

## Full paper

### Hepatitis B Virus prevalence and serological profiles in a hospital in Southern Italy

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**Running title:** Southern Italy hepatitis B Virus serological profiles.

#### SUMMARY

Viral hepatitis still represents a significant worldwide public health issue, being an important cause of morbidity and mortality. The aim of our study is to evaluate the prevalence of Hepatitis B virus (HBV) markers from serologic analysis of hospitalized patients at University Hospital of Campania “Luigi Vanvitelli” and also to investigate the prevalence of HBV/HCV coinfection. We screened serum Anti-Hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), and antibody to Hepatitis C Virus (Anti-HCV) from January to December 2020.

Analyses of HBV serological profile based on age showed that the 51-60 age group was the most numerous and with the highest cases of HBsAg. The 61-70 age group recorded the highest prevalence of anti-HBc while age groups 0–10 years and 31–40 years the highest cases of anti-HBs. Antibody levels decline with time. In subjects older than 20 years, compared to vaccinated cohort individuals, anti-HBc seropositive prevalence increased linearly. This study underlined, in our geographic region, the decreased incidence of hepatitis B and high immunogenicity in the young population. Therefore, administration of HBV vaccine booster dose should be considered for the population rather than vaccination in the first year of life. In conclusion, our findings reaffirm the importance of health surveillance in hospitalized subjects, stressing the need to improve immunized subjects to increase the general population's health.

**Key words:** Viral hepatitis, hepatitis B virus, HBsAg, anti-HBs, anti-HBc, vaccination.

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## INTRODUCTION

Viral hepatitis still represents a significant worldwide public health issue, being an important cause of morbidity and mortality. Viral hepatitis is caused by five different types of viruses, including hepatitis virus A, B, C, D and E. Hepatitis B virus (HBV) and Hepatitis C virus (HCV), are among the leading causes of chronic liver diseases such as cirrhosis, liver failure and hepatocellular carcinoma [Lavanchy, 2005]. The World Health Organization (WHO) estimates that 296 million people live with chronic HBV infection and 58 million people have chronic HCV infection. In 2019, hepatitis B caused an estimated 820,000 deaths and approximately 290,000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer) [WHO, 2021]. In Italy, time-series data on the incidence of viral hepatitis B and C cases have been reported since 1985. The Integrated Epidemiological System of Acute Viral hepatitis-SEIEVA, coordinated by the Istituto Superiore di Sanità (ISS), describes the epidemiology of acute and chronic hepatitis. Data are presented annually and enable analysis of trends over time to underline the main incidence of HBV infection in the Italian population [<https://www.epicentro.iss.it/epatite/dati-seieva>]. HBV with an incomplete double-stranded DNA genome of 3.2 kb belongs to the *Hepadnaviridae* family [Van Damme et al., 2013]. Hepatocytes are the primary site for HBV replication [Gerlich, 2013]. The viral genome encodes core and surface proteins [Liang, 2009]. Hepatitis B can be diagnosed by serological dosage of specific viral markers like proteins produced by the virus or antibodies produced by the host [Dienstag, 2008]. Hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc) represent the specific HBV virus markers detected in the serum during the different phases of HBV infection [Dienstag, 2008]. Specifically, HBsAg indicates an ongoing infection and is used to evaluate the prevalence of acute or chronic HBV infection. Anti-HBc show previous or present infection, while anti-HBs reveal immunity after either infection or vaccination [Ifeorah et al., 2017]. The serologic prevalence of the HBsAg, in the defined geographic area of the general population, reflects active HBV infection endemicity. In industrialized West European countries and in North America, HBsAg positivity prevalence is less than 2% (low endemicity); in most countries in the Mediterranean, East Europe, and Asia it ranges from 2 to 8% (intermediate endemicity), whereas over 8% of individuals in some developing countries in Far-East Asia and Sub-Saharan Africa (high endemicity) are exposed to the virus at birth or in the first decade of life [Romano' L et al., 2011]. In the first group of low endemicity HBV countries, transmission occurs mainly in adulthood due to unprotected sexual contact or the sharing of syringes [Sagnelli et al., 2014]. In Italy, a low endemic country, HBV infection is under 2% and incidence has progressively decreased due to demographic socio-economic changes and to preventive measures adopted. Since 1991, HBV vaccination has been recommended especially for

