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Seroprevalence of HBsAg in Patients with Chronic Liver Disease and Hepatocellular Carcinoma at the Earliest Endpoint of treatment: A Cross-Sectional Study

Seroprevalence of HBsAg in Patients with Chronic Liver Disease and Hepatocellular Carcinoma

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Abstract:- Background: The ideal goal of therapy of Hepatitis B virus (HBV) infection should be host sterilization which corresponding to clearance of HBsAg from serum and of covalently closed circular DNA (cccDNA) from hepatocytes. Good combination of actual therapies may allow achievement of HBV sterilization in host. The aim of this study was to highlight seroprevalence of HBsAg in patients with chronic liver disease and hepatocellular carcinoma (HCC) at the earliest endpoint of treatment.

Methods: During a cross-sectional study, consenting participants with chronic liver disease and HCC recruited between December 2020 and March 2022 at the Yaoundé General Hospital (YGH) and the University Teaching Hospital of Yaoundé (UTHY) provided five milliliters of blood sample. A qualitative search for HBsAg was performed by Rapid Diagnostic Tests (RDTs) and Monolisa™ HBs Ag ULTRA. Data analysis were performed using SPSS Version 25.0 software.

Results: Out of the 135 patients tested for HBsAg, 69 samples were positive simultaneously with RDTs and Monolisa™ HBs Ag ULTRA, 37 (100.0%), 19 (100.0%), 13 (100.0%) respectively in participants with chronic hepatitis, cirrhosis, and HCC (P<0.000 1).

Conclusion: Our results showed a high prevalence of HBsAg in patients with chronic liver disease and hepatocellular carcinoma at the 24th week of their treatment.

Keywords:- *HBsAg, Chronic Hepatitis, Cirrhosis, Hepatocellular Carcinoma.*

I. INTRODUCTION

The World Health Organization (WHO) estimates that approximately 354 million people are living chronically with hepatitis B virus (HBV) worldwide (WHO, 2022) and this chronic infection can lead to cirrhosis and hepatocellular carcinoma (HCC) (Andoulo et al., 2013). Epidemiological data estimate that 50% of HCC cases are attributable to HBV infection worldwide (Mbaga et al., 2022) and that the number of HBV-related deaths from cirrhosis or HCC has increased considerably over the past 20 years (Harris et al., 2019). In Africa, HBV is endemic and take up considerable proportions among the 96% of mortality attributable to viral hepatitis in people living with cirrhosis and those with HCC (OMS regional Afrique, 2018). In Cameroon, the overall prevalence of HBV is around 11.2% (Bigna et al., 2017) and vary considerably across different types of Cameroonian population (Andoulo et al., 2013; Bigna et al., 2017; Djuidje Ngounoue et al., 2018; Eyong et al., 2019; Ndifontiayong et al., 2021; Ngoupa et al., 2019; Njouom et al., 2018; Noah et al., 2016; Tsague et al., 2019). To date, treatments against HBV infection focus on achieving the virological response which manifests by an undetectable viral load at the 24th or 48th week of treatment (Aljumah et al., 2019) and normalization of aminotransferase levels which allows the host immune system to gain control over the infection (Tang et al., 2018). But this is rarely achieved, either spontaneously or through treatment [16]. Following the suppression of polymerase activity by Direct Acting Antivirals (DAA), serum HBV DNA is rapidly reduced without directly affecting HBsAg production (Soriano et al., 2020) however, obtaining HBsAg seroclearance during treatment marks a functional cure (Tout et al., 2021) and this can lead to the elimination of HBV (Soriano et al., 2020). Indeed, the ideal goal of therapy against HBV infection should be host sterilization corresponding to clearance of HBsAg from serum and covalently closed circular DNA (cccDNA) from hepatocytes (Hutin et al., 2018). Current treatment for HBV infection such as nucleos(t)ide analogues or immune modulators like pegylated interferon alpha were proved to effective decreasing viral replication (Choi et al., 2022; Xu et al., 2018) HBV treatment aims to prevent or significantly delay liver-related morbidity and mortality (Martinez et al., 2021). Because a complete cure with the eradication of cccDNA is not a realistic goal with the current therapeutic options, a functional

