

Research Article

## **Prevalence of Antigens/Antibodies Against Hepatitis B and C Viruses in A Cohort of Italian Patients with Pancreatic Adenocarcinoma Admitted to Two Hospital Wards in Italy: A Pivotal Retrospective Study**

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**Abstract**

**Background/Objectives:** Pancreatic adenocarcinoma (PAC) is a disease with a poor prognosis. Hepatitis B (HBV)/Hepatitis C (HCV) viruses are hepatotropic pathogens with pro-carcinogenic properties able to attack also the pancreas. Although several trials, mainly carried out in the USA and in the Eastern Countries, strongly suggested that HBV/HCV exert a role in PAC development, no study on this topic was still performed in Italy. Through this present work, we aimed to assess HBV antigens/antibodies and anti-HCV antibodies prevalence in a small cohort of Italian patients with PAC, irrespective of the other risk factors for PAC development, like smoking, alcohol drinking, and diabetes.

**Methods:** This pivotal-retrospective-study was led both at Surgery Unit of Maggiore Hospital, (Bologna) and at Unit of Gastroenterology and Digestive Endoscopy of Sandro Pertini Hospital, (Rome). Data concerning age, sex, pancreatic cancer localization (head, body, tail) and serum HBV/HCV profiles of subjects with a histological/radiological/biochemical diagnosis of PAC were collected from files concerning pancreatectomy and endoscopic-retrograde-cholangiopancreatography (ERCPs).

**Results:** It was found that 4 patients were HBsAg positive and 28 were HBsAb/HBcAb-positive, with a prevalence equal to 1% and 7.5%, respectively. Sixteen patients were HCV positive, with a prevalence equal to 4.3%.

**Conclusions:** Our observational study describes, for the first time in our Country, HBsAg, HBsAb/HBcAb and HCV prevalence in a small-sized cohort of patients suffering from PAC. Despite no definitive conclusions on the association between HBV/HCV infection and PAC may be drawn, our research could represent the basis for additional epidemiological/histological nationwide trials in Italy.

**Keywords:** HBV; HCV; pancreatic adenocarcinoma; Hepatitis viruses; virus

## Introduction

Pancreatic adenocarcinoma (PAC) is one of the most aggressive human malignancy entailing a very dismal prognosis [1], with about 459,000 cases and 432,000 deaths worldwide in 2018, according to the Global cancer statistics estimates [2]. Despite the improvement in the understanding of molecular mechanisms and events involved in the development of this neoplasm, the overall five-year survival is < 5%. Therefore, this neoplasm ranks as the 14th most common malignancy and the 7th highest cause of tumor-related mortality in the world. The poor prognosis of PAC depends on several factors, such as its aggressive biological behavior, its early ability to spread locally as well as to metastasize at distance and its asymptomatic course in the initial stages of carcinogenesis [3]. Despite several efforts in the past and even in the last times, the diagnosis of this cancer is very often performed at an advanced stage, when the available therapeutic options, both surgical and pharmacological ones, are ineffective or useless. At the time of diagnosis, most PACs have already disseminated beyond the pancreas [4, 5] and recurrence rates of this tumor are very high (about 85%), even if a curative resection is performed [6]. Only a few risk factors are known to be associated with its development, including smoking, chronic pancreatitis, familial cancer syndromes, diabetes and alcohol [7]. Since a substantial enhancement in PAC incidence was progressively observed over recent years and it is still expected to increase in the next decades, at least in the most developed Countries for several reasons [8], the identification of modifiable or treatable risk factors of this severe and lethal disease has become

a pressing need [9]. An approach intended to the development of screening programs, targeted at high-risk people for prevention or early detection of PAC is strongly required, like this strategy, if adopted, could contribute to increase survival rates and improve the quality of life of people suffering from this pathology. In our clinical and scientific study, we mean to evaluate the possible factors associated with pancreatic carcinogenesis. As multiple pieces of evidence from epidemiological and basic research studies suggest that about 15-20% of all malignancies are somehow linked to a viral infection [10], in the last years we have turned our attention to investigate the potential role of Hepatitis B (HBV) and C (HCV) viruses in PAC development. These hepatotropic pathogens own well-known carcinogenic properties for the liver, but their antigens and genomes may be detected also in extra-hepatic tissues, including the pancreas. HBV antigens and genome, as well as intermediate HCV genome replication forms, have been found in exocrine and endocrine pancreatic cells [11-13]. Taking advantage of these evidence, in the last years, some studies have been carried out with the aim to investigate the possible mechanisms involved in the processes of pancreatic carcinogenesis. Several reports have shown the possible intra- and extra-cellular targets of some viral proteins, including Hepatitis x protein (HBx) for HBV, non-structural 3 (NS3), 4 A (NS4A), 5A (NS5A) and 5B (NS5B) for HCV. Most of these binding sites have been shown in animal and human cell culture systems derived mainly from hepatocytes or hepatoma cell lines, but also from pancreatic cancer cells [14]. HBx, NS3, NS5A and NS5B have been demonstrated to dysregulate the expression profile of some cellular cytoskeletal genes [15] or to interact with distinct elements of cytoskeleton (microfilaments, microtubules, intermediate filaments and actin stress fibers) [16-18] of different elements of

intracellular signalling pathways, like Rat-sarcoma/proto-oncogene serine/threonine-protein kinase/mitogen-activated protein kinases (Ras/Raf/MAPK) [19], proto-oncogene tyrosine-protein kinase Src (Src-) [20] and Focal adhesion tyrosine-kinase (FAK)-pathways [21-24] and of cell to cell and cell to extracellular-matrix adhesion molecules [25], such as Integrins [26, 27], E-Cadherin,  $\beta$ -catenin [28] and CD 44 [29, 30]. In addition, it has reported that HBx may directly modulate the activities of a lot of

nuclear transcription factors, influencing their function. These events lead to a perturbation of cell and stroma composition as well as of their structure, shape, disposition and of their physical- and chemical-properties [31-34]. This complex series of modification induces deep deregulation of normal cell and extracellular matrix function and promotes the malignant transformation of the cells and of the matrix surrounding them. Some of these events are described in Table 1 and Figure 1, 2 and 3.