high-risk groups – e.g., family members of HBsAg carriers, healthcare professionals, etc., and became mandatory for all new-borns and for twelve-year-old adolescents [Mele et al., 2008]. This measure has led to progressive reduction in hepatitis B incidence (from 5.1/100,000 cases in 1991 to 0.85/100,000 in 2012) [Sagnelli et al., 2017]. Despite this national data, Southern Italy records the highest rates of liver cancer in Europe, and about 90% of hepatic tumours are a consequence of HBV and/or HCV infection [Mavilia, Wu, 2018]. Moreover, because of their similar transmission modes, coinfection HBV with HCV is frequent, particularly in areas where the viruses are endemic. This type of HBV/HCV coinfection may further increase the severity of liver disease [Mavilia, Wu, 2018]. The aim of our study is to evaluate the prevalence of HBV markers from serologic analysis of hospitalised patients at University of Campania “Luigi Vanvitelli” but also to investigate the prevalence of HBV/HCV coinfection. Knowledge of local situations could help in the estimation of current susceptibility rates and immune/carrier subjects thirty years after universal HBV vaccination. The determination of serological markers in combination with clinical assessment enables the evaluation of natural history and individual risk of progressive liver disease.

## **MATERIALS AND METHODS**

With data collected from January to December 2020, we developed a retrospective study on hepatitis virus infection in hospitalized patients at University Hospital of Campania “Luigi Vanvitelli.” We screened serum hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), and antibody to Hepatitis C Virus (Anti-HCV) with enzyme immunoassays (The Ortho Clinical Diagnostics VITROS® 3600 Immunodiagnostic System, Rochester, NY, USA). Before being further analysed, the samples were centrifuged to remove cells, cell deposits and fibrin. HBsAg result  $\geq 1.00$  indicated a reactive sample and the possible presence of HBsAg. A result  $<0.90$  indicated a non-reactive sample, negative for HBsAg. A result  $\geq 0.90$  and  $<1.00$  indicated a borderline sample. A sample found to be borderline or reactive in the HBsAg test will be subjected to further analysis in duplicate to verify its actual status. For anti-HBs, samples with results  $<10$  mIU /mL were labelled "antibody negative," samples with results between 10 and 12 mIU/mL were labelled "borderline," and samples with results  $>12$  mIU/mL were labelled "positive to the antibody." A "borderline" result was indicative of an antibody level within 20% of the WHO-recommended cut-off for immunity of 10 mIU/mL. An anti-HBc result of  $<1.00$  indicated a positive sample. A result of  $\geq 1.00$  and  $<1.20$  indicated a borderline sample. A result of  $\geq 1.20$  and  $<4.80$  indicated a non-reactive sample, negative for Anti-HBc. A result  $\geq 4.80$  indicated a sample that needs to be diluted and retested. An anti-HCV result of  $\geq 1.00$  indicated a reactive sample and the possible presence of anti-HCV. A result of  $<0.90$  indicated a negative sample and a result of  $\geq 0.90$  and  $<1.00$  indicated a borderline sample. For the purposes of this analysis, positive results for anti-

HBc indicated past or ongoing HBV infection, while positive results for anti-HBs indicated only immunity vaccination or past infection. Negative patients for analysed HBV serological markers were considered non-immune or non-infected.

#### Statistical Analysis

The data were collated and analysed using IBM SPSS 22.0 for IOS (SPSS Chicago, IL, USA). Category variables were presented as frequencies and percentages. Qualitative data was compared using the Chi-square test or Fisher exact probability method.  $P < 0.05$  indicated that the difference was statistically significant.

### RESULTS

Of the total of 1816 patients, 50.9% (925) were male. In the study population 49.1% were among the 21-60 age group followed by the over-61 age group. Most of the patients were enrolled from General Medicine (37.5%) and General Surgery (23.8%) (**Figure 1**).

Serological data showed 1.5% HBsAg positive subjects, 27% anti-HBc and 39.4% anti-HBs positive subjects. Males had the highest percentage in all serological assays. As reported in the anti-HBc table, there were 33.9% positive males were compared to 19.7% positive females (**Table 1**).

Analysing HBV serological profile according to age, the data showed the 51-60 age group was the most numerous (339) with the highest HBsAg positive percentage (5.3%). Age group 61-70 reported 47.8% of anti-HBc prevalence, while the 0-10 and 31-40 age groups recorded the highest rates of anti-HBs cases: 60.6% and 60.2% respectively (**Table 2**).

