile duct cancer and increase the risk of PLC, though only during the first year after surgery (38). The role of gallstones in the etiology of CCC is controversial. Intrahepatic stones, hepatolithiasis, is common in Asia (39). An etiologic relationship between hepatolithiasis and CCC is generally accepted with chronic proliferative cholangitis resulting from chronic mechanical irritation or repeated bacterial infections as a causative factor (10, 39). However, gallstones are reported in only 2–21% in most studies concerning CCC (7, 11, 13). The incidence of gallstones is reported to increase with age in the population and may be as high as 30% in patients over 70 years of age (40). The corresponding figure in our CCC patients (81%) could thus not be explained by the age factor alone. As previously reported, the finding of cholelithiasis in CCC is also significantly more common ($p < 0.01$) than in HCC cases with the same mean age (18). It has been postulated that cholelithiasis may develop as a consequence of CCC, since stenosis of the bile duct caused by CCC could lead to retention of bile flow, resulting in the formation of stones (10). However, involvement of the biliary tract in our study was unusual. Thus, cholelithiasis may be an important etiologic factor in CCC in a low-incidence area of PLC.

The most common histologic features were tubular pattern of growth and relatively abundant fibrous stroma, which is in accordance with other reports (4, 21).

The massive and the multinodular types of CCC are reported to predominate. With the exception of one report (41), the massive type is usually reported as the most common one (4, 13, 42), which is in accordance with our findings.

Vascular invasion of portal and hepatic veins is not regarded as a prominent feature of CCC, in contrast to HCC, which frequently shows extensive vascular tumor thrombi (43). However, in a Japanese study, 32% of the CCC cases had portal vein thrombi at autopsy (25). This finding was not unusual in our study either.

There was no metastatic growth at death in a minority of our case cohort, which is in accordance with other studies (4, 7, 9, 13, 25). However, the finding that it is more common with metastases in HCC than in CCC contradicts the opinion that CCC is more prone to metas-

tases than HCC (21). With the exception of the skeleton, the relative frequency of metastases in different sites in our study is in accordance with most reports, where metastasis of CCC is generally found in lymph nodes and lungs (4, 7, 9, 13, 25). Even if we included tumor emboli without metastases in the lung parenchyma, which is not necessarily synonymous with metastatic growth (44), the skeleton was still a more common site of metastases than the lungs. This discrepancy with other studies may, at least in part, be explained by the fact that these reports usually consider selected and relatively small samples of patients from populations with a higher incidence of PLC than is found in Sweden. On making a comparison with HCC cases from the same population (22), we have been able to confirm the suggestion that the pattern of spread in CCC and HCC is quite similar (21), with, in our study, the lymph nodes, lungs, and skeleton being the main sites. As expected, an increase in the weight of the liver was found to be associated with the presence of metastases.

Since the autopsies disclosed tumor involvement in both lobes and/or metastatic disease in more than two-thirds of the patients with CCC in our study, it is hardly surprising that the main cause of death was directly or indirectly related to CCC. It has to be stressed, however, that the presented results are derived from CCC patients in the terminal stage of the disease. Thus, at the time of diagnosis the metastatic growth would be less pronounced. Nevertheless, in the choice of cases eligible for therapy and evaluation of a possible metastatic spread, it should be especially important to examine lymph nodes, lungs and skeleton.

We conclude that:

- CCC in our study population is a rare disease, which afflicts older people, and with female preponderance.
- CCC has an extremely poor prognosis and in most cases remains undiagnosed before death.
- Most patients present with malaise, weight loss, abdominal pain and hepatomegaly.
- There is evidence to suggest an excess risk of CCC among patients with cholelithiasis, but the data available do not permit any conclusions to be drawn about other suggested risk factors.
- The most common sites of metastatic growth are lymph nodes, skeleton, and lungs.

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