	<b>Targets</b>	<b>HBV (HBx)</b>	<b>HCV (NS3, NS4A, NS5A, NS5B)</b>	<b>Effects</b>
<b>Intracellular Microenvironment</b>	<b>Adhesion Molecules</b>	<ul style="list-style-type: none"> <li>-Rearrangement of cellular adhesion molecule CD44;</li> <li>-Decreased E-cadherin synthesis and interaction of <math>\beta</math>-catenin via HBx;</li> <li>-Reduced E-cadherin link to cytoskeleton by means of HBx;</li> <li>-Decreased production of <math>\beta</math>1 integrins and dysregulation in the processes controlling cell adhesion/de-adhesion status</li> </ul>	<ul style="list-style-type: none"> <li>-Direct binding of <math>\alpha</math>-actinin and NS5B;</li> <li>-Altered localization of tight junction-bound proteins, such as Occludin, Claudin and Zonula Occludens protein-1 throughout HCV-Envelope 2 glycoprotein</li> </ul>	Alteration in: <ul style="list-style-type: none"> <li>-cellular cytoskeletal structure</li> <li>-cellular adhesion molecules</li> <li>-cellular form</li> </ul>

	<b>Cytoskeleton Components</b>	No studies available	Direct interaction of HCV replication complexes with some components of cytoskeleton (microtubules and actin filaments) modulated by NS3 and NS5A	Changes in architecture and dynamics of cytoskeleton
	<b>Signalling Pathways:</b>			
	<b>a) Ras-Raf-MAP-Kinase pathway</b>	Increased KRAS activity, mediated by HBx	Up-regulation of K-Ras activity, stimulated by HCV core and NS5A	-Transduction of stimuli originated from ECM to cellular signaling cascades;
	<b>b) Src-pathway</b>	Increase of K-Ras activity, mediated by HBx	Increase of K-Ras activity, mediated by NS5A	-Interaction with some crucial intracellular signaling paths, including Src- and Notch-cascades
	<b>c) FAK-pathway</b>	Increase of K-Ras activity, mediated by HBx	Increase of K-Ras activity, mediated by NS3A and NS5A	-Direct or indirect induction of Ras-Raf-MAP-Kinase cascade -Adherens junctions disruption of throughout Src dependent

				<p>phosphorylation</p> <ul style="list-style-type: none"> <li>-Enhanced activity of cellular adhesion molecules;</li> <li>-Rearrangement in cellular cytoskeleton;</li> <li>-Modification in cell contractility and motility;</li> <li>-Modification of cellular form and DNA transcription</li> </ul>
	<b>Genome</b>	<p>Altered expression of 7 genes, including:</p> <ul style="list-style-type: none"> <li>-microtubule tubulin-<math>\beta</math>2,</li> <li>-microtubule tubulin-<math>\beta</math>3,</li> <li>-microtubule tubulin-<math>\beta</math>6,</li> <li>-periplakin,</li> <li>-keratin-8,</li> <li>-keratin-18,</li> <li>-actin-<math>\gamma</math></li> </ul>	No available studies	<p>Perturbation of cytoskeletal structure, disposition and motility</p>
<b>Extracellular Microenvironment</b>	<b>Stellate Cells</b>	<p>Paracrine HBx-mediated stimulation and proliferation</p> <p>Perturbation in activity of genes, encoding some extracellular matrix proteins, like</p>	<p>Paracrine NS3- and NS5A-mediated stimulation and proliferation</p> <p>Perturbation in activity of genes, encoding some extracellular matrix proteins, like collagen</p>	<p>Modification in:</p> <ul style="list-style-type: none"> <li>-Cellular cytoskeletal structure,</li> <li>-ECM components,</li> <li>-ECM stiffness</li> </ul>