According to the serological profile, subjects were divided into five subgroups to evaluate infection stages, vaccinated subjects and NO markers population. 7.8% of patients had anti-HBc alone; 17.7% had both anti-HBc and anti-HBs positive. 1.5% of the other subgroups of had anti-HBc and HBsAg positive. 394 patients (21.7%) were anti-HBs positive, while 51.3% of the study population showed NO markers and so were susceptible to infection (**Table 3**).

Figure 2 shows HBV seromarker distribution according to age group, and reports the 41-50 age groups more susceptible to infection, with 68.9% NO markers, followed by the 11-20 and 21-30 age groups with 65.4% and 60.4% NO markers rates. Data showed that the 0-10 and 31-40 age groups had the highest percentage of immunized subjects with anti-HBs alone of 56.7% and 54.7%, respectively. On the other hand, the 51-60 age group had ongoing HBV infection with 5.4% of HBsAg+ and Anti-HBc+. The majority of patients with resolved HBV infection were in the 61-70 and 71-80 age groups, with 33% and 28.3% anti-HBc+ and anti-HBs+ rates (**Figure 2**).

The HBV/HCV co-infection rate was found to be more common in patients over 50 years of age but increased linearly with age. Co-infection rates were observed not only in the 51-60 age group

(45.4%) but also 61-70 (42.1%) and up to the 81-90 age groups (54.5%). No statistical differences were observed between male and females in HBV/HCV co-infection (**Table 4**).

## **DISCUSSION**

The last decade has seen a substantial change in the epidemiology of viral hepatopathies in Italy, determined by a reduction in the incidence of new cases due to spontaneous healing, reduction of mortality and efficacy of therapy. Virological control of blood and blood derivatives, the use of disposable tools and accessories, as well as the reduction of intravenous drug abuse have changed the scenario of viral hepatitis infection, with reduced cases of HBV and HCV [Franceschi et al., 2006]. Another reason for the change in HBV epidemiology in Italy was the improvement of socio-economics conditions, especially in the domestic environment, as well as educational campaigns and, in parallel, the introduction of HBV vaccination. Data confirm the national trend, with decreasing incidence of new cases of HBV infection. Our study was carried out from January to December 2020 among hospitalized patients at University Hospital of Campania “Luigi Vanvitelli.” There was no difference between males and females in the patient cohort; the 21-60 age group was the most prevalent and the majority of patients were enrolled from General Medicine and General Surgery. The data from Surgery are related to blood screening of serological markers as prevention systems for professionals and patients [Candotti, Laperche, 2018]. The HBV study population serological profile according to gender revealed that the risk of infection is higher in males than in females, as noted by Khan *at al.* The HBsAg serological profile showed a seroprevalence rate of 1.5%. Data was relevant compared to previously reported data in Italy, a low endemicity country, with HBsAg carrier prevalence below 2% [Stroffolini et al., 2022]. Analysis by age found the HBsAg seroprevalence rate higher in the 51-60 age group than in the other groups. The lowest HBsAg positivity rate was observed in the under-30 age groups. Similar findings were observed in Guclu *at al.* (2016) where, assessed by age, HBsAg positivity was found to be lowest in the 0-12 age group and highest in the 51-60 age group. However, in our region, high percentages of 11-50-year-old patients were HBV seronegative. Accordingly, a percentage of individuals were still susceptible to HBV infection. A singular anti-HBs positivity reflected HBV vaccination and was detected in the 0–10 and 31–40 age groups, as it had serology compatible with vaccination [Boccalini et al., 2013]. Xaydalasouk *et al.* (2021) reported about 63% of subjects aged < 30 years were anti-HBs positive compared to about 25% in older subjects, with no differences between genders. Our vaccine coverage data in the 31-40 age group was in agreement with the fact that in 1991, Italy introduced a program of universal vaccination against hepatitis B for all new-borns and for twelve-year-old adolescents [Morisco et al., 2017]. In almost all subjects in the 11-20 and 21-30 age group that were included in the 1991 Italian vaccination program, the seroepidemiological data show a low rate of

