



Hepatitis C in Laos: A 7-Year Retrospective Study on 1765 Patients

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Received: 20 April 2018 / Accepted: 25 May 2018 / Published online: 13 June 2018
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Abstract

Hepatitis C virus (HCV) is a global health concern, notably in Southeast Asia, and in Laos the presentation of the HCV-induced liver disease is poorly known. Our objective was thus to describe a comprehensive HCV infection pattern in order to guide national health policies. A study on a group of 1765 patients formerly diagnosed by rapid test in health centres was conducted at the Centre of Infectiology Lao Christophe Merieux in Vientiane. The demographic information of patients, their infection status (viral load: VL), liver function (aminotransferases) and treatments were analysed. Results showed that gender distribution of infected people was balanced; with median ages of 53.8 for men and 51.6 years for women (13–86 years). The majority of patients (72%) were confirmed positive (VL > 50 IU/mL) and 28% of them had high VL (> 6log₁₀). About 23% of patients had level of aminotransferases indicative of liver damage (> 40 IU/mL); but less than 20% of patients received treatment. Patients rarely received a second sampling or medical imaging. The survey also showed that cycloferon, pegylated interferon and ribavirin were the drugs prescribed preferentially by the medical staff, without following any international recommendations schemes. In conclusion, we recommend that a population screening policy and better management of patients should be urgently implemented in the country, respecting official guidelines. However, the cost of biological analysis and treatment are significant barriers that must be removed. Public health resolutions should be immediately enforced in the perspective of meeting the WHO HCV elimination deadline by 2030.

Keywords Hepatitis C virus (HCV) · HCV incidence · Antiviral agents · Laos

Introduction

Infection with Hepatitis C virus (HCV) is one of the major causes of death and morbidity especially in low and middle income countries (Stanaway *et al.* 2016). According to Lim *et al.* (2017) the prevalence of Hepatitis C in the general population ranges from 0.1% to 4.7% in the Asia–Pacific region. In Laos, among Vientiane Capital blood donors, the prevalence of anti-HCV is 1.1% (Jutavijittum *et al.* 2007). Overall, the epidemiology of Hepatitis C remains poorly defined in Laos, despite its location in the centre of the HCV genotype-6 distribution area (Lee *et al.* 2017). Most notably, it is unknown in Laos whether HCV-infected patients correspond to an age-driven or are merely the result of on-going HCV transmission.

Chronic infection with HCV is a well-recognized risk factor for liver cirrhosis and hepatocellular carcinoma. Therefore it is important to (1) detect and (2) carefully follow infected subjects to propose adequate disease management. For this purpose, the Centre d’Infectiologie

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Lao-Christophe Mérieux (CILM) in Vientiane capital has been receiving patient samples since 2010. In the present study, we examined the course of HCV infection in 1765 patients who attended CILM between January 2010 and November 2016. Several parameters, such as gender, age, treatment allocation, HCV RNA viral load (VL) and liver damage [through aspartate (AST) and alanine aminotransferases (ALT) serum levels] were assessed. The ultimate aim of our work was to better characterize key epidemiologic features of HCV infections in Lao population and to identify unforeseen high-risk groups that should be primarily targeted by proactive screening campaigns.

Our results aim to help health authorities to apply cost-effective prevention and treatment measures to resolve the HCV infection burden as recommended by the World Health Organization in its 2030 agenda for sustainable WHO (2017).

Materials and Methods

Sample Collection

The study was conducted retrospectively by analysing the data of 1765 individuals positive for anti-HCV visiting CILM between January 2010 and November 2016. Anti-HCV antibody detection was performed using rapid point-of-care diagnostic tests according to manufacturer instructions in hospitals and private clinics before patients arrived at CILM. Patients never came to CILM on their own initiative; they were diagnosed in health care centres during routine health check-ups or during specialized consultations related to symptoms they experienced. Patients' blood was collected on EDTA, plasma harvested and stored at -20°C prior further analysis. Age, gender, geographic origin, aspartate (AST) and alanine (ALT) aminotransferases serum levels, and treatments were registered.