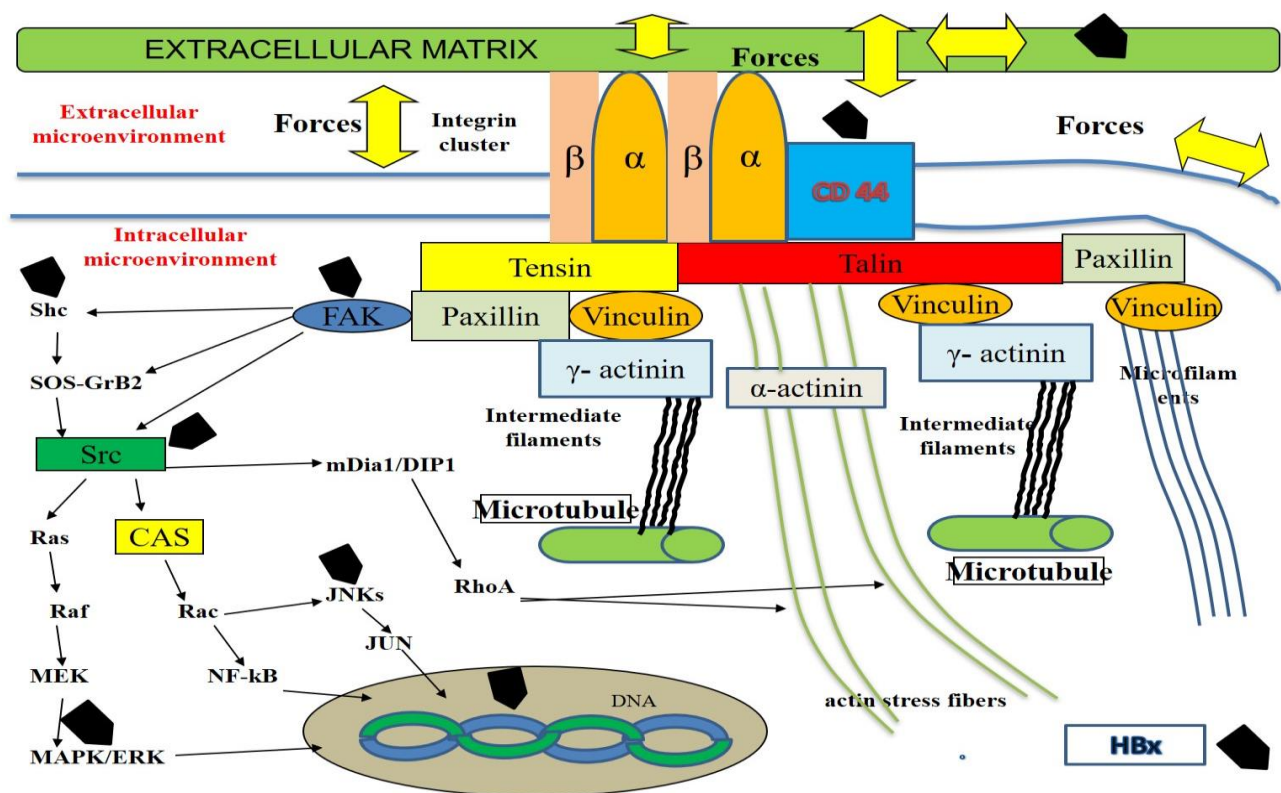
The scheme shows the cellular membrane, some intracellular micro-organelles (nucleus and microtubules), the focal adhesion molecules (integrin receptors and signaling proteins like talin, paxillin, vinculin, tensin,  $\alpha$ -actinin and  $\gamma$ -actinin), the elements constitutive of intracellular cytoskeleton (actin stress fibers, intermediate filaments and microfilaments) and several components of the intracellular signaling cascades. According to Ingber's hypothesis, in normal conditions, several tightly-regulated mechanisms cooperate to maintain homeostasis within the extracellular and intracellular compartments and contribute to preserve their function. Cells with their microorganelles and cytoskeleton as well as the stroma with its constitutive elements, such as structural- (Collagens, Proteoglycans and Glycosaminoglycans) and non-structural- (Regulatory proteins like Matricellular Proteins) components surrounding cells constitute a tensed network of elements. These components self-stabilize their structure, form, spatial disposition and activity throughout the generation of micro-tensional and micro-compressive forces. Cells sense the mechanical properties in the extracellular matrix via Integrins and offset these stimuli, by means of some responses including the transient rearrangement of cytoskeleton and the transitory modification of its tension. Integrins are involved in cell-matrix interactions, providing a bi-directional contact between extracellular- and intracellular microenvironments. A tightly regulated loop exists among the stroma components, the intracellular elements and the tensional/compressive forces generated in this microenvironment. In particular, according to the current knowledge, the direct or indirect action of the above-mentioned forces on the cell membranes and on the extracellular stroma causes the transient activation of focal adhesion structures in the cell membranes and

the association of integrin receptor monomers ( $\alpha$  and  $\beta$ ) with the consequent clustering of the integrin receptors and the recruitment of the signalling proteins. These focal adhesion structures bind to cell actin cytoskeletal filaments, microfilaments, intermediate filaments and microtubules. This event induces the rearrangement of intracellular cytoskeleton, modifying its disposition and structure and, as consequence, modulating the activities of several intracellular microorganelles including microtubule, mitochondria, endoplasmic reticulum and nucleus as well as the function of several enzymatic transduction pathways in the cytoplasm such as c-Jun N-terminal Kinase (JNK), mitogen-activated protein-Kinase (MAPK), mitogen-activated protein kinase kinase (MEK), extracellular signal-regulated kinase (ERK) and non-receptor tyrosine kinases family, known as Src Family Kinases (Src), Focal Adhesion Tyrosine-Kinase (FAK). The elements of these cascades are in direct or in indirect connection with focal adhesion molecules, via additional elements, including the components of the Rho Family of GTPases. Src may directly activate Ras, Raf, MEK, MERK, MAPK or indirectly, throughout the induction of Crk-associated substrate (CAS) and Rac. These events lead to the stimulation of JNK, JUN and several nuclear factors, including nuclear factor  $\kappa$ B (NF- $\kappa$ B). In addition, ERK/MAPK pathways activity may be induced by FAK, by means of the involvement of several proteins, such as son-of sevenless (SOS) and growth factor receptor-bound-2 (GRB-2). In normal conditions, this very complex system is able to transfer stimuli from extracellular- to intracellular-compartments and to cause changes in both chemical and physical properties of intracellular microenvironment. The stiffness of intracellular microorganelles is modified and this event is associated with changes in the activity of intracellular metabolic machinery, in the genome replication and



transcription as well as in the processes of protein translation. Pathological processes, such as inflammation, may induce significant changes in the quali/quantitative composition of extracellular matrix, in its structure and in its chemical and physical properties. One of the most important effects of this event is generally represented by an increase of the extracellular matrix rigidity (stiffness) and of the whole tissue. The consequence of these processes is represented by an

unbalanced stimulation of the enzymatic-pathways, by a dysregulated and persistent modification in the disposition and shape of nucleus, mitochondria, microtubules and endoplasmic reticulum with the alteration of cellular and stroma function and a chronic perturbation of homeostasis both at a microscopic- and macroscopic level. On the whole, this model may contribute to explain the process of pancreatic carcinogenesis.