cure, defined as durable loss of HBsAg with or without seroconversion, is considered as the optimal treatment endpoint, and it is associated with significantly improved patient outcomes (French et al., 2020). The efficacy of antivirals has markedly improved the long-term outcomes of patients with HBV infection (Lin & Kao, 2016). However, clearance of HBsAg is only achieved in a small portion of HBV patients therefore, a cure of HBV infection is still a daunting challenge (Baltayiannis & Karayiannis, 2014). Good combination of actuals HBV therapies may allow achievement of HBV sterilization in host (Tout et al., 2021). We have led this study to highlight proportion of patients with HBV infection capable to get seroclearance of HBsAg at the earliest endpoint of treatment.

II. METHODS

A. Study design and setting

We conducted a cross-sectional study at the hepato-gastroenterology departments of the Yaoundé General Hospital (YGH) and internal medicine of the University Teaching Hospital of Yaounde (UTHY) between December 2020 and March 2022.

B. Study population and sampling

The study population was composed of participants with Chronic Liver Disease and those with Hepatocellular Carcinoma (HCC). The aim of the study was explained to all consent participants before their inclusion. All participants were HBe Ag positive at the beginning of their treatment 24 weeks ago. Participants were divided into 3 groups. Group 1 consisted of participants with chronic hepatitis (CH) who were negative for cirrhosis and HCC. Group 2 consisted of participants with cirrhosis who were negative for HCC. Group 3 was composed of participants with HCC. The participants who were 21 years of age or older were chosen, regardless of their sex, age, or nationality. Participant having hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV) and those under or upper 24th weeks of treatment, were excluded from the analysis.

C. Procedure for collecting socio-demographic and clinical data

Data collection from each participant was done consecutively and prospectively. For each consenting participant, qualitative and quantitative variables were collected by interviewing the participant and by blood samples analysis. The qualitative variables collected were: Sex, marital status, scarification, tattoos and piercing, alcohol consumption before the discovery of the disease; tobacco consumption before the discovery of the disease; surgical history; history of blood transfusion; vaccination against HBV. The quantitative variables collected were Age; ALT (GPT); AST (GOT); AFP;

D. Collection and fate of blood samples

Five milliliters of blood samples were taken in EDTA tubes. The samples were divided into three aliquots of the same volume each. The first aliquot was used for the search for HBsAg, HCV and HIV the second aliquot was used for the quantification of ALT, AST, and AFP. The third aliquot was stored for further analysis.

E. Biological analysis of samples

- *Search for HBs Ag*

We carried out Rapid Diagnosis Tests (RDTs) to detect Hepatitis B virus surface antigen (HBsAg), HCV and Anti-HIV respectively by HEXAGON HBsAg, Monolisa™ HCV Ag-Ab ULTRA and Alere DetermineTM for HIV-1/2 manufactured respectively in Japan, USA and Germany according to the instructions of the designers. Positive HBsAg results were confirmed by Monolisa™ HBs Ag ULTRA for the detection of HBsAg manufactured in the USA.

- *AFP, ALT, and AST quantification*

The quantification of AFP and transaminases (ALT and AST) was carried out only on HBs Ag positive samples with Monolisa™ HBs Ag ULTRA. Thirty participants positive to HBsAg were randomly selected by using a random number generator in Microsoft Excel. The codes corresponding to the aliquots were all used in analyses. AFP quantification was performed using Finecare™ Rapid AFP Quantitative Strip (made in China) which uses fluorescence immunochromatography Assay and Finecare™ FIA Meter device. Quantification of transaminases (ALT and AST) was performed using SPRINREACT reagents (made in Spain) and a spectrophotometer.

F. Ethical considerations

To respect the ethics of medical research, the study was authorized by the University Teaching Hospital of Yaoundé (N° 3235/AR/CHUY/DG/DGA/CAPRC), Yaoundé General Hospital (N/Ref: 213-21/HGY/DG/DPM/MA-TR). The Centre for the Study and Control of Communicable Diseases of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé. Each patient who participated in this study read the information sheet then gave their signed consent and finally an anonymity code was assigned to them.