protective antibody against HBV. From data in the literature, it is evident that antibody levels have declined over time. The Verso et al. (2019) study reported that subjects vaccinated at birth or during adolescence maintain, in most cases, a protective antibody titre in the following 10 years, while the percentage drops 20 years after the primary vaccination schedule, especially if immunization was performed during the neonatal age [Verso et al., 2019]. Several studies have shown that subjects vaccinated in the first year of life were less protected than subjects vaccinated at age 12 [Coppola et al., 2015; Bini et al., 2018; Coppeta et al., 2019; Sartorelli et al., 2022]. Data obtained in the present study are consistent with these findings; in fact, the 21-30 age group corresponded to subjects that were immunized in the first year of life. In subjects older than 20, compared to individuals in vaccinated cohorts, the prevalence of seropositive individuals for anti-HBc increased linearly from 0% in the 0-10 age group to 14.3 % in the 81-90 age group. It is likely that those subjects were infected in the past. In line with the acquired immunity due to vaccination, the younger generation show the absence of or low anti-HBc rates, whereas anti-HBc seroprevalence rates were detected in older generations, as stated in several reports [Sagnelli et al., 2008] [Mavilia Wu, 2018]. The last part of our analysis reported HCV co-infection in patients with resolved/occult and with ongoing HBV infection. Only 8.1 % have anti-HCV antibodies. The HBV/HCV co-infection rate was found to be more common in patients over 50 but increased with age [Daw, El-Bouzedi, 2014]. As reported in Libya, the percentage of the population with HBV and HCV increases according to age. The prevalence of anti-HCV was stable at 0.8–0.9% until the age of 30, after which it rose steadily, reaching 2.7% in those older than 50 [Daw, El-Bouzedi, 2014]. Our data confirm that anti-HCV prevalence was stable in younger ages, increasing steadily after the age of 30 and reaching a peak in the 51-60 age group. A similar picture was also found in Tunisia and Morocco, where the rates ranged from 1.7 to 2.9%, with higher rates in some regions [Benouda et al., 2009]. Subjects with HBV/HCV infection were more likely to be older than 51. Our findings show that the prevalence rates of HBV and HCV in our region are relatively low, and this could be a result of the implemented national control policies. Continuous surveillance and maintenance of the national program against hepatitis are essential, and vaccination is the most effective way to control and prevent HBV infection, as demonstrated by the absence of HBsAg and anti-HBc in younger age groups. Our study revealed that a significant proportion of the study population is at risk of infection with HBV because of the absence of detectable markers of infection or immunity, but also reported decreased incidence of hepatitis B and high immunogenicity of the young population in this geographic region.

Future studies could be conducted to analyse the evolution of serological markers in the pre-pandemic and pandemic period. The current HBV seroprevalence situation clearly demonstrates the need for continuously improved vaccination because of lower immunogenicity in adolescence or adulthood. Immunological memory persists for years after HBV vaccination but there remains the possibility

that individuals vaccinated in childhood presenting a low anti-HBs titre and could be infected. Estimation of antibody titre 5 and 10 years post-vaccination determines the need for a booster dose in subjects over 20. In conclusion, our findings reaffirm the importance of health surveillance in hospitalized subjects and emphasise the need to improve vaccination rates to limit the number of non-immunized subjects, in order to increase the general population's health.

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**Table 1:** HBV serological profile of study population according to gender

HBV Markers		HBsAg+	Anti-HBc+	Anti-HBs+
Total	1816	28 (1,5)	490 (27,0)	715 (39,4)
Males	925	28 (2,9)	314 (33,9)	384 (41,5)
Females	891	0	176 (19,7))	331 (37,1)

**Table 2:** HBV serological profile of study population according to age.

Age group (Years)	Not tested	HBsAg+ n(%)	Anti-HBc+ n (%)	Anti-HBs+ n (%)
0-10	127	0	5 (3,9)	77 (60,6)
11-20	133	0	1 (0,7)	45 (33,8)
21-30	149	2 (1,3)	6 (4,0)	53 (35,6)
31-40	181	0	14 (7,7)	109 (60,2)
41-50	235	2 (0,85)	42 (17,9)	62 (26,4)
51-60	339	18 (5,3)	130 (38,3)	113 (33,3)
61-70	324	4 (1,2)	155 (47,8)	135 (41,7)
71-80	237	2 (0,8)	100 (42,2)	90 (38,0)
81-90	91	0	37 (40,6)	31 (34,1)
Total	1816	28 (1,5)	490 (27)	715 (39,4)