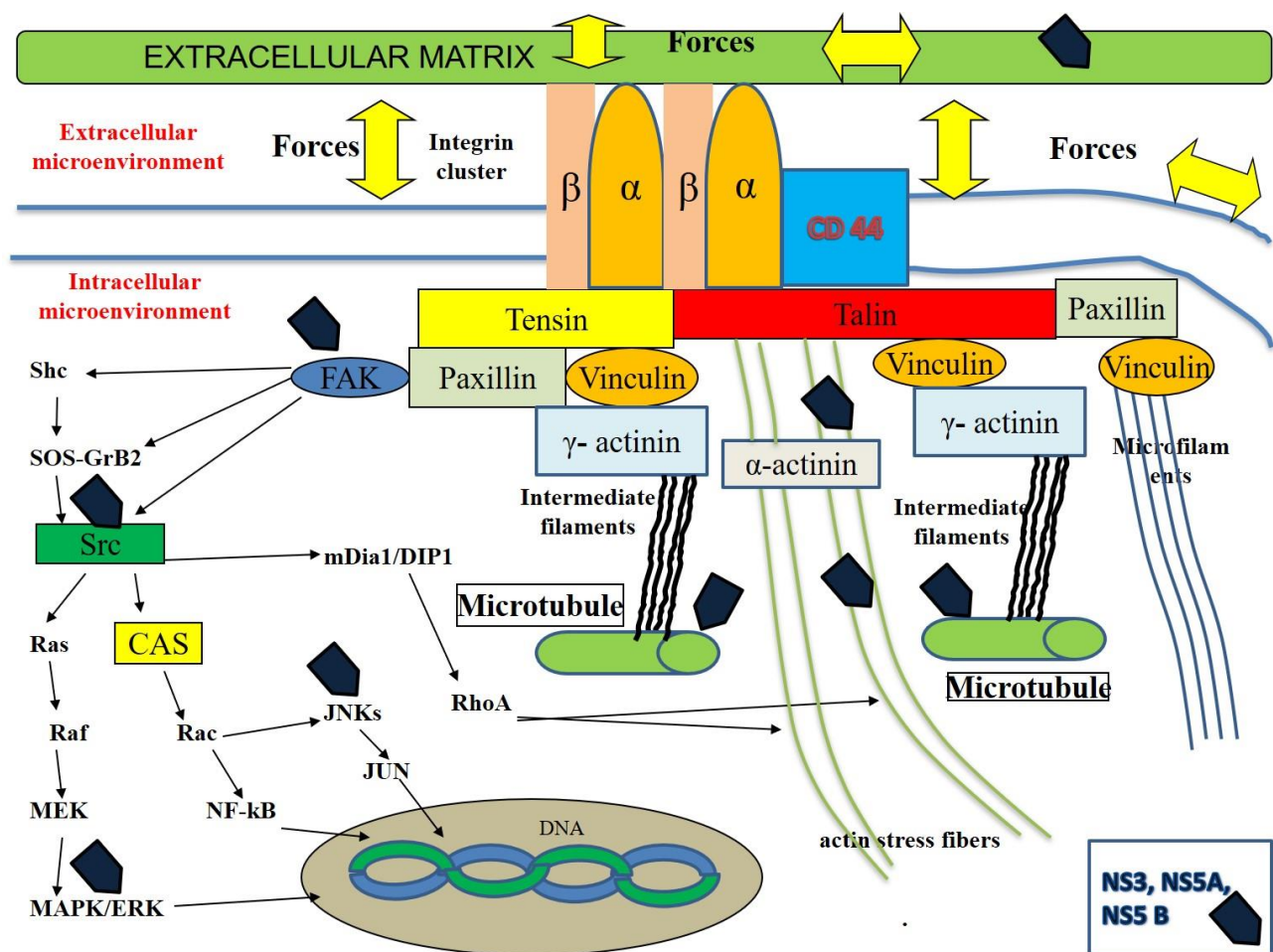
**Figure 2:** Intracellular and extracellular targets of HBx.

In cells HBx may act both in the nuclear and cytoplasmic compartments. In nucleus HBx may stimulate the function of 7 genes, which encode several proteins with a regulatory role in the organization of distinct cytoskeletal elements. These components include:  $\gamma$ -actin, periplakin, keratin-8, keratin-18, microtubule tubulin- $\beta$ 2, tubulin- $\beta$ 3 and tubulin- $\beta$ 6. In

cytoplasm, HBx is able to influence the activity of: a) some cellular transcription factors, regulating the function of Src family kinase- and Ras-Raf-MAP kinase-paths; b) some adaptor proteins, like GRB-2, SOS and Shc, promoting the association of these elements; c) FAK with the stimulation of some intracellular proteins, including paxillin and  $\alpha$ -actinin;

d) some cellular membrane adhesion molecules, including CD44. The results of all these events is the disruption of the system of focal adhesion structures, via the Src-mediated phosphorylation of  $\beta$ -catenin and its subsequent detachment from E-cadherin and the down-regulation in the synthesis of wild-type  $\beta$ -catenin and E-cadherin. These changes are associated with the modification of the cytoskeleton architecture and of its tensional activity, with the alteration of cellular

morphology, shape, motility, polarization and adhesion ability as well as of processes of energy production by mitochondria. Furthermore, the HBx-induced alteration of cellular nuclear factors causes the perturbation of the processes of cell DNA transcription and duplication. HBx is able to induce paracrine activation of and proliferation of stellate cells in extracellular matrix and promote the expression of genes encoding extra-cellular matrix proteins, like collagen type I.