G. Sample Size calculation

The minimum study sample size was 208 participants. The calculation of this sample size was made using 11.2% HBV prevalence in Cameroon (Bigna et al., 2017) and according to GLOBOCAN data from 2020 which provided a prevalence of 3.7% for liver cancer in Cameroon (GLOBOCAN, 2020). This calculation was made using the following formula: $n=P(1-P)(Z_{1-\alpha})^2/i^2$ (Wang & Ji, 2020) with $Z_{1-\alpha}= 1.96$; $i= 0.05$; P =prevalence of outcomes.

H. Statistical analysis

For each participant recruited, qualitative and quantitative variables were collected by either interviewing the participant, or analyzing blood samples. The collected data were recorded and processed using the Excel Version 2016 (Microsoft Corp., USA). Analyses were done using the biostatistical software Statistical Package for Social Sciences (SPSS) Version 25.0. The Chi-square test allowed us to compare the proportions between the different groups and the non-parametric Kruskal Wallis test allowed us to compare the means ± standard deviations between the different groups. A p-value of < 0.05 was considered statistically significant.

III. RESULTS

A. Participants

We offered the study to 619 patients who came for consultation at the UTHY or the YGH, respectively 331 patients at the UTHY and 288 patients at the YGH. Of these 619 patients, 41 refused to participate to the study, which gave a non-response rate of 6.6%, and 93 were excluded because they were on their first medical visit. One hundred and ninety-two patients were excluded because they were infected with HCV. Of the 294 HBV patients submitted to treatment, 48 were excluded because HIV. Among the remaining 246 HIV-negative participants, 80 participants were at less than 24 weeks of treatment, 135 were at 24 weeks of treatment and 31 were at more than 24 weeks of treatment. Of the 135 eligible participants, only 97 participants were positive for HBsAg with the HEXAGON HBsAg RDTs, respectively 65 participants with CH, 19 with cirrhosis, and 13 with HCC. Of these only 69 samples were positive for HBsAg by Monolisa™ HBs Ag ULTRA, respectively 37 CH, 19 cirrhosis, and 13 HCC among which we randomly selected 10 CH, 10 cirrhosis, and 10 HCC samples, to quantify the AFPs, transaminases (ASAT, ALAT), **(Figure 1)**.

