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Meaning of elevated CA 19-9 serum levels in chronic hepatitis and HCV-related cirrhosis

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Aim. **Carbohydrate 19-9 antigen (CA 19-9) has been used in the diagnosis and follow-up of gastrointestinal tumors. However, a remarkable reduction of specificity has been described in subjects with chronic diseases. Elevated CA 19-9 serum levels have been described in non neoplastic liver diseases, such as hepatic cirrhosis, where they correlate with the fibrosis grade and the disease severity. The aim of the study is to evaluate CA 19-9 levels in chronic hepatitis patients (CH) and hepatic cirrhosis patients, Hepatitis C Virus (HCV)-correlated. Our goal was to establish whether elevated CA 19-9 levels can be considered a non casual event in chronic liver disease and whether a correlation can be found between CA 19-9 levels and the severity of the disease.**

Methods. **116 patients have been recruited (76 m, 40 f, average 54 years); 56 patients were affected by CH and 60 by hepatic cirrhosis (Child A). All patients were HCV+, genotype 1b. Patients positive to CA 19-9 high levels were subjected to abdominal echography, EGDS, colonscopy, abdominal CT.**

Results. **Fifty two percent presented high levels of CA 19-9. None was affected by intestinal or pancreatic neoplasia, or colestatic icterus. CA 19-9 levels were elevated in 46% of patients with chronic hepatitis, and in 54% in patients with hepatic cirrhosis. Furthermore, CA 19-9 levels in hepatic cirrhosis compared to CA 19-9 levels in chronic hepatitis was statistically significant (P>0.007).**

Conclusion. **Increased serum levels of CA 19-9 are frequent in chronic viral hepatitis; this often does not indicate a contemporary neoplastic disease and correlates in a statistically significant way (P>0.007) with the severity of the disease.**

Key words: **CA 19-9 Antigen - Liver cirrhosis - Hepatitis C.**

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Carbohydrate 19-9 antigen (Ca 19-9) is mucine glycoprotein used in the diagnosis and follow-up of gastrointestinal tumors. Its levels are elevated in patients with gastric adenocarcinoma, in colon, biliary duct and pancreatic carcinomas.[1-7] The cut off value (100 UI/mL) grants a sensibility and specificity of 96% and 90% respectively. A significant reduction of its specificity is seen in patients affected by chronic diseases such as pacreatitis, renal failure and chronic liver disease. Moreover, some works in literature

report an increase of CA 19-9 serum levels in non neoplastic liver disease such as simple liver cysts, severe steatosis, autoimmune hepatitis, chronic alcoholic hepatitis [8-11] and hepatic cirrhosis, where CA 19-9 levels seem to correlate with the grade of fibrosis.[12, 13]

The aim of this study is to evaluate serum CA 19-9 levels in a cohort of subjects affected by chronic HCV-correlated hepatitis (CHC), in order to verify whether elevated CA 19-9 serum levels can be considered a non casual event, and whether a correlation can be found between CA 19-9 serum levels and the severity of the disease.

Materials and methods

One hundred sixteen patients have been recruited (76 males, 40 females); the age ranged from 35 to 78 years (average 54 years). Clinical evaluations, haematho-chemical, virological, instrumental and histological analysis were performed on these patients. None of the patients made excessive use of alcohol or hepatotoxic drugs. The following analysis are reported in details.

Hepatic function tests

Aspartate Aminotransferase and Alanine Aminotransferase (AST and ALT), gamma Glutamil Tranferase (gGT), Alkaline Phosphatase, total, conjugated and unconjugated bilirubin, cholinesterase, serum proteins, prothrombin, fibrinogen, and Protombine Time (PT) analyses were performed.

Virological analysis

In all patients Hepatitis B surface Antigen (HBsAg), Hepatitis C Virus antibody (anti-HCV), and Hepatitis C Virus RNA (HCV-RNA) were searched, and the HCV genotype was determined. Anti-HCV antibodies were determined via (Enzime-Linked immunosorbent assay) ELISA assay (Ortho Diagnostic System Inc., Raritan, NJ, USA).

Quantitative pretreatment HCV-RNA was searched by means of the Polimerase Chain Reaction (PCR) analysis of the region 5'-UTR of HCV-RNA, using Cobas Amplicor, Monitor Test 2.0, Roche Diagnostics. Viral genotypes were identified by restriction analysis of the amplified 5'-UTR, and the Simmons classification was applied.

Autoimmunity

Organ-specific and non organ-specific autoantibodies (Antinuclear Antibodies: ANA, Antimitochondrial Antibodies: AMA, Smooth Muscle Antibodies: SMA, Liver Kidney Microsomal Antibodies Tipe 1: LKM1) were searched by immunofluorescent technique and semiquantitative ELISA.