Viral Load Determination

VL determination was performed through Multiplex Real-Time PCR by TaqMan[®] technology (Biorad, France) with a detection threshold of 50 IU/mL (see <http://www.fast-trackdiagnostics.com/> for details).

Aminotransferases Quantification

ALT and AST concentrations were measured on HumaStar 600 machine (CoreLab, Germany), according to manufacturer's instructions. De Ritis ratio (De Ritis *et al.* 2006) or AST/ALT ratio was also calculated as in chronic liver disease, and especially in case of infection with HCV, as

when the ratio is even slightly above 1.0 (e.g. 1.09) it often correlates with the presence of fibrosis.

Statistics

Data were inputted using Microsoft Excel software (14.4.7) and analysed with Minitab software (17.3.1). Comparisons between groups (numerical data) or proportions (categorical data) were performed using Student's *t*-test, Fisher's exact test, Chi squared test, ANOVA or non-parametric tests (e.g. Mann–Whitney, Kruskal–Wallis), as appropriate. Two-sided *P*-values lower than 0.05 were considered as statistically significant.

Results

Samples

Our laboratory has received a total of 3102 blood samples, corresponding to 1765 different individuals seropositive for anti-HCV between January 2010 and October 2016 previously diagnosed through rapid tests in health care structures (see Tables 1 and 2 for details). A large majority of patients ($n = 1233$; 70%) had a single viral load measurement, while 220 (12%) had two, 129 (7.3%) had three VL tested and the others more than 3 (one patient had been tested 18 times). At the beginning of the activity of CILM in 2010, only 44 patients were received annually (Fig. 1). Then the number of patients increased to 378 in 2015 and the number of collected samples was about 2 times greater. Since 2013 the number of patients and samples have been almost stable.

Whole Group and Sub-Groups Configuration

The 1765 patients who presented at the CILM for VL measurement were sub-divided into 3 sub-groups: true positive (the largest group with 1278 people), true negative treated and not treated (487) and true negative not treated (407). The sex-ratio of the whole group of patients (and also of true positive and true negative non-treated) was 1.2. The age ranged from 13 to 86 years, with median ages of 53.8 for men and 51.6 years for women ($P = 0.006$). Patients with an on-going infection (true positive) were displaying higher median values for aminotransferases ($P < 0.0001$) than negative patients. A subset of 407 naive patients (28.4%) were negative for HCV RNA (called "true negative and non-treated" in Table 1). These naive patients could reflect the natural course of HCV infection in Lao populations with spontaneous cleared infection (if any). However, among this sub-group, 42 benefited from a second VL measurement and 7 (16.7%) of them turned out to

Table 1 Global results on the whole group and sub-groups.

At first sampling	Number of patients	Sex ratio	Median age of men	Median age of women	Mean VL log ₁₀	Number of patients with VL > 6	Median AST IU/mL	AST > 40	Median ALT IU/mL	Number of patients with AST and ALT > 40	Number of treated patients
Whole cohort	1765	1.2	53.8*	51.6*	4.04 ± 2.7	487 (28%)	55 (n = 620)	405 (23%)	55 (n = 615)	349 (19%)	329 (18%)
True positive	1278	1.2	53.3*	51.5*	5.6 ± 1.4	487 (38%)	60 (n = 468)	327 (70%)	59 (n = 464)	285 (61%)	249 (19%)
True negative (treated and not)	487	1.2	51.6	50.4	-	-	41 (n = 152)	78 (51%)	42 (n = 151)	64 (42%)	80 (16%)
True negative (non treated)	407	1.08	49	48.4	-	-	42 (n = 127)	65 (51%)	41 (n = 126)	52 (41%)	-

*Statistical difference ($P < 0.001$)

have VL > 50 IU/mL suggesting that HCV loads were possibly oscillating around detection threshold in a sizable proportion of patients. Individuals with apparent spontaneous recovery at initial sampling were significantly younger than RNA(+) patients (48.4 ± 12.7 vs. 52.4 ± 11.3 ; $P < 0.0001$). No gender difference was noticed in this sub-group. Remarkably, despite the absence of circulating HCV genomes, 41.0% (n = 52) of HCV RNA(-) subjects tested for aminotransferases displayed levels above the upper limit of normal.