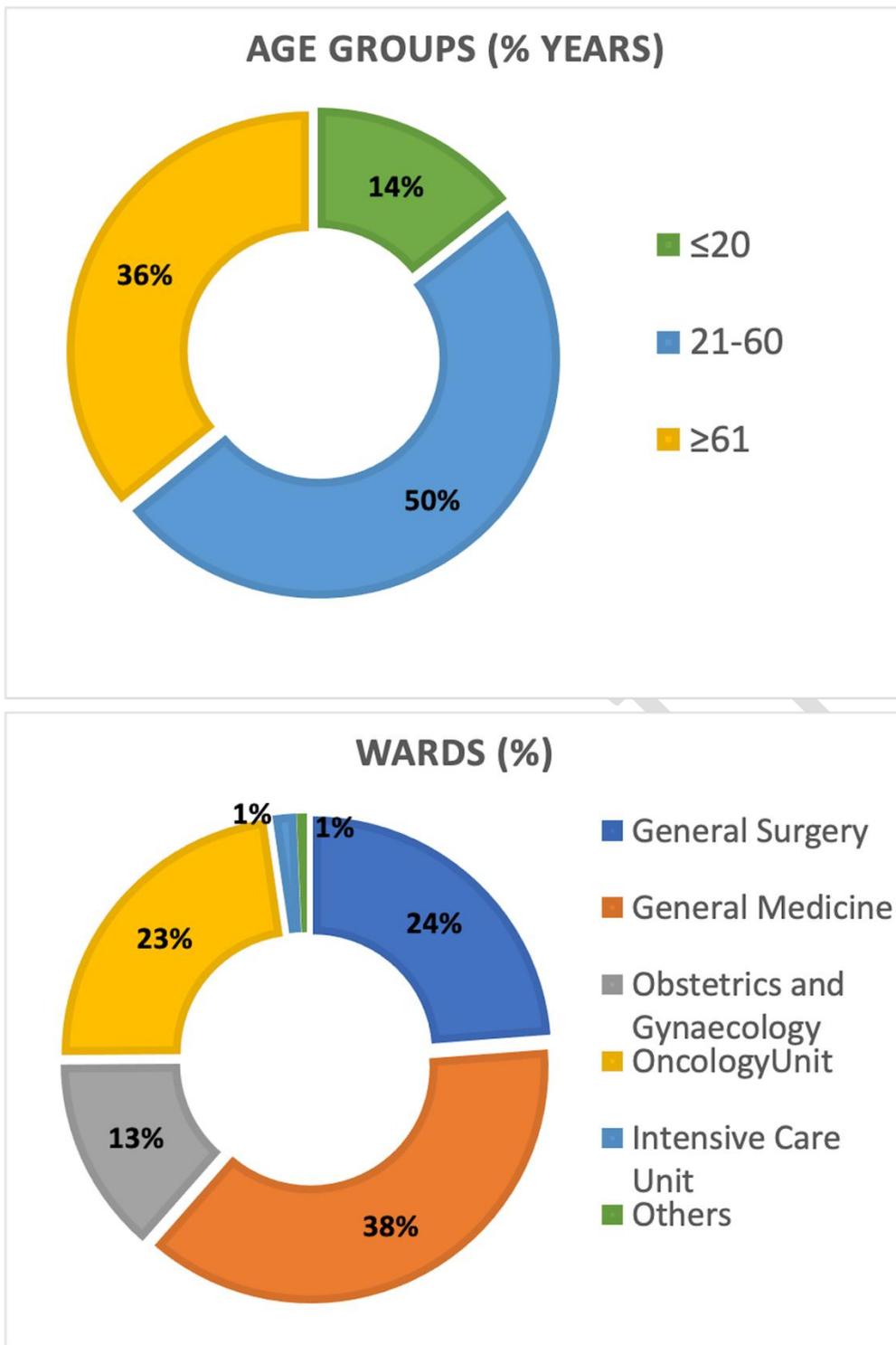
**Table 3:** Serological profile HBV infection in study population

HBV Positive Markers	No. (%)	Interpretations
Anti-HBc alone (Anti-HBs-; HBsAg-)	142 (7,8)	Resolved or occult HBV infection
Anti-HBc+ and Anti-HBs+	321 (17,7)	Resolved HBV infection; immunity
HBsAg+; Anti-HBc+	28 (1,5)	Ongoing HBV infection
Anti-HBs+ alone	394 (21,7)	Immunity due to the vaccination or resolved HBV infection
NO Marker	931 (51,3)	Susceptible to HBV infection

