STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	Epidemiology, therapy and outcome of hepatocellular carcinoma between 2010 and 2019 in south-western piedmont
		BACKGROUND: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and the second leading cause of cancer deaths worldwide. It is often diagnosed at an advanced stage and therefore its prognosis remains poor, with a low 5-year survival rate. HCC patients have increasingly complex and constantly changing characteristics and therefore updated and comprehensive data are increasingly needed. AIM: To analyze the epidemiology and main clinical characteristics of HCC patients in a North-West Italy referral center hospital between 2010 and 2019. METHOD: In this retrospective study, we analyzed clinical data of all consecutive patients with a new diagnosis of HCC evaluated at "Santa Croce e Carle" Hospital in Cuneo (North Italy) between 1st January 2010 and 31st December 2019. To highlight possible changes in HCC patterns over the ten-year period, we split the population into two five-year groups, according to the diagnosis period (2010-2014 and 2015-2019). RESULTS: 328 HCC patients were included (M/F 255/73; mean age 68.9 ±11,3 years), 154 in the first period and 174 in the second. Hepatitis C virus infection was the most common HCC risk factor (41%, 135 patients). Alcoholic etiology rate was 18%, hepatitis B virus infection etiology was 5% and non-viral/non-alcoholics etiology rate was 22%. Child-Pugh score distribution of the patients was: class A 75%, class B 21% and class C 4%. The average MELD score was 10,6 ± 3.7. 55 patients (17%) were affected by portal vein thrombosis and 158 (48%) by portal hypertension. HCC had average nodule size of 4.6 ± 3.1 cm. 204 patients (63%) had a number of nodules <3 and 92% (305 patients) had non-metastatic stage. Barcelona Clinic Liver Cancer staging distribution of all patients was: 4% very early, 32% early, 23% intermediate, 34% advanced and 7% terminal. Average survival rate was 1.6 ± 0.3 years. Only 20% of the patients underwent curative treatment. Age, presence of ascites, BCLC stage and therapy were predictors of better prognosis (p<0,01). Comparing the two five-year groups we observed a statistically significant difference only in global etiology (p<0,05) and AFP levels (p<0,01). CONCLUSION: In this study analyzing patients with a new diagnosis of HCC between 2010-2019, Hepatitis C virus infection was the most common etiology. Most of patients presented with an advanced stage disease with poor prognosis. Comparing the two five-year groups we observed a statistically significant difference only in global etiology (p<0,05) and AFP levels (p<0,01).
Introduction		
Background/rationale	2	Primary liver cancer is a relevant disease with more than 850,000 new cases per year worldwide1. With a 5-year survival of 18%, this neoplasm is currently the second leading oncological cause of death globally (after pancreatic cancer) and this figure is constantly increasing. Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for 90% of cases. The World Health Organization (WHO) estimates that more than 1 million patients will die of HCC in 2030. In Italy, the diagnosis of about 12,800 new cases of HCC was estimated in 2018, about 3% of all new cases of cancer, with a 5-year survival rate of 20% and a 10-year survival rate of 10%. Various risk factors for the development of HCC are well defined, such as liver cirrhosis (regardless of its etiology), chronic hepatitis B virus (HBV) and chronic

		hepatitis C virus (HCV), alcohol abuse and metabolic syndrome. Universal HBV vaccination and the extensive implementation of HCV-action antiviral agents are likely to change the etiological landscape of HCC. However, the increase in non-alcoholic fatty liver disease (NAFLD), along with metabolic syndrome and obesity, will soon become one of the most important HCC causes in Western countries. Significant discoveries have been made in understanding the epidemiology, risk factors and molecular profiles of HCC in recent decades. Furthermore, rational approaches to prevention, diagnosis and treatment have been developed. Where these approaches have been applied to high-incidence populations, they have demonstrated their effectiveness in preventing HCC and reducing its overall mortality. The fact that risk factors can be eliminated, however, does not always translate into global improvements due, for example, to the suboptimal implementation of treatments in underdeveloped areas; Similarly, despite the fact that surveillance is cost-effective in HCC, the global implementation of such programs is still suboptimal and is estimated to involve <50% of the target population in the Western countries. For this reason, the incidence and specific mortality for HCC continues to increase in many countries and in most patients this neoplasm still occurs at an advanced stage. New diagnostic approaches and recent therapeutic advances could achieve a reduction in HCC morbidity and mortality within the next few decades. In the light of all the information set out above, HCC patients have increasingly complex and constantly changing characteristics, which require an individualized and evidence-based approach. This implies the need for recent and exhaustive literature data on this type of patient. The impact of changing risk factors, demographics and new therapies remain unclear to date with few studies in the literature dedicated to these areas.