Treated Patients

Treatment prescription reflected the availability of drugs in Laos and economic status of the patients; as a result, international guidelines were seldom followed by the prescribing physicians.

Ten drugs were used in mono to quadruple therapy (Table 3). In total, 329 patients (18.7% of the whole group) received an antiviral treatment including a subset of 80 (24.4%) subjects negative for HCV RNA(-) who were receiving or had already received antiviral treatment at the time of initial VL determination (Tables 1 and 2). We then compared treated individuals (those with confirmed presence of the virus), who had been cured of the virus with those patients who remained chronically infected. Gender distribution was somewhat skewed towards men in patients who displayed a sustained viral response to treatment, albeit without reaching the level of significance ($P = 0.0906$, ns). As for untreated patients, disappearance of HCV RNA in treated patients was the characteristic of younger age (49.8 ± 11.1 vs. 52.6 ± 9.3 , $P = 0.031$).

The De Ritis ratio (AST/ALT ratio) was similar in RNA(+) and RNA(-) groups with 38% of the latter displaying a value above 1.1, indicative of significant liver fibrosis.

When we considered treatments received, we observed that patients who had recovered from viral infection received Pegylated interferon significantly more often than other drugs.

Among infected patients, VLs were significantly higher in treated than in naive patients (6.4 ± 1.7 vs. 5.4 ± 1.3 , $P < 0.0001$). Furthermore, an important subset (64.3%) of treated patients had VL > 6 log₁₀, a threshold considered by some Asian authors as a predictor for liver disease progression (Noh et al. 2016). This rate was significantly lower in treated naive patients (31.8%, $P < 0.0001$). Concerning liver damage, treated patients displayed decreased enzyme values when compared with untreated ones.

Table 2 Compared characteristics of the whole group of patients and HCV RNA \mp sub-groups.