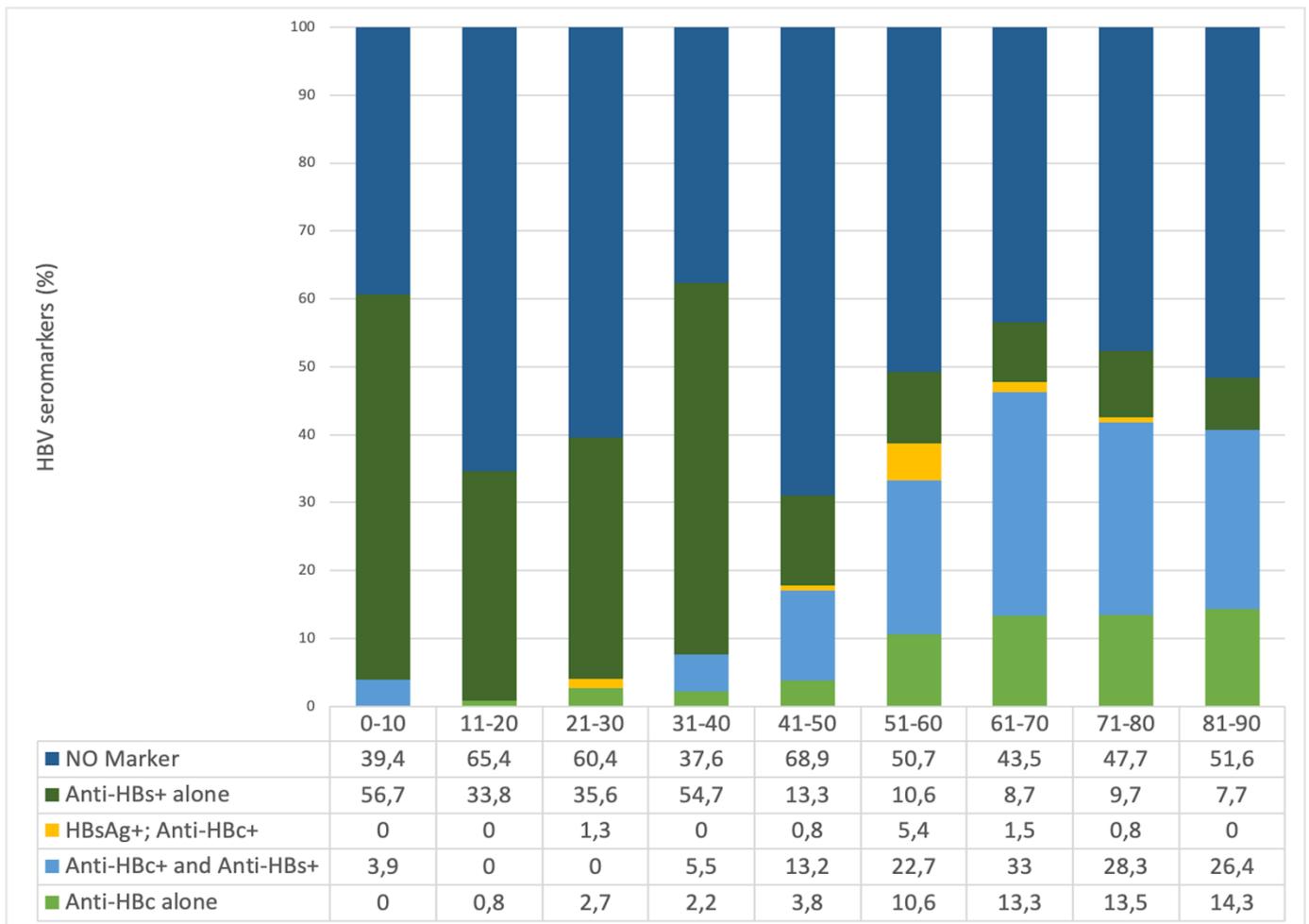
**Table 4:** HCV Serological profile and coinfection according to age

<b>Age group (years)</b>	<b>HCV <i>n</i> (%)</b>	<b>Anti-HBc+ and HCV <i>n</i> (%)</b>	<b>HBsAg+ and HCV <i>n</i> (%)</b>
<b>0-10</b>	0	0	0
<b>11-20</b>	0	0	0
<b>21-30</b>	9 (8,2)	1 (11,1)	0
<b>31-40</b>	13 (11,9)	1 (7,7)	0
<b>41-50</b>	19 (17,4)	2 (10,5)	0
<b>51-60</b>	22 (20,1)	10 (45,4)	5 (22,7)
<b>61-70</b>	19 (17,4)	8 (42,1)	1 (5,3)
<b>71-80</b>	16 (14,7)	6 (37,5)	0
<b>81-90</b>	11 (10)	6 (54,5)	0
<b>Total</b>	109 (6)	34 (31,2)	6 (5,5)

**Figure 1:** Age group and wards distribution of study population.



**Figure 2:** Distribution of HBV seromarkers according to age group



Ahead