Objectives	3	This study therefore aims to analyze the epidemiology, presentation and main clinical characteristics and therapeutic management of HCC patients in the geographical area of north-western Italy in the decade 2010-2019.
Methods		
Study design	4	Retrospective cohort study.
Setting	5	In this retrospective analysis, the clinical data of 328 newly diagnosed HCC patients evaluated at the "Santa Croce e Carle" General Hospital of Cuneo (Piedmont, Italy) between 1st January 2010 and December 31st 2019 were analyzed. To highlight possible changes in HCC patterns compared to the decade-period, we divided the population into two five-year groups, according to the diagnosis period (2010-2014 and 2015-2019).
Participants	6	We selected patients by searching the clinical information system and medical records database of the hospital with the codes for HCC (according to the ICD-9M classification system).
Variables	7	HCC patients were etiologically classified according to the most likely anamnestic cause as follows: 1. HBV: if patients had a history of hepatitis B virus infection with a positive test for the hepatitis B surface antigen (HbSAg). Patients with negative HbSAg but with positive anti-HBc antibodies and a history of antiviral drug therapy were also considered in this group; 2. HCV: if patients had a history of hepatitis C virus infection with positive anti-HCV serum antibodies and HCV-RNA titers. Patients with positive serum anti-HCV antibodies but negative HCV-RNA and history of eradicating drug therapy were also considered in this group; 3. alcoholic: in case of anamnestic history of daily intake of ethanol> 60 g for women

		and> 80 g for men for more than 10 years; 4. multi-etiology: if there was a combination of causal factors (viral + alcoholic); 5. NAFLD: in case of demonstration of steatosis on ultrasound and / or biopsy and absence of significant alcohol consumption; 6. hereditary hemochromatosis: in case of alterations in transferrin saturation (> 45%) and positivity to the genetic test for one of the HFE / HJV and HAMP / TFR2 / SLC40A1 genes; 7. not known / idiopathic: in case of absence of known recognized causes and / or unspecified etiology.
Data sources/ measurement		Laboratory findings were documented at the time of initial HCC diagnosis. If they were not available on the exact date, results closest to the date of diagnosis within 90 days were considered. The severity of liver dysfunction was assessed according to the Child-Pugh classification. The MELD score, chronic liver disease severity score system, was calculated to predict survival at three months. It was specified where the diagnosis of HCC was confirmed by biopsy and histological examination. HCC was classified as unifocal, paucifocal (≤ 3 nodules), multifocal (> 3 nodules), infiltrative and / or massive (infiltrating growth pattern and / or a mass of> 10 cm diameter and an indefinite limit). The tumor size of the expanding nodules was also measured (in cases of multinodular tumors, the largest was measured). The HCC stage was assessed according to the BCLC (Barcelona Clinic Liver Cancer) staging system, updated to 202212. Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) score. Regarding treatment, patients were divided into patients who received curative therapy for HCC after diagnosis (liver transplant, surgical resection or percutaneous ablation) and patients who received non-curative therapy (TACE - Trans-Arterial Chemo Embolization, SIRT - Selective Internal Radiation Therapy, Sorafenib chemotherapy or palliation).