	Whole group n = 1765				HCV RNA(+) = 1278				
	All n = 487	Untreated n = 407	Treated n = 80	Naive (never treated before and after) n = 1029	Treated n = 249	Treated before VL n = 118	Treated after 1 st VL n = 95	Treated before transaminases n = 65	Treated after transaminase n = 39
<i>Sex ratio M:F</i>	1.21 (267/220)	1.10 (213/194)	2.08 (54/26)	1.18 (556/473)	1.31 (141/108)	1.15 (63/55)	1.26 (53/42)	0.97 (32/33)	1.60 (24/15)
<i>Age</i>									
Mean \pm SD	48.7 \pm 12.5	48.4 \pm 12.7	49.8 \pm 11.1	52.4 \pm 11.3	52.6 \pm 9.3	53.0 \pm 9.4	51.8 \pm 9.1	51.7 \pm 8.5	52.5 \pm 8.4
Median (range)	50.6 (13–85)	50.4 (13–85)	51.3 (18–73)	53.5 (17–86)	53.4 (19–78)	53.5 (22–78)	52.6 (19–70)	53.3 (30–69)	52.3 (33–65)
<i>HCV RNA load (log₁₀IU/mL)</i>	<50	<50	<50	> 50	> 50	> 50	> 50	> 50	> 50
Mean \pm SD	na	na	na	5.4 \pm 1.3	6.4 \pm 1.7	6.3 \pm 1.8	6.5 \pm 1.6	6.3 \pm 1.8	6.3 \pm 1.2
Median (range)	na	na	na	5.4 (1.8–12.8)	6.5 (2.0–15.3)	6.4 (2.1–14.9)	6.6 (2.0–15.3)	6.4 (3.3–15.3)	6.6 (2.0–8.0)
<i>VL > 6log₁₀ (in %)</i>	na	na	na	31.8	64.3	60.2	68.4	60	71.8
<i>Delay between ARV and VL [1] or transaminases [2] (days)</i>									
Mean \pm SD	na	na	na	na	na	271 \pm 401	108 \pm 185	402 \pm 470	262 \pm 383
Median (range)	na	na	na	na	na	106 (1–2601)	42 (0–1182)	208 (0–1983)	92 (1–1765)
<i>AST (IU/mL)</i>	(n = 152)	(n = 127)	(n = 25)	(n = 360)	(n = 108)	(n = 44)	(n = 23)	(n = 65)	(n = 39)
Mean \pm SD	73 \pm 127	72 \pm 133	78 \pm 91	99 \pm 198	89. \pm 107	91 \pm 112	93 \pm 58	76 \pm 96	111 \pm 125
Median (range)	41 (2–1413)	41 (2–1413)	43 (12–430)	61 (12–3216)	58 (11–770)	54 (11–710)	84 (22–253)	41 (11–710)	79 (22–770)
<i>ALT (IU/mL)</i>	(n = 151)	(n = 126)	(n = 25)	(n = 360)	(n = 104)	(n = 42)	(n = 23)	(n = 65)	(n = 39)
Mean \pm SD	74 \pm 118	75 \pm 125	69 \pm 73	97 \pm 189	76 \pm 58	75 \pm 57	95 \pm 73	68 \pm 53	89 \pm 64
Median (range)	42 (5–933)	42 (5–933)	40 (10–311)	61 (10–2942)	50 (13–293)	56 (13–254)	50 (29–293)	47 (13–254)	60 (25–293)
<i>De Ritis ratio</i>	(n = 151)	(n = 126)	(n = 25)	(n = 360)	(n = 104)	(n = 42)	(n = 23)	(n = 65)	(n = 39)
Mean \pm SD	1.2 \pm 1.2	1.2 \pm 1.3	1.3 \pm 0.9	1.2 \pm 0.9	1.2 \pm 0.8	1.2 \pm 0.7	1.1 \pm 0.5	1.1 \pm 0.6	1.3 \pm 1.1
Median (range)	1.0 (0.1–12.8)	1.0 (0.1–12.8)	1.1 (0.4–5.3)	1.0 (0.1–14.7)	1.0 (0.4–7.1)	1.0 (0.4–4.4)	1.1 (0.5–2.5)	1.0 (0.4–4.4)	1.1 (0.4–7.1)
<i>Treated patients (in %)</i>	16.4	na	100	na	100	100	100	100	100
<i>Cycloferon (%)</i>	2.7	na	16.3	na	47	58.5	40.0	55.4	38.5
<i>Ribavirin (%)</i>	6.2	na	37.5	na	47	40.0	54.7	60.0	51.3
<i>Pegylated interferon (%)</i>	8.4	na	51.3	na	28	17.8	37.9	24.6	30.8
<i>Monotherapy</i>	na	na	56 (70%)	na	145 (58%)	83 (70%)	41 (43%)	34 (52%)	22 (56%)

Table 2 (continued)

	Whole group n = 1765		HCV RNA(+) = 1278					
	HCV RNA(-) n = 487		Naive (never treated before and after) n = 1029	Treated n = 249	Treated before VL n = 118	Treated after 1 st VL n = 95	Treated before transaminases n = 65	Treated after transaminase n = 39
All n = 487	Untreated n = 407	Treated n = 80						
Dual therapy	na	21 (26%)	na	95 (38%)	34 (29%)	50 (53%)	28 (43%)	15 (38%)
Triple therapy	na	3 (4%)	na	6 (3%)	1 (1%)	2 (2%)	2 (3%)	1 (3%)
Quadruple therapy	na	0	na	3 (1%)	0	2 (2%)	1 (2%)	1 (3%)

Impact of Treatment as Seen on VL and Transaminases

Among the 249 HCV RNA(+) treated patients (detailed in Table 2), 108 have AST and 104 have ALT measurements; 65 patients were treated before aminotransferases determination and 39 were treated subsequently. AST and ALT medians were significantly lower (respectively $P = 0.005$ and $P = 0.026$) when the treatment was received before transaminases evaluation. Among the 39 patients treated after the first transaminase sampling, 17 had a second determination after treatment. Only the AST median was significantly lower after treatment ($P = 0.014$). However, time intervals between samplings were extremely variable and this group rather small. A group of 197 infected treated patients had 2 serial VL determinations (data not shown). We observed in them a significant decrease of the mean and median VL ($P < 0.0001$), but there was no difference between therapeutic schemes regarding the magnitude of VL decrease.