**Figure 3:** Intracellular and extracellular targets of NS3A, NS5A and NS5B are reported in the picture.

In cells HCV may act both in the intracellular- (cytoplasm) and in the extracellular microenvironment. HCV-RNA synthesis requires the polymerization of microtubules and actin filaments. In cytoplasm HCV

replication complexes directly interact with these components of cytoskeleton. NS3 and NS5A regulate this process. Furthermore, these proteins stimulate cytoplasmic FAK, Src, MAPK/ERK and JNKs pathway.

In particular, the up-regulation of FAK modulates the activity of several proteins, which are involved in the regulation of important cellular function. NS3A, NS5A and NS5B may directly or indirectly interact with paxillin and  $\alpha$ -actinin, promoting the dysregulation in the form, in the disposition and in the organization of cellular Tight-Junctions. These events lead to the perturbation of the cellular homeostasis with very important effects on its morphology, shape, motility, polarization, adhesion ability as well as on the processes of energy production in the mitochondria and of genome transcription and duplication in the nucleus.. In addition, NS3A, NS5A and NS5B also promote the paracrine activation and proliferation of stellate cells in extracellular matrix and induce the expression of genes encoding extra-cellular matrix proteins, like collagen type I.

In addition to studies performed in pancreatic tissue, some epidemiological trials and meta-analyses have also suggested that HBV and HCV infections are associated with an increased risk of PAC occurrence. Before carrying out our research in this field, in June 2019, according to the methods used in our previous meta-analysis on the same topic [35], we performed a preliminary systematic review of available evidence concerning the association between HBV/HCV infection and risk of PAC development. We considered the papers published on this subject until the first quarter of 2019.

In particular, based on these criteria, we obtained the following results:

1. concerning HBV infection and risk of PAC development, we identified 6 case-controls (in USA [36], Taiwan [37], Korea [38] and China [39-41]), 9 cohort studies (in Taiwan [42, 43],

USA [44, 45], Australia [46, 47], Sweden [48], Denmark [49] and Japan [50]) and 7 meta-analysis (in China [51-55], USA [56] and Italy [35]). Most of case-controls, cohort trials and all meta-analyses registered an increased risk of PAC development;

2. as regards HCV infection, 4 case-controls (in China [41], USA [36], Korea [38] and Taiwan [37]), 7 cohort studies (in USA [57, 58], Australia [46, 47], Denmark [59], Sweden [48] and Japan [50]) and 3 meta-analyses (in China [54, 55] and in Italy [35]) were considered. By examining the most of case-control, cohort-studies and all meta-analyses a higher probability of PAC development emerged.

Since 2014 no additional meta-analyses about this topic were published and before most trials or meta-analyses were performed mainly in the USA, in China and in the South-Eastern Countries. Only three studies carried out in Europe are detectable in literature. To our knowledge, no data are available among the Italian population about the possible relationship occurring between HBV/HCV infection and the risk of PAC. Therefore, additional studies in Italy are needed to clarify this point. From an epidemiological point of view, the annual pancreatic cancer incidence in this Country is equal to 9.2/100,000 [60]. Prevalence of serum patterns of HBV markers and anti-HCV antibodies implied important changes in the general population in Italy, with a progressive reduction of their prevalence in the latest times. According to some recent surveys, the HBsAg prevalence in the Italians is estimated to be largely below 2%. This percentage decreased from 3-5% in the 1980s, settling down to about 0.8-1% in 2010 [61], so that, to date, Italy may be considered as a low HBV endemic Country, such as United States of America or Northern

Europe [62], where most of the individuals with a previous contact with HBV show a serum pattern characterized by absence of HBsAg and presence of specific antibodies against two antigens of this pathogen agent represented by HBcAb and HBsAb. It is also well-known that coexistent serum HBsAb and HBcAb antibodies in some individuals, HBsAg-/HBcAb+/HBsAb- or HBsAg- /HBcAb+/HBsAb+ in association, are the evidence of a previous exposure to HBV, not necessarily related to an underlying vaccination. In the past, both patterns were considered to be specific markers of a complete recovery from a previous exposure to HBV. However, the progressive improvement of molecular biology techniques for the study of this pathogen afforded to demonstrate, in some circumstances, its persistence and replication at low levels even in subjects with HBsAg-/HBcAb+/HBsAb- or HBsAg- /HBcAb+/HBsAb+ patterns. This condition, characterized by the presence of HBV-DNA in liver tissue and its absence in serum [63], is defined as “occult” HBV infection and its clinical impact in the human pathology, as well as in pancreatic carcinogenesis, is still unknown.

Only a few trials were performed to calculate the percentages of isolated anti-HBs+ or anti-HBc+, as well as anti-HBs+/anti-HBc+, in Italian people. In a survey conducted on the general population in Northern Italy [64] their prevalence resulted to be 23.8%, 4.2% and 8.4%, respectively. In an analogous trial, performed in Southern Italy, HBcAb+ prevalence in general people dropped from 66.9% to 7.6% in a period ranging from 1978 to 2006 [65]. In both studies, the decline of HBsAg+ and anti-HBc+ was even more striking in younger individuals (aged from 15 to 24 years). On the other hand, in a recent study, the overall prevalence of anti-HCV antibodies in the Italian general people is

estimated to range between 1% and 2.2-2.7% [66], although an existing wide variability, depending both on distinct geographical areas (about 1.6% in North, 2.6% in Centre and 2.4% in South Italy) and on different age classes, reaching the highest values in individuals born between 1935 and 1944 (until 7%), as well as in subjects born before the year 1935 (about 4.2%), regardless of the geographical areas in which they are settled [67]. Based on all these preliminary results and considerations, we first aimed to perform a retrospective pivotal work to assess HBV antigens/antibodies, as well as anti-HCV antibodies prevalence in a cohort of Italian patients with PAC admitted to two hospital wards in Italy, without taking into account the other well known risk factors for the development of PAC, such as smoking, alcohol drinking, and diabetes. Therefore, these variables have not been collected and included in our analysis. We have organized our data, stratifying our patients both in birth cohorts and in groups of age to assess the HBsAg, HBsAb/HBcAb and anti-HCV prevalence in these different classes, according to Fabris' and Andriulli's trials. These two Authors have evaluated the prevalence of HBV and HCV serum markers in two large Italian populations [64, 67].