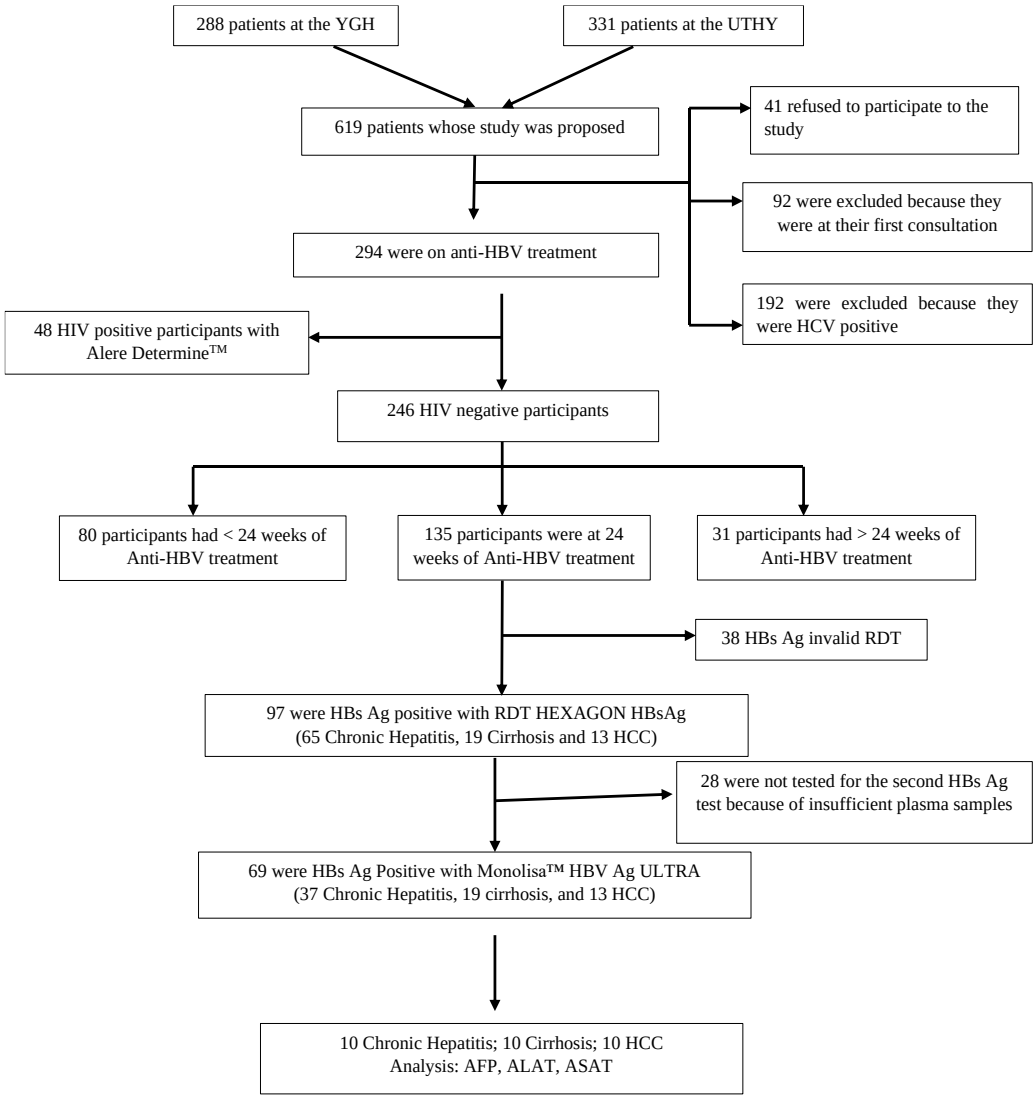

Figure 1: Flow Chart of selection of participants with Chronic Hepatitis, Cirrhosis, and Hepatocellular Carcinoma between December 2020 and March 2022

B. Sociodemographic and clinical characteristics of study participants

Overall, the age of the participants was between 26 and 71 years old. Depending on the group considered, the participants with HCC had the most advanced mean age ± SD of 65.08 ± 3.9 years. The 60-69 age group was the most represented with 8 (12.3%); 4 (21.05%) and 11 (65.5%) participants with chronic hepatitis, respectively; Cirrhosis, and HCC. The male sex was in the majority regardless of the group considered with 11 (84.6%); 13 (68.4%) and 41 (63.07%) respectively for participants with HCC, Cirrhosis, and CH. **(Table 1)**.

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Table 1: Sociodemographic and clinical characteristics of the HBsAg positive participants at 24 weeks of treatment

	CH n=65	Cirrhosis n=19	HCC n=13
	n(%)	n(%)	n(%)
Age			
Mean±**Standard Deviation**	46.6±13.7	45.8±16.1	65.08±3.9
20-29	4 (6.1)	4 (21.05)	//
30-39	8 (12.3)	5 (26.3)	//
40-49	7 (10.7)	//	//
50-59	9 (13.8)	5 (26.3)	//
60-69	8(12.3)	4(21.05)	11(84.6)
70-79	1(1.5)	1(5.2)	2(15.3)
Gender (Male)	41 (63.07)	13 (68.4)	11(84.6)
Scarification	6 (9.2)	3(15.7)	4(30.7)
Tattoo	9(13.8)	5 (26.3)	//
Surgical history	3 (4.6)	1(5.2)	1(7.6)
Blood transfusion	8(12.3)	1(5.2)	3 (23.07)
Alcohol consumption before illness	10 (15.3)	3(15.7)	2(15.3)
Smoking before illness	//	1(5.2)	2(15.3)
Vaccination against HBV (No)	65 (100.0)	19 (100.0)	13 (100.0)
HBV treatment			
Lamivudine	23(35.3)	3(15.7)	2(15.3)
Tenofovir	13(20.0)	4(21.05)	5(38.4)
Tenofovir+ Emtricitabine	29(44.6)	12(48.0)	6 (46.1)