58% of this group received monotherapy with acridone-acetic acid (Cycloferon) predominantly employed. Therefore we compared Cycloferon impact on VL in patients who received it in mono or dual therapies (Table 4). We noticed that the viral response to Cycloferon was stronger when monotherapy was used ($P = 0.0001$).

We also analysed the impact of dual therapy on true positive patients with 2 samplings. A subset of 88 patients received dual therapy: 31 ribavirin + Cycloferon (group 1), 30 ribavirin + Pegasys (group 2) and 27 “others” (group 3). We found that VL were more strongly impacted in group 2, while groups 1 and 3 were similar (same decrease of the VL) $P < 0.0001$.

The global impact of monotherapy versus dual therapy on VL was also assessed. We observed that there was no difference between these therapeutic schemes regarding viral response.

Evolution of Medications Administered to Patients

As shown in Fig. 2, 10 different medicines were used between January 2010 and November 2016. The drug classes included adenosine analogues (adefovir and tenofovir), guanosine analogue (ribavirin), cytidine analogue (lamivudine), immunostimulant (cycloferon), and Interferon-like (Interferon, Pegasys), NS5A replication complex inhibitors (ledipasvir, daclatasvir) and nucleoside NS5B polymerase inhibitors (sofosbuvir). Ribavirin, cycloferon and pegasys were the most prescribed drugs; they peaked in 2012, 2014 and 2015 respectively. The least used drug was sofosbuvir.

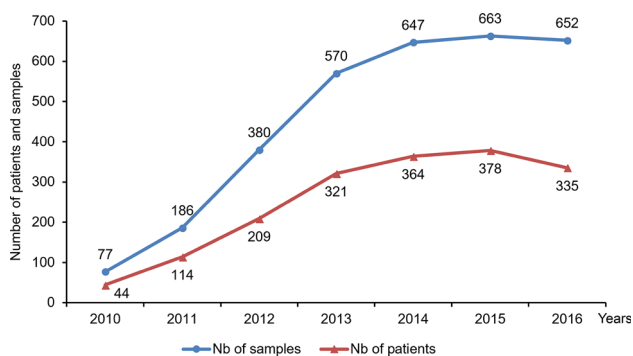


Fig. 1 Number of patients received (blue line) and samples processed at CILM (orange line) from January 2010 to November 2016.

Discussion

In recent years, worldwide, almost half of the patients with HCC have been diagnosed positive for HCV (Chassagne *et al.* 2016; El-Serag and Mason 1999). This has been confirmed in a study we conducted at the Calmette hospital in Cambodia on around 500 patients, where HCV was associated with half the cases of HCC, with higher prevalence in women (Chassagne *et al.* 2016). In Laos, comprehensive epidemiological data are lacking but those

obtained by Jutavijittum *et al.* (2007), aggregated to ours, are a source of concern. Between 2010 and 2016, nearly 2000 patients diagnosed in Lao public or private health care structures came to CILM for the determination of their VL. Of course, this figure does not reflect the prevalence of the disease, as there is no systematic screening for HCV in the Lao population. Patients usually come to be diagnosed at CILM following the advice of a physician. The cost of HCV viral load measurement is around 45 USD in Vientiane, which is a substantial amount of money for most people in the country (minimum salary is 111 USD per month). It is probable that such expense may deter many Lao people to visit diagnostic laboratories. Overall, the fact that HCV infection is commonly associated with low income status both in developed and developing countries implies that the problem in Lao PDR is much more serious than the situation we can describe with the evidence at our disposal (Mahajan *et al.* 2017; Alter *et al.* 1994).