Bias	9	The retrospective observational nature of the study did not allow the evaluation of some very interesting variables (such as body mass index, for example). Furthermore, the exclusion of patients for whom it was not possible to derive the variables useful for the study exposed to potential selection bias. Finally, the uniqueness of the study, relating to a population belonging to a center with a specific geographical origin, exposed our data to possible reference bias.
Study size	10	the clinical data of 328 newly diagnosed HCC patients evaluated at the "Santa Croce e Carle" General Hospital of Cuneo (Piedmont, Italy) between 1st January 2010 and December 31st 2019 were analyzed. To highlight possible changes in HCC patterns compared to the decade-period, we divided the population into two five-year groups, according to the diagnosis period (2010-2014 and 2015-2019). We selected patients by searching the clinical information system and medical records database of the hospital with the codes for HCC (according to the ICD-9M classification system).
Quantitative variables	11	age, date of diagnosis of HCC, laboratory data (bilirubin, AFP, creatinine, INR, albumin), scores (Child-Pugh, MELD, BCLC, ECOG), surveillance and survival.
Statistical methods	12	Continuous variables were presented as mean and deviation standard, and categorical variables as absolute and relative frequencies. Group comparisons were performed using the ANOVA test for continuous variables and the Pearson $\chi 2$ test for categorical variables. Survival was calculated from the time HCC was diagnosed to death; the data was censored after 5 years of follow-up. The Kaplan-Meier method was used for the survival analysis and the difference between the survival curves was assessed

		using the Log-Rank test. A Cox proportional risk model was adopted to test the role of prognostic factors associated with the probability of death. Statistical significance was assumed by accepting an alpha<0.05 error. All statistical analysis was performed using Rstudio 2022.02.0 software based on R version 4.1.2.
Results		
Participants	13*	The study included 328 patients with HCC, 154 in the first period (2010-2014) and 174 in the second period (2015-2019).
Descriptive data	14*	The mean age at diagnosis was 68.9 years, with no difference between the two groups, while the mean age at death was 69.7 years in the first period and 72.4 years in the second period (p<0.05). The male/female ratio was 255/73.
Outcome data	15*	cause of liver disease, Child-Pugh's classification , MELD score, BCLC system, therapeutic procedures, overall survival.
Main results	16	The most common cause of liver disease was HCV infection (135 cases, 41%), followed by alcoholic etiology (58 cases, 18%). HBV infection was identified in only 15 cases (5%), while multiple etiology (viral + alcoholic) was found in 36 patients (11%). 7 patients (2%) had NAFLD and 4 patients (1%) had hereditary hemochromatosis. In 73 patients (22%), the etiology was unknown/idiopathic. The likelihood of portal thrombosis was statistically higher in patients with more advanced BCLC stage (100% of cases in BCLC C-D stage) (p<0.01), with AFP>200 ng/dL (83% of cases) (p<0.01), with a non-unifocal HCC (78% of cases) (p<0.01) and with portal hypertension (85% of cases) (p<0.01). According to Child-Pugh's classification, 247 patients (75%) were class A, 68 patients (21%) class B and 13 patients (4%) class C. The mean MELD score was 10.6 (±3.7). Patients were grouped into five different stages according to the BCLC system: 12 patients (4%) belonged to BCLC stage 0, 105 (32%) to BCLC stage A, 74 (23%) to BCLC stage B, 113 (34%) to BCLC C stage and 24 (7%) to BCLC stage D; 117 patients (36%), 74 patients (23%) and late stage (C-D) 137 patients (42%) were at the early stage (0-A). 64 patients (20%) underwent curative therapeutic procedures: surgical resection in 8 cases (2%) and percutaneous radiofrequency ablation in 56 cases (17%); 264 patients (80%) underwent non-curative therapeutic procedures: TACE in 124 cases (38%), SIRT in 46 cases (14%) and systemic chemotherapy with Sorafenib in 17 cases (5%). 