## Material and methods

### Study Population

In this pivotal bi-Centre retrospective study we collected data of patients to which a primary diagnosis of PAC was made, consecutively admitted and treated in the following Units of two large-sized Italian Hospitals:

- a) Surgery Unit A, Azienda USL- Bologna, Maggiore Hospital, Bologna (Bologna group);
- b) Unit of Gastroenterology and Digestive Endoscopy, Sandro Pertini Hospital, Rome

(small center with low case volume)  
(Rome group).

Sources included records of inpatients extracted from the pancreatectomy files and from the ERCP files available in Hospital Units in Bologna and in Rome, respectively.

This study was carried out in accordance with the ethical principles of the Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>).

In the Surgery Division A, Bologna, Maggiore Hospital, data concerning patients with a diagnosis of PAC were pulled from the pancreatectomy files, whose database includes 839 subject underwent, for several reasons, a surgical partial or total pancreatectomy in this Unit between January 1, 2005, and December 31, 2017. Inclusion criteria for this group were aged 16 years or older and PAC diagnosis, confirmed by histology on pancreatic surgical specimens.

In the Unit of Gastroenterology and Digestive Endoscopy, Sandro Pertini Hospital, Rome, data of individuals with a PAC diagnosis were collected from the ERCP File. This database includes 212 patients to whom was made an endoscopic retrograde cholangiopancreatography (ERCP), between January 1, 2014, and August 31, 2018, owing to various causes. The inclusion criteria for this group were: 16 years of age or older and PAC diagnosis, confirmed either on pancreatic microbiopsy specimens or diagnosed by symptoms, signs, biochemical tumor markers tests (CA 19.9) and at least two types of imaging tools,

represented by abdominal Computed Tomography and Endoscopic Ultrasonography (EUS).

The parameters of data recording in the two groups were: age, sex, pancreatic cancer localization (head, body, tail) and serum HBV/HCV profiles (HBsAg, HBsAb/HBcAb, and HCVAb). Data on viral markers were withdrawn by medical records or by the archives of Laboratories in both Hospitals if the first ones were not available. Taking into account that HBV (antigens and antibodies), as well as anti-HCV antibodies prevalence, in Italian population is variable, depending on the different classes of age, we stratified our patients both in a birth cohort and in groups of age to assess the HBsAg, HBsAb/HBcAb and anti-HCV prevalence in them. According to the studies of Fabris and Andriulli, we first considered the following classes of age or birth cohort: 0-14, 15-24, 25-34, 35-44, 45-54, 55-64, 65-74,  $\geq 75$ , and  $>1984$ , 1975-1984, 1965-1974, 1955-1964, 1945-1954, 1935-1944,  $<1935$ . Then we summarized our results in some synthetic tables, together with data on the prevalence of HBsAg, HBsAb/HBcAb and anti-HCV.

Continuous variables were expressed as mean  $\pm$  standard deviation and compared to two sample t-test; categorical data were indicated as numbers (percentages) and p-value less than 0.05 was significant.

## Results

The distribution of demographic characteristics and the selected variables of the patients analyzed are reported in Table 2.



Variables	Total of 373 patients
Age (years)	68.2 ±10.1
Sex (pts: 373)	
Male	200 (53.6%)
Female	173 (46.4%)
Cancer localization	
Head	289 (77.5%)
Body/tail	67 (18.0%)
Head + Body/Tail	17 (4.5%)
HBsAg	
Positive	4 (1.1%)
Negative	369 (98.9%)
Both HBsAb/HBcAb positivity	28/373 (7.5%)
Anti-HCV	
Positive	16/373 (4.3%)
Negative	357/373 (95.7%)

**Table 2:** Main characteristics of patients included in the study with PAC and HBV/HCV markers status.

In the Bologna group, among 839 patients included in the pancreatotomy files, we collected 311 patients with a proved histological diagnosis of PAC (37.1%). The remaining 528 patients, whose the mean age was  $68.3 \pm 9.3$  years, were not taken into account because suffering from other pathological conditions, including main biliary duct cancers, neuroendocrine pancreatic tumors, Vater's papilla cancer, metastasis to pancreas from different sites (carcinomas of breast, of kidney and of colorectal, sarcoma in the lower limb).

In the Rome group, among 698 subjects included in the ERCPs File, we found 62 patients with a PAC diagnosis (8.9%). The main indication to perform ERCP in these patients was represented by the endoscopic palliation of jaundice. The remaining 636 patients were left out as not affected by pancreatic cancer, but by other diseases, such as stones in the main biliary duct, cancer of main biliary tract, Vater's papilla cancer. In this group, the mean age of patients was  $67.9 \pm 13.2$  years.

Merging the two results arising from the two groups, the overall mean age was  $68.2 \pm 10.1$  years.

### HBV markers prevalence

In our study population, four patients turned out to be HBsAg positive, with a prevalence equal to 1% in 373

patients. In the overall Italian population, the available surveys report similar values. In 28 individuals we observed the coexistence of HBsAb and HBcAb in their serum. The prevalence of HBsAb/HBcAb was equal to 7.5% in 373 subjects (Tables 3 and 4).