Nevertheless, the number of patients attending CILM to control their HCV RNA VL increased constantly from 2010 to 2016. This increase could reflect increased economic status of the patients, the effectiveness of public health prevention campaigns for hepatitis, the high reputation of the CILM (meaning that less patients cross the border to

Table 3 Percentages of drugs used in mono, multi therapy and all together.

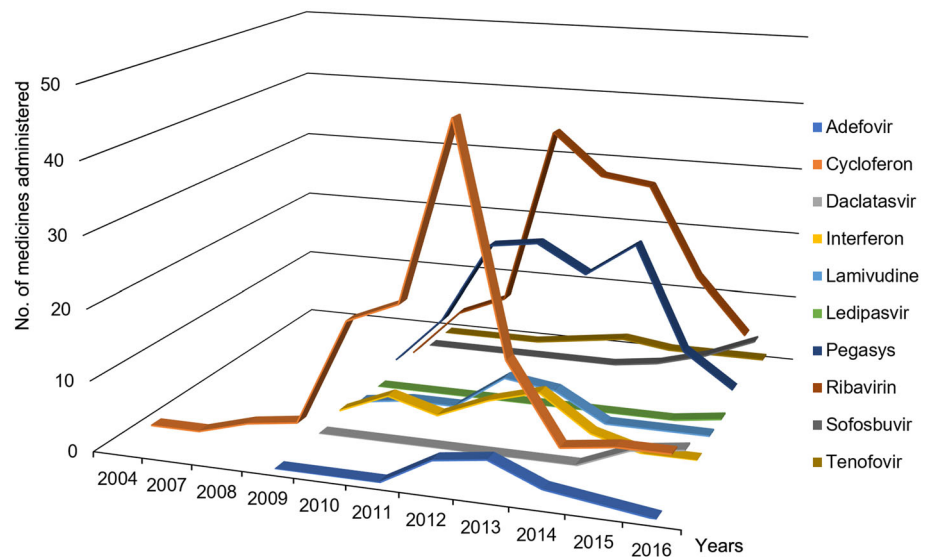
Drugs	All therapeutic schemes		Mono therapy		Dual therapy		Triple therapy		Quadruple	
	Nb used	%	Nb used	%	Nb used	%	Nb used	%	Nb used	%
Adefovir	13	2.8	11	3.3	2	1.6	–	–	–	–
Cycloferon	131	27.8	103	31.3	26	20.3	2	16.7	–	–
Daclatasvir	8	1.7	–	–	7	5.5	1	8.3	–	–
Interferon	23	4.9	16	4.9	5	3.9	–	–	2	66.7
Lamivudine	17	3.6	12	3.6	5	3.9	–	–	–	–
Ledipasvir	1	0.2	–	–	1	0.8	–	–	–	–
Pegasys	113	23.9	96	29.2	13	10.2	4	33.3	–	–
Ribavirin	149	31.6	77	23.4	66	51.6	5	41.7	1	33.3
Sofosbuvir	10	2.1	9	2.7	1	0.8	–	–	–	–
Tenofovir	7	1.5	5	1.5	2	1.6	–	–	–	–

Table 4 Comparison of the medians of VL at first sampling (1) and second sampling (2) in true positive treated patients with (+) or without (–) cycloferon in monotherapy versus dual therapy.

Drugs	Monotherapy (n = 101)		Dual therapy (n = 88)	
	Median 1 log ₁₀	Median 2 log ₁₀	Median 1 log ₁₀	Median 2 log ₁₀
Cycloferon+	6.57 (n = 41)	5.45 (n = 41)	6.69 (n = 39)	5.27 (n = 39)
Cycloferon–	6.11 (n = 60)	5.02 (n = 60)	6.52 (n = 49)	Negative (n = 49)

True positive treated patients, n = 197 true positive and receiving treatment before or after first VL; dual therapy, in total 459 patients were true positive with 2 samplings; cycloferon, the most used drug in the study.

Fig. 2 Evolution of medications administered to patients received at CILM between January 2010 and November 2016 (all therapies cumulated when the date of treatment is known).



be diagnosed in Thailand), or last but not least because the hepatitis epidemic is spreading in the country.