HCC: Hepatocellular Carcinoma; CH: Chronic hepatitis; HBV: Hepatitis B Virus

C. Virological profile of study participants

After carrying out the RDTs for the search of HBsAg on the 135 participants at the 24th week of treatment, we obtained 97 positive participants for HBsAg, respectively 65 (73.6%) CH participants, 19 (63.3%) cirrhosis and 13 (76.4%) HCC positive for HBsAg (P <0.0001) **(Table 2)**. With the Monolisa™ HBs Ag ULTRA, HBs Ag was carried out on 97 patients positive for HBs Ag by TDR. Of these, 28 were not tested because of insufficient plasma samples. All the remaining 69 samples had a ratio > 1. Samples with ratio ≤1 were considered negative and samples with ratio > 1 were considered positive. These were declared positive for HBV with Monolisa™ HBs Ag ULTRA, respectively 37 (56.9%), 19 (100.0%), and 13 (100.0%) for participants with chronic hepatitis, cirrhosis, and HCC (P<0.0001) **(Table 3)**.

Table 2: Profile of participants screened for HBsAg by RDTs at 24 weeks of treatment

Virological marker		HC n(%)	Cirrhosis n(%)	HCC n(%)	P-value
HBsAg	**Invalid RDT**	23(26.1)	11(8.1)	4(23.5)	
	Positive RDT	65(73.6)	19(63.3)	13(76.4)	<0.0001

HCC: Hepatocellular Carcinoma; CH: Chronic hepatitis; HBV: Hepatitis B Virus

Table 3: Profile of participants screened for HBsAg by Monolisa™ HBs Ag ULTRA at 24 weeks of treatment

Virological markers		HC n (%)	Cirrhosis n (%)	HCC n (%)	P-value
HBsAg	**Positive ELISA**	37(100.0)	19(100.0)	13(100.0)	<0.000 1

HCC: Hepatocellular Carcinoma; CH: Chronic hepatitis; HBV: Hepatitis B Virus

D. Biochemical markers of HBsAg positive participants simultaneously with RDT and Monolisa™ HBs Ag ULTRA.

The biochemical profile of the participants consisted of the search for transaminases and AFP on the samples of the participants positive for the RDTs and Monolisa™ HBs Ag ULTRA tests and chosen randomly. Overall, the biochemical profile of participants positive for HBsAg was carried out on 30 samples distributed as follows: 10 samples from participants with chronic hepatitis (CH), 10 samples from participants with cirrhosis, and 10 samples from participants with HCC. It appears that only HCC patients had an abnormal average ALT (GPT) and AST (GPT) of 76.9 ± 10.1 IU/L and 73.7 ± 11.2 IU/L respectively compared to the participants. with CH and Cirrhosis P <0.0001 **(Table 4).**

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Table 4: Biochemical markers of randomly selected HBsAg-positive participants

	CH (n=10)	Cirrhosis (n =10)	HCC (n =10)	p-value
ALT (GPT) IU/L	28.2±12.7	57.6±9.6	76.9 ±10.1	<0.000 1
AST (GOT) IU/L	23.3±4.0	53.3±7.9	73.7 ±11.2	<0.000 1
AFP ng /ml	75.6±29.5	95.5±38.8	203.7 ±45.2	<0.000 1

HCC: Hepatocellular Carcinoma; CH: Chronic hepatitis; HBV: Hepatitis B Virus

IV. DISCUSSION

This study aimed to estimate the seroprevalence of HBsAg in participants with chronic liver disease and HCC at the 24th week of their treatment. Of the 135 patients tested, 69 samples were positive for HBsAg simultaneously with RDTs and Monolisa™ HBs Ag ULTRA, 37 (100.0%), 19 (100.0%), 13 (100.0%) respectively in participants with CH, cirrhosis, and HCC (P<0.000 1). The main objective of the antiviral treatment currently administered to patients is the complete suppression of HBV replication, which has little or no effect on HBsAg levels. Indeed, HBsAg is an envelope protein of HBV which is associated with covalently closed circular DNA (cccDNA) found inside hepatocytes and cccDNA is a structure with high resistance to the activity of currently available antiviral drugs (Lai et al., 2014). In fact, there is growing evidence that serum HBsAg, usually assessed as a qualitative marker for the diagnosis of HBV, could also be an additional tool to monitor the effects of treatment of HBV infection (Yoshida, 2014). The current research challenge is to find treatments that can increase the elimination of HBsAg, but this first requires the identification of predictive factors for treatment failure which can be combined with immunological factors in the prediction of HBsAg elimination (Tout et al., 2021). This therefore places HBsAg as a good indicator of therapeutic efficacy for preliminary evaluation of the effect of treatment on cccDNA (Huang et al., 2022). Although the induction and maintenance of long-term HBV suppression remain the primary goal of antiviral treatment, the elimination of HBsAg remains an optimal treatment endpoint (Gramenzi et al., 2011).