Thyroid function

Measurement of Thyroid-Stimulating Hormone (TSH) (WHO 2nd IPR 80/558-ECL), free-Triiodothyronine T3 (free-T3), free-Tetraiodothyronine T4 (free-T4) Enanced Chemiluminescence (ECL), Thyroid Perossidase-Antibodies (TPO-Ab) and Thyroglobulin-Antibodies (TG-Ab) (ECL) was performed.

Iron indexes

Blood iron (normal value [n.v.] 55-160 mg/dL in men, 45-150 mg/dL in women), iron-saturated transferrin (n.v. 2-36 g/L), of the total transferrin (Total Iron Binding Capacity: TIBC n.v. 218-411 mg/dL), unsaturated transferrin (UIBC n.v. 110-290 mg/dL), transferrin saturation (Fe/TIBCx100, n.v. 0-40), serum ferritin (n.v. 18-370 ng/mL).

CA 19-9 measurement

CA 19-9 measurement has been performed in all patients by ECL method (n.v. 0.0-39.0 UI/mL).

All hepatic function tests, haematochemical and hormonal measurements and virological analysis were performed in the laboratory of our hospital with automated and standardized methods, in conformity to the quality certified standards EN ISO 9001:2000.

Instrumental tests

Patients who resulted positive to high levels of CA 19-9 were subjected to abdominal

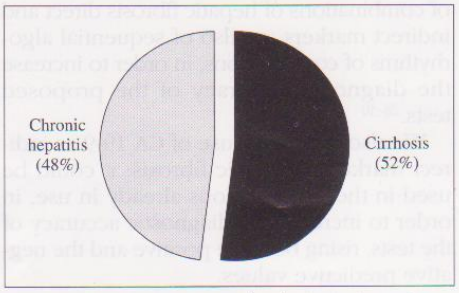

Figure 1.—The figure shows 56/116 (48%) chronic hepatitis (CH) cases, 60/116 (52%) cirrhosis Child A HCV 1b correlated.

echography and esophagogastroduodenoscopy (EGD), colonscopy and abdominal spiral CT.

Hepatic biopsy

Hepatic tissue specimens were taken from all patients, by echo-driven biopsy by means of Menghini's method. Histological evaluation of necro-inflammatory activity and fibrosis according to METAVIR score, and semi-quantitative evaluation of severity and distribution of iron deposit according to Brissot *et al.* Intrahepatic iron was evaluated by Perls coloration with Prussia blue.

Statistical analysis

Continuous variables were presented with mean values and standard deviation. Dicotomic variations were expressed as frequencies and examined by non parametric statistical methods (χ^2, Fisher's exact test). In the case of two groups comparison, couples of data and single data were examined by t Student test.

Results

Fifty-six out of 116 patients resulted affected by chronic hepatitis (CH) (48%), and 60 out of 116 by hepatic cirrhosis HCV-correlated, genotype 1b (52%). Sixty patients (40 males, 20 females [52%]) presented high serum levels of CA 19-9 (mean value 76.8 UI/mL, SD:42.3 UI/mL). Clinical, instrumental and laboratory investigations allowed to exclude any intestinal, pancreatic, ovarian and mammalian tumors; inflammatory intestinal diseases or colestatic icterus were absent too (Figure 1). CA 19-9 serum levels were elevated in 46% (28/60) of CH patients, with a METAVIR score ranging from F1 to F3, and in 54% (32/60) of hepatic cirrhosis patients, with a F4 METAVIR score. The increase of CA 19-9 in hepatic cirrhosis resulted statistically significant in comparison to the increase in chronic hepatitis (P>0.007), as shown in Figure 2.

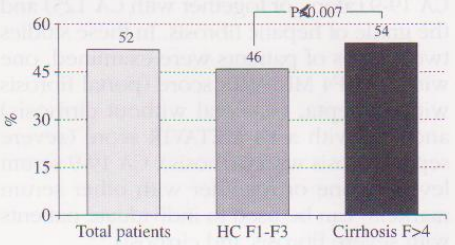

Figure 2.—Elevated CA 19-9 serum levels.

Discussion and conclusions

Our data demonstrate that high levels of CA 19-9 are a frequent event in viral chronic hepatitis (52%); they often cannot be considered as a sign of neoplasia, and are statistically correlated with the severity of the disease (P>0.007) and with an increase of fibrotic process.

The first outcome of our study is that it is necessary to careful evaluate the use of CA 19-9 as a tumoral marker in the presence of a chronic liver disease, because a high percentage of false positives can occur.