All patients presenting at CILM were diagnosed anti-HCV(+) using rapid point-of-care tests with questionable efficacy according to our experience (Paboriboune *et al.* 2018). This diagnosis was established during routine health check-ups or specialized gastroenterology consultations. Patients never come to CILM on their own initiative.

Among them 18.6% had undetectable virus. In the small subset of initially HCV RNA(−) patients tested twice, a substantial proportion (16.7%) turned out to be actually infected with HCV. Thus, a sizeable subset of anti-HCV(+)/RNA(−) was, in fact, positive. This observation questions the sensitivity of the initial analysis that was performed in the presence of undetectable VL according to our standards but could also reflect the fluctuation of the viral load. Moreover, despite the absence of circulating HCV genomes, a large section among the untreated anti-HCV(+)/RNA(−) subjects displayed increased levels of AST and ALT > 40 with a De Ritis ratio value above 1.1 considered as a predictor of liver fibrosis. This suggests that additional liver injury such as chronic high alcohol intake, occult B infection or infection by liver flukes commonly found in Laos were actually on-going in these patients (Sripa *et al.* 2012).

The significance of the VL is source of debate as oscillation in HCV RNA levels has been reported as a “stamp” of the viral dynamics in the early stages of HCV infection, and was described by some investigators as a “yo–yo” pattern (Aberle *et al.* 2006; Smith *et al.* 2010). We also observed this phenomenon in patients who had multi samplings. Noh *et al.* (2016) showed that the cumulative risk of HCC development was higher in patients with high titer of HCV RNA than patients with low

titer. These authors also stated that VL is “high” when > 6log₁₀. In our hands 27% of patients have VL > 6 log₁₀ and among them 105 patients have VL > 6 log₁₀ associated with liver injury evidenced by AST and ALT > 40 IU/mL (6% of the whole group of patients). A subset of 38 patients among the 105, received treatments (36% of this sub-group i.e. only 3% of the RNA(+) group). According to Botros and Sikari (2013) the De Ritis ratio reflects the time course of acute viral hepatitis and is generally a vital clue to the patient’s prognosis. We found that 218 patients who had one sampling had high fibrosis risk (AST/ALT = 1.09–2.0), but only 46 received treatments. We also observed that 39 patients had acute viral hepatitis flares (AST/ALT > 2.0) but only 10 received treatments (with means AST around 200 and ALT > 50 IU/mL). Only 74 on 615 patients had a second sampling and AST/ALT measured; 50% of them scored > 1.09, and less than 1/3 were treated.

Some authors have demonstrated that HCV genotype 1b was more often related to the development of HCC than non-1b genotypes (Ishiguro *et al.* 2011). Unfortunately, we did not have the opportunity to perform genotyping on the sample-set described herein. Nevertheless, it could be considered that genotype determination could soon become obsolete since drugs such as Sofosbuvir (and many other direct-acting antivirals) are effective on all known genotypes (Linaz *et al.* 2015). The cost of these therapies is, however, shamefully prohibitive for the vast majority of infected people in the world. Fortunately, affordable alternatives exist (although less effective than Sofosbuvir, used in only 2.1% of patients in this study).

Ten different medicines and four therapeutic schemes (mono, dual, triple, and quadruple therapy) were used. Curiously, we observed that anti-hepatitis B antivirals were

prescribed to a small subset of HCV patients ($n = 37$). This may be due to the fact that hepatitis B is much more frequent in Laos than HCV and that medical staff prescribe drugs in a probabilistic manner when they consider the treatment necessary.

Ribavirin, Cycloferon and Pegasys were the most prescribed drugs of all therapies. Nonetheless only 30 patients received the WHO-validated therapeutic scheme (Ribavirin + Pegasys) with 2 samplings to ascertain the efficacy of the therapy (WHO 2014). As expected, we showed in these cases that the combination was effective on the VL.