Birth Cohort/ Number of patients	HBsAg (373 pts)	HBsAb/HBcAb (373 pts)
>1984	0	0
1975-1984	0/1	0/1
1965-1974	0/12	1/12 (8.3%)
1955-1964	1/45 (2.2%)	4 /45 (8.9%)
1945-1954	2/95 (2.1%)	5/95 (5.2%)
1935-1944	1/157 (0.6%)	10/157 (8.9%)
<1935	0/63	8/63 (12.6%)
<b>Total</b>	<b>4/373 (1.1%)</b>	<b>28/373 (7.5%)</b>

**Table 3:** Birth cohort prevalence of HBV markers profile in the series of patients with PAC from Bologna and Rome.

Classes of age (years)/ Number of patients	HBsAg (373 pts)	HBsAb/HBcAb (373 pts)



<b>0-14</b>	0	0
<b>15-24</b>	0	0
<b>25-34</b>	0	0
<b>35-44</b>	0/8	1/8 (12.5%)
<b>45-54</b>	1/37 (2.7%)	2/37 (5.4%)
<b>55-64</b>	2/70 (2.8%)	7/70 (10.0%)
<b>65-74</b>	1/167 (0.6%)	8/167 (4.8%)
<b>≥ 75</b>	0/91	10/91 (11.0%)
<b>Total</b>	4/373 (1.1%)	28/373 (7.5%)

**Table 4:** Prevalence of HBV markers (HBsAg, HBsAb/HBcAb), according to the age groups in of patients with PAC from Bologna and Rome

In his survey on the general Italian people, Fabris showed that HBsAb/HBcAb overall prevalence was 8.4% and it progressively increases with the age, ranging from 0% to 17.8% in over 65 years subjects.

#### HCV markers prevalence

Sixteen patients were HCV positive, with a prevalence in our study population equal to 4.3%. The prevalence of anti-HCV positivity in our study population was variable, depending on the different age - as well as birth – classes, as also reported in trials by Andriulli and Fabris (Table 5).

<b>Age-groups (year of birth)</b>	<b>HCV positive patients/ HCV negative patients/Percentage in Andriulli's study</b>	<b>HCV positive patients with PAC/ HCV negative patients with PAC /Percentage</b>
<b>&gt;1984</b>	1/524 (0.2%)	0
<b>1975-1984</b>	8/652 (1.2%)	0/1
<b>1965-1974</b>	14 /868 (1.6%)	0/12
<b>1955-1964</b>	12/1010 (1.2%)	2/45 (4.4%)
<b>1945-1954</b>	21/972 (2.2%)	3/95 (3.1%)
<b>1935-1944</b>	47/669 (7.0%)	5/157 (3.2%)

<b>&lt;1935</b>	9/212 (4.2%)	6/63 (9.5%)
<b>Total</b>	112 /4907 (2.3%)	16/373 (4.3%)

**Table 5:** Birth cohort prevalence of anti-HCV positivity in the general population of five Italian urban areas from Andriulli's study, in the series of patients with PAC from Bologna and Rome.

In our research, the mean age of HCV positive patients was higher than the one of HCV negative subjects ( $73.1 \pm 12.4$  years vs  $68.0 \pm 9.9$  years,  $p = 0.0471$ ). The prevalence of anti-HCV positivity according to the age groups and birth-classes in the series of patients with PAC from Bologna and Rome is shown in Table 5 and Table 6.

<b>Age-groups (years)</b>	<b>HCV positive patients with PAC/ HCV negative patients with PAC /Percentage</b>
<b>0-14</b>	0
<b>15-24</b>	0
<b>25-34</b>	0
<b>35-44</b>	0
<b>45-54</b>	2/37 (5.4%)
<b>55-64</b>	2/70 (2.8%)
<b>65-74</b>	4/167 (2.4%)
<b>≥75</b>	8/91 (8.8%)
<b>Total</b>	16/373 (4.3%)

**Table 6:** Prevalence of anti-HCV positivity according to the age groups in the series of patients with PAC from Bologna and Rome (third column).

## Discussion

Our observational study assessed, for the first time in Italy, the prevalence of HBV and HCV antigens/antibody patterns in an Italian cohort of patients with PAC, admitted to two hospital wards in Italy. In our purpose, we just had the opportunity to refer only to a very small number of trials, assessing HBsAg and HBsAb/HBcAb and anti HCVAb prevalence in the general Italian population. In particular, we considered the works of Fabris and Andriulli [64, 67]. In our research HCV prevalence came out to be equal to 4.3% in all patients, whereas this value in the general Italian population-based survey by Andriulli is 2.3%. Similar results have been shown in an additional study by Fabris and colleagues [64], carried out also among the general Italian people. Furthermore, in our research HCV prevalence reached a value of 4.4% in the class of age, ranging from 1955 to 1964, whereas in Andriulli's research it was fixed to 1.2%. On the other hand, in our study the high HCV prevalence in birth class <1935 seems to be in accordance with the more elevated one in older people, at least in Italy, as reported by the epidemiological studies performed to date. Although our study met several limitations, such as the retrospective design and the small number of the enrolled patients, which are expression of a peculiar case series (patients admitted to two hospital wards) and not of general Italian population, the observed data seem to provide some useful outcomes and stimulate the interest in this research field. Considering that most of the available studies are signed in the USA and in the South-Eastern Asian Countries, their results may differ from the ones emerged in Italy and in Europe, currently lacking consistent trials. In order to fill such this gap, several factors could be usefully evaluated in the future, including the different ethnical characteristics of

patients enrolled in the trials, not without focusing on their geographical areas of provenance. However, we succeeded in establishing the prevalence of anti-HCV antibodies in patients with PAC at 4.3%, despite the heavy constraints above reported, whereas the ranges described in the general Italian population are forecasted between 2.2 and 2.7%. This evidence could stimulate the researchers to keep going on since if our data and assumptions will be confirmed in additional trials, it might come out that both the viruses would be able to promote pancreatic carcinogenesis, whose process is mostly sped up in the young.