Our data also focus on the possibility to use CA 19-9 as indirect marker of hepatic fibrosis. Some works in literature report a statistically significant correlation between CA 19-9 serum levels and some standard parameters of hepatic function: a positive correlation can be shown with levels of AST, ALT, Alkaline Phosphatase and bilirubin.[13]

Other studies show a correlation between

CA 19-9 (alone or together with CA 125) and the grade of hepatic fibrosis. In these studies two groups of patients were examined, one with a F3-F4 METAVIR score (portal fibrosis without septa, or septal without cirrhosis) and one with a F4 METAVIR score (severe septal fibrosis with cirrhosis). CA 19-9 serum levels (alone or together with other serum markers) can be used to individuate patients with severe fibrosis and cirrhosis.[14]

Several direct and indirect hepatic fibrosis markers were studied in the last years. Ialuronic acid, type IV collagen, metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase (TIMP-1) were tested as direct markers. Fibrospect and the European Liver Fibrosis study group panel were used as tests with a combination of markers.

Transaminase enzymes were the first indirect hepatic fibrosis markers. They were eventually associated to each other in the Aspartate aminotransferase-Alanine aminotransferase ratio (AAR) index.[15] Wai and other authors proposed a further evolution of AAR index, by combining AST to platelet count: Aspartate aminotransferase Platelet Ratio Index (APRI).[16-22] Forns *et al.* proposed a simple test which combines age, cholesterol, γGT and platelets (Forms' index).

The most studied non invasive markers combination is Fibrotest, which associates total bilirubin, aptoglobin, apolipoprotein A1 and μ-macroglobulin.[24] Fibrotest is nowadays not only the most studied non invasive markers combination but also the most validated, with more than 30 reports in literature.[24, 25] In HCV hepatitis it showed a significant area under curve (AUC) ranging from 0.74 to 0.87 for the fibrosis (F>2) and from 0.71 to 0.87 for the cirrhosis. The present data suggest, thus, that Fibrotest is efficient in the diagnosis of the extreme conditions of hepatic fibrosis (F0-F1 and F4), but is rather less efficient for intermediate stages. Furthermore, in some studies Fibrotest did not result as accurate as in the first works.[26, 27]

These and other data in literature show how in the last years many researchers concentrated their efforts on the identification of combinations of hepatic fibrosis direct and indirect markers, or also of sequential algorhythms of combinations, in order to increase the diagnostic accuracy of the proposed tests.[28-30]

We also report the use of CA 19-9 as indirect marker of hepatic fibrosis; it could be used in the combinations already in use, in order to increase the diagnostic accuracy of the tests, rising both the positive and the negative predictive values.

Riassunto

Significato di elevati livelli sierici di CA 19-9 nell'epatite cronica e nella cirrrosi HCV correlata

Obiettivo. L'antigene carboidratico 19-9 (CA 19-9) è utilizzato come marker neoplastico nella diagnosi e nel follow-up di tumori gastrointestinali. Tuttavia la sua specificità si riduce nelle malattie croniche. Incrementi del CA 19-9 sono stati segnalati in patologie epatiche non neoplastiche, tra cui la cirrosi epatica, in quest'ultimo caso i suoi livelli sembrano essere correlati con il grado di fibrosi e la severità della malattia. Lo scopo di questo lavoro è valutare e confrontare i livelli plasmatici di CA 19-9 in pazienti affetti da epatite cronica (CH) e in pazienti con cirrosi epatica HCV-correlata, per verificare se in corso di CH il riscontro di valori elevati di CA 19-9 sia un evento frequente e se vi sia correlazione tra i livelli di CA 19-9 e la severità dell'epatopatia.

Metodi. Sono stati arruolati 116 pazienti, di cui 76 maschi e 40 femmine, di età media 54 anni, 56 con epatite cronica, 60 con cirrosi epatica Child A HCV-correlata, genotipo 1b. I pazienti con incremento del CA 19-9 sono stati sottoposti a ecografia addominale, esofagogastroduodenoscopia, colonscopia, TAC spirale addominale.

Risultati. Il 52% (60/116) dei pazienti presentava aumento del CA 19-9. Nessuno era affetto da neoplasie intestinali, pancreatiche o ittero colestatico. I livelli sierici di CA 19-9 erano elevati nel 46% (28/60) delle epatiti croniche e nel 54 % (32/60) delle cirrosi epatiche. L'incremento del CA 19-9 nella cirrosi epatica rispetto all'epatite cronica risultava statisticamente significativo (P>0.007).

Conclusioni. L'aumento dei livelli sierici di CA 19-9 è frequente nell'epatite cronica virale (52%); spesso nella CH non è spia di una concomitante neoplasia ma si correla (p>0.007) con la severità della malattia.

Parole chiave: Antigene carboidratico 19-9 - Fibrosi epatica - Epatite cronica - Epatite C.

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