Ribavirin is part of the World Health Organization's list of essential medicines (WHO 2016). It has been extensively used in dual therapy (51.6%) but for unknown reason we found that its use was declining since 2012. Cycloferon is considered as an early inductor of types 1 and 2 Interferons. This particularly cheap medicine (10 US\$/month) is imported from Russia into Laos and was the most used drug in monotherapy scheme. It was used extensively when Russian clinicians were present in the public health system. Pegasys (Pegylated interferon alfa-2a) has been used in a stable and continuous manner since 2011, and was almost as used as Cycloferon in monotherapy schemes. The other drugs were used in less than 5% of all therapeutic schemes (direct acting antivirals, Sofosbuvir and Ledipasvir were used in only 11 patients).

A meta-analysis of controlled trials has shown that antiviral therapy reduced the rate of HCC development in patients with liver cirrhosis (Craxì and Cammà 2005). Moreover, it has been shown that in patients with chronic hepatitis C (with normal aminotransferases level) interferon therapy improves liver histology (Voronkova *et al.* 2002). It is then of utmost importance to make "curative" drugs affordable to infected patients and to encourage physicians to follow international guidelines as soon as possible in Laos.

Nevertheless, we showed that among true positive patients who were treated and sampled twice, those receiving monotherapy had a greater VL decrease than those treated with dual therapy. This is in contradiction with the therapeutic schemes recommended by the World Health Organization who advocate for a combination of Interferon and Ribavirin, effective against all the genotypes of HCV (WHO 2014).

Our results also suggest that the use of Cycloferon should be examined in combination. In other studies it was shown to increase other antiviral drugs' efficacy, providing earlier normalization of clinical and biochemical parameters; it reduces the incidence and degree of adverse reactions and increases the quality of life in treated patients (Romantsov *et al.* 2008, 2010; Sologub *et al.* 2010; Malyí *et al.* 2013). Last but not least it is economically

sustainable in a country like Laos. Therefore, clinical trials should be conducted in Laos to re-define the most effective and affordable drugs to be recommended by health authorities, including Cycloferon.

The biological follow-up of patients should be also updated. As a matter of fact, meta-analysis regression demonstrated that advanced stages of fibrosis were significantly correlated with platelet count, AST/ALT ratio and AFP levels but there was no correlation with viral load. Fanning *et al.* (1999) observed correlation between VL and the degree of inflammation while they did not detect any correlation between VL and the degree of fibrosis nor between serum viral load and ALT, although there was a correlation between ALT and the degree of inflammation. Su *et al.* (2009) showed that 42% of patients achieving sustained virological response following peg-interferon alpha and ribavirin therapy showed delayed biochemical normalization (persistently abnormal transaminase levels). Delayed biochemical response was associated with the baseline hepatic fibrosis stage and liver cirrhosis. All these data suggest that we should consider other alternatives to VL for the follow-up of HCV infected patients. According to Durante-Mangoni *et al.* (2013), hepatitis C virus core antigen (HCVcoreAg) levels are significantly correlated with HCV-RNA, ALT levels and liver necroinflammatory activity. Although these findings must be confirmed by additional studies, the determination of HCVcoreAg could be useful for the monitoring of chronic hepatitis C patients in the future.

In conclusion, it is urgent to challenge on-going diagnostic and therapeutic strategies in Lao PDR, with better follow-up of patients, in order to optimize the reduction in the burden of HCV-related diseases. Field studies should be conducted to determine the routes of infection in the Lao population, which population groups are the most vulnerable and why preventive campaigns have failed in some areas of the country.

Acknowledgements We would like to express our sincere thanks to medical staff and patients. Phimpha Paboriboune was supported by CILM, Institut de Recherche pour le Développement, Campus France and the Fondation Mérieux. We are grateful to Elizabeth Elliott for editing assistance.

Author Contributions P. Paboriboune, TV, PS, ED made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. SB, CT, PD, FXB, NS, P. Pineau were involved in drafting the manuscript or revising it critically for important intellectual content.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement The study was approved by the Ethic Committee of the National Authority. All participants provided written informed consent. Written consents were obtained from all children's parents involved in the study.

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