This hypothesis, if viable at least in patients with HBV infection, would prove to be in line with a previous Editorial by Sherman and with a large-sized USA cohort-study [44, 68]. In these trials, the cohort of patients with PAC and HBV infection was composed of young individuals, predominantly male, black and Asian, in comparison with controls without a history of exposure to this pathogen. Patients aged 26 to 40 years occupied the greater pool of pancreatic cancer-HBV group population. It is difficult assuming the possible mechanisms involved in this process, but some years ago several epidemiological studies highlighted that a persistent inflammation [69, 70] becomes a predisposing factor to carcinogenesis in the tissues, where it occurs [71]. Both HBV and HCV might promote an inflammatory environment in the pancreas, leading to malignant transformation [70]. A previous paper proposed a potential model of HBV/HCV mediated pancreatic carcinogenesis [72]. In synthesis, both these pathogens, by means of their proteins, such as HBx and Hepatitis B surface antigen (HBsAg) and HCV NS3, NS4A, NS5A and NS5B might modulate and modify transcriptional cell genome activities both directly, such as HBx protein, and indirectly, such as

HBsAg, HBx, NS3, NS4A, NS5A and NS5B via interaction with components of cytoplasmatic enzymes and cell cytoskeleton. This interaction results in an alteration of the normal balance existing among intracellular and extracellular tensional forces, originated by cytoskeleton and extracellular matrix, respectively, in which it is possible to see a de-regulation of the normal intracellular cytoskeletal architecture and modifications of the disposition of the cell microorganelles, as well of the shape of the nucleus and the cell conformation. These events take so long to determine quali/quantitative alterations of crucial intracellular biochemical activities regarding the transcription, the translation, the transduction of nuclear genes and may also cause chromatin de-arrangement. The macroscopic effects of these microscopic modifications in the cells and in the stroma surrounding them are represented by the development of tissue with biochemical and biophysical differences in comparison with a normal one. In particular, as previously reviewed [73], a modified pattern in the deposition and disposition of several tissue structural (such as collagens or proteoglycans), as well as non-structural proteins (such as matricellular proteins), emerges. One of the most important effects of these complex processes is the increase of the tissue stiffness, a well-known factor promoting carcinogenesis (see Table 6 and Figure 1, 2 and 3).

Therefore, our previous and current studies put our attention once again on the possible role of hepatotropic viruses in pancreatic cancer development and seem to suggest that pancreas is not merely a reservoir of both hepatotropic viruses, but it may support their replication. Taking into account both epidemiological and etiological available studies concerning pancreatic carcinogenesis, it is conceivable that HBV and HCV act

more probably in this process as cofactors in cooperation with other actors (smoking, diabetes, alcohol) than as the only cause of this malignancy, although the action of both viruses may accelerate the development and the course of this neoplasm. In addition, as in the USA, also in Italy it is unknown what is the potential role and the clinical impact of "occult" HBV infection in PAC development [74, 75]. In several countries with decreased HBsAg prevalence, such as Italy, owing to long-term vaccination programs a significant number of subjects with markers of past exposure to HBV (serum HBsAb and/or HBcAb positivity) persists and these individuals might present a higher risk of PDAC [76].

Unfortunately, only a small number of trials have been carried out with the aim to understand this important point and the results are not univocal.

In particular, in the past two case-control studies detected an increased risk of PDAC in HBsAg-/HBcAb+/HBsAb- individuals [36, 39], whereas one study did not do it [40]. On the other hand, concerning HBsAg-/HBcAb+/HBsAb+ profile, two case-control studies showed an enhanced probability to develop this malignancy [36, 40] whereas one study did not do it [39]. The recent large sized cohort studies in the USA provided no specific contribution to clarify this important matter of debate. However, two meta-analyses, including these studies, have argued that a significantly increased risk occurs in HBcAb+/HBsAb+ vs HBsAg-/HBcAb- individuals, with RR equals to 1.41 (95% CI: 1.06-1.87) [35, 54] and in HBcAb+/HBsAb- vs HBsAg-/HBcAb- subjects, with OR equals to 1.76 (95% CI: 1.05-2.93). In any case, further studies are necessary.

## Conclusion

Even if our observational study describes the prevalence of HBsAg, HBsAb/HBcAb as well as HCV in a small-sized cohort of patients with PAC from two Italian Hospitals, it was not designed to investigate whether HCV or HBV infection is associated with an increased risk of PAC development in Italy. Therefore, this pivotal study represents the basis for additional well-designed and well-sized epidemiological and histological nationwide trials in Italy, with the aim to: i) confirm or deny the potential role in pancreatic carcinogenesis; ii) clarify which is the real impact of HBV and HCV in Italy and in other European Countries and whether it differs from the other nations, where a large body of data are already available in literature, such as China, North America, and Eastern Asia.

## Author Contributions

Study concepts: SF

Study design: SF, MZ

Data acquisition: SF, MZ, CB; ALdQ, MM, MFL, AL, RL, MZ, LM, SA, GDS, MC, LD, SN, GA, MV, AF, AT, PEO, GO; GC; PL

Quality control of data and algorithms: MLBR, PA, FB, SB,

Data analysis and interpretation: MLBR, PA, EG, IC,

FB, SB

Statistical analysis: MLBR

Manuscript preparation: SF, DdB, PL, PA, FB, EJ

Manuscript editing: DdB, PA, FB, EJ

Manuscript review: SF, PL, PA, FB, EJ, DdB

Final approval of the version: SF, MZ, CB; ALdQ, MM, MFL, AL, RL, MZ, LM, SA, GDS, MC, LD, SN, GA, MV, GC, AF, AT, PEO, GO, PL, PA, FB, EG, LR, IC, SB, MLBR, DdB, EJ

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## Conflict of interest

Nothing to declare

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