77 patients (23%) received palliation or no therapy at all. No patients underwent liver transplantation. The likelihood of curative therapy was statistically higher in patients with unifocal HCC (73% of cases) (p<0.01), early BCLC stage (62% of cases in BCLC stage 0-A) (p<0.01), good performance status (81% of cases with ECOG score 0) (p<0.01), AFP<199 ng/dL (77% of cases) (p<0.05), without ascites (91% of cases) (p<0.05). A statistically significant difference in the likelihood of curative or non-curative therapy between the etiological groups was not evaluated. Among patients with viral etiology, 21 out of 91 (23%) patients in the first period and 20 out of 95 patients (21%) in the second period underwent antiviral therapy. Overall, among patients with viral etiology, 41 patients (22%) underwent antiviral therapy. Overall 1-year survival was 60.4% [95% CI 55.3-65.9], while 5-year overall survival was 17.1% [95% CI 13.5-21.7]. The median overall survival was 576 days [95% CI 476-695]. In the first period, 1-year survival was 56.5% [95% CI 49.1-64.9%], while in the second period 63.8% [95% CI 57.0-71.3%]. The median survival of the first period was 517 days [95% CI 361-757], while in the second period it was 603 days [95% CI 476-757] (p<0.08)
Discussion		
Key results	17	The aim of this study was therefore to analyze the clinical characteristics of a large

		cohort of HCC patients treated at a second level hospital. We noted an increase in newly diagnosed HCC cases in more recent years (174 in the 2015-2019 period vs 154 in the 2010-2014 period). We found that the mean age of newly diagnosed HCC patients increased from 67.6 to 70.0 years ($p<0.1$). In our study there was also a significant proportion (22%) of HCC apparently of idiopathic etiology. We also found a low rate of etiology related to a clear diagnosis of NAFLD (2%). Regarding tumor stage, during the entire study period, the most common HCC presentation pattern was a single nodule with a diameter between 3 and 5 cm. Regarding liver function, the majority of our population was in Child A class, in line with other national studies. In the second period (2015-2019) we found a trend (to the limits of significance) regarding the decrease in the multifocal pattern at diagnosis and a frank significance about lower AFP values at diagnosis. Regarding the staging of HCC, we highlighted two incidence peaks in BCLC stage A (early) and stage C (advanced) at the time of diagnosis. As many as 42% of the total sample was either in the advanced or terminal stage (BCLC stage C-D). By dividing this data into two five-year-olds, this percentage dropped from 47% of the first to 37% of the second (this trend also bordering on significance). From a therapeutic point of view, we found an adoption of curative therapies (surgical resection / ablation with RF) in 20% of cases, however underutilized compared to the fact that 36% of cases had 0-A (early) BCLC staging. Among the curative therapies, the most frequently used was percutaneous ablation with radiofrequency was the most frequently used. Our patients' prognosis was characterized by modest overall survival values at one year (60%) and very low at 5 years (17%).
Limitations	18	The retrospective observational nature of the study did not allow the evaluation of some very interesting variables (such as body mass index, for example). Furthermore, the exclusion of patients for whom it was not possible to derive the variables useful for the study exposed to potential selection bias. Finally, the uniqueness of the study, relating to a population belonging to a center with a specific geographical origin, exposed our data to possible reference bias.
Interpretation	19	this study presents detailed information on periodic changes in risk factors and liver function characteristics, tumor stage and treatment modalities performed at diagnosis in a large cohort of patients with HCC in a referral center in the area of the Northern Italy. The approach to HCC is far from adequate and HCC remains undertreated or inappropriately treated despite the positive changes that have occurred in recent years. This should therefore be a stimulus for a better implementation of the surveillance of patients at risk according to the guidelines of the main national and international scientific societies, with subsequent better use of the various therapeutic opportunities available.
Other information		
Funding	20	No funding received.