Depending on the three groups studied, the chronic hepatitis group was in the majority with 37 participants respectively, but the HCC group had the highest average age with 65.08 ± 39 years, a sex ratio of 1.9 in favor of men, and a predominance of HCC in the 60-69 age group. Our results are similar to those reported in previous studies conducted on participants with liver disease and HCC in Cameroon and some African countries such as Gabon, Gambia, and Nigeria (Amougou et al., 2016; Andoulo et al., 2013; Echejoh et al., 2008; Mboto et al., 2005; Perret et al., 2002) HCC participants had the highest ALT (GPT), AST (GOT) and AFP levels with respectively 76.9±10.1 IU/L, 73.7±11.2 IU/L, 203.7±45.2 ng/mL compared to participants with CH and Cirrhosis (P<0.0001). The transaminase test (ALT and AST) is simple, practical, inexpensive and non-invasive but not specific to liver disease. Indeed, transaminases are present respectively in the cytoplasm and mitochondria of hepatocytes and muscle cells. When hepatocytes are damaged by the cytopathic effects of oncogenic hepatitis viruses, transaminases are released into the bloodstream resulting in increased serum levels of ALT and AST in the peripheral blood. It is also possible that inflammatory stimulation due to tumor progression evidently stimulates hepatocytes to produce abundant amounts of transaminases compared to what will be found in patients with early liver disease (Ayuso et al., 2018; EASL, 2017; W. Wang & Wei, 2020). Besides its role in the diagnosis of HCC, we observed elevated serum AFP levels in participants with HCC. AFP is currently the most widely used serum biomarker for screening and early diagnosis of HCC, as well as for evaluating the efficacy and prognosis of HCC treatment. However, not all HCC tumors contribute to an increase in AFP levels. It is therefore important to explore the search for new biomarkers capable of overcoming the shortcomings of transaminases and AFP to complement x-ray examinations (Ghenea et al., 2021).

The results obtained in our study can be used to update and add epidemiological data to influence policy makers in HBV infection control policy. Lifelong treatment can be a burden on health systems in some developing countries, therefore it becomes imperative that research for the development of sterilization treatments for HBV infection be effective in countries with high morbidity of HBV (Hall et al., 2020). In this study we excluded all participants with comorbidities such as diabetes, and HIV because immunosuppressive diseases may increase the proportion of participants with HBsAg at the 24th week of treatment. We did not search Hepatitis D and E Viruses (HDV) and (HEV) in these study participants yet HDV is known to be a satellite virus of HBV (Amougou et al., 2016) and in 2017, Amougou Atsama et al reported a high prevalence of HEV in Cameroonian patients with HCC (Amougou Atsama et al., 2017). Only plasma samples were considered in this work, we could have obtained more revealing results if we had used serum, whole blood or biopsy fragments. We were unable to screen study participants for the concurrent presence of HBsAg and other serum HBV markers such as HBeAg, HBcrAg, Anti-HBs, Anti-HBe and Anti-HBc. As other limitations, we can note the lack of staging of cirrhotic participants and those with HCC. To achieve the WHO goals to eliminate HBV by 2030, sub-Saharan Africa will need to actively prioritize the implementation of several elimination strategies such as the introduction of free HBV treatment by national policies. Identify groups at high risk of treatment failure in order to take steps to limit the spread of antiviral resistance.

V. CONCLUSION

Our results showed a high prevalence of HBsAg in patients with liver disease and hepatocellular carcinoma at the 24th week of their treatment. This indicates a relatively very short time to assess the loss of HBsAg which is a good serological marker for evaluating functional cure.

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COMPETING INTERESTS
The authors declare no competing interest.

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