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Serum CA 19.9 Levels in Patients With Benign and Malignant Disease: Correlation With the Serum Protein Electrophoretic Pattern

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Abstract. *Aim: To confirm that the carbohydrate antigen 19.9 (CA 19.9) protein can be evaluated by determining changes in the β2 zone in protein electrophoresis. Materials and Methods: A total of 75 patients (64 with cancer, 11 with benign diseases) with abnormal serum CA 19.9 level were included. Results: Patients with cancer had significantly higher serum CA 19.9 concentrations than those with benign diseases (p<0.001). Similar CA 19.9 levels were observed in patients with normal (median 1129 U/ml), weakly positive (median 699 U/ml) and positive (2333 U/ml) β2 fraction in protein electrophoresis. In contrast, changes in the protein pattern of the β2 fraction were related to an inflammatory pattern with significantly higher C-reactive protein concentration (p<0.0001), independently of serum CA 19.9 level. Conclusion: The intensity of the β2 fraction in protein electrophoresis is related to inflammation and not to CA 19.9 in patients with cancer or other diseases.*

Carbohydrate antigen 19.9 (CA 19.9) is a sialylated Lewis (Le), *a* blood group antigen (Le^a) (1-4) clinically used as a tumor marker. Different publications have clearly demonstrated its diagnostic value in different malignancies, mainly in patients with gastrointestinal cancer, mucinous ovarian carcinoma and especially in pancreatic cancer or cholangiocarcinoma (5-14). Nonetheless, CA 19.9 is not specific and abnormal levels may be found in different benign diseases, mainly associated with cholestasis (9-11,15-16). Abnormal levels of CA 19.9 have been reported in more than 75% of patients with pancreatic cancer or cholangiocarcinoma (17-21). However, patients with the Le(a–b–), phenotype are unable to release CA 19.9, and CA 19.9 in these patients is usually undetectable in serum in any condition, including cancer (2-3, 21-22). These data indicate that CA 19.9 is not useful in around 10% of patients with biliary tract tumors (23-25).

Grubb *et al*. have suggested that the CA 19.9 protein can be detected in all patients, including those with a Lewis antigen-negative phenotype (26). Likewise, Delanghe *et al*. have shown that changes in CA 19.9 concentrations are reflected by changes in the β2 fraction in electrophoresis of serum proteins. In a patient with metastatic pancreatic adenocarcinoma, they reported very high CA 19.9 concentrations correlated with the presence of a peak in the β2 fraction on serum protein electrophoresis (27). Confirmation of these results would indicate the possibility of increasing the diagnostic sensitivity of CA 19.9 in cancer, mainly in patients with biliary tract tumors, and establish its use in the differential diagnosis or follow-up of these patients.

The aim of this study was to confirm correlation between the β2 fraction and serum CA 19.9 levels, and secondarily, the use of serum protein electrophoresis for the evaluation of patients with a Lewis antigen-negative phenotype and suspicion of digestive tract tumors.

Materials and Methods

Seventy-five patients (age=35-90 years, median=70 years), 41 men and 34 women, were referred with abnormal CA 19.9 levels, including 64 patients with active malignancy (49 stage IV, 15 stage III) and 11 patients with benign diseases (eight of them with liver diseases). Patients with cancer included: 16 with lung cancer, 15 with colorectal tumors, 11 with pancreatic cancer, 10 with cancer of unknown primary origin, three with mucinous ovarian malignancy, six with primary liver cancer, two with esophageal cancer and 1

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with bladder cancer. Serum CA 19.9 levels ranged from 375 to 1278 U/ml in those with benign diseases and from 120 to 977,700 U/ml in those with malignant diseases.

Blood serum samples were collected after diagnosis (before treatment) by venous puncture using a gel separator tube. After centrifugation, the serum samples underwent routine biochemistry evaluation. CA 19.9 was analyzed in an Elecsys 411® autoanalyzer (Roche Diagnostics, Basel, Switzerland), considering 37 U/ml as the cut-off level. Serum protein electrophoresis was performed using a commercial Capillarys 2 system (SEBIA, Lisses-Evry Cedex, France), following the manufacturer's guidelines: All fractions were calculated, and α1, α2 and β fractions of 3.7-7.8%, 5.2-10.7%, and 8.5-13%, respectively, were considered as normal. In relation to serum protein electrophoresis, we mainly considered the β2 fraction, which was classified as normal (typical electrophoresis pattern, β2 lower than β1), weakIy positive (when β2 reached approximately the same level as β1), or positive (when β2 was higher than β1; abnormal).C-Reactive protein (CRP) was determined by an immunoturbidimetric assay enhanced by latex in an Advia Centaur (Siemens Medical Solutions Diagnostics, Erlangen, Germany) with serum levels less than 1 mg/dl being considered as normal. An increase in α1 and α2 fractions in the serum electrophoretic pattern was considered as representing an inflammatory profile. This protocol was approved by the Ethical Committee of the Hospital Clinic in 2017.

Statistical methods. Descriptive data are expressed as the median with 95th percentile. Categorical variables are described using frequency distributions. All the data were analyzed with SPSS statistical software (version 17.0; SPSS Inc. Chicago, IL, USA). CA 19.9 levels were compared using non-parametric (Wilcoxon, Mann–Whitney *U*, Kruskal–Wallis) or parametric tests (Student's t-test). A *p*-value of 0.05 or less was considered statistically significant.

Results

Table I shows the CA 19.9 results obtained in the different study groups. Patients with cancer had significantly higher concentrations of this antigen than those with benign diseases ($p<0.001$). Table II and Figure 1 show the serum CA 19.9 levels in three groups of patients according to the intensity of the β2 fraction. No differences in serum CA 19.9 level were found among the different groups studied. Figure 2 shows the electrophoretic pattern found in one patient with very high CA 19.9 concentration but with a normal β2 fraction.

Significantly higher CRP concentrations were found in patients with an increase in the β2 fraction ($p<0.0001$) (Figure 3). Table III shows the serum CRP and CA 19.9 levels according to β2 fraction in serum electrophoresis. Significantly higher concentrations of both parameters were found in patients with cancer than in those with benign diseases. Likewise, protein electrophoresis showed that compared to CA 19.9 levels, CRP concentrations were significantly higher in patients with cancer with an abnormal β2 fraction ($p<0.001$).

Table IV shows the serum CRP and CA 19.9 levels subdivided according to inflammatory profile (based on α1 and α2 fractions) and β2 fraction in protein electrophoresis. It is of note that an inflammatory profile was clearly related to the serum CRP level in all situations for the whole group ($p=0.0001$), and in patients with normal β2 ($p=0.04$) or abnormal β2 fraction in protein electrophoresis ($p=0.006$). In contrast, serum CA 19.9 levels were not related to any of these parameters in this analysis.

Table I. *Serum carbohydrate antigen 19.9 levels in the different study groups.*

Diagnosis	Patients (n)	Median (U/ml)	Range (U/ml)
Primary liver cancer	6	674	120-9,690
Colorectal cancer	15	2909	599-40,598
Mucinous ovarian cancer	3	1841	1,120-8,972
Pancreatic cancer	11	2649	244-977,700
Lung cancer	16	978	545-7,972
Bladder cancer	1	1891	-
Esophageal cancer	2	21633	2039-4,1227
CUP	10	1920	186-2,1095
Liver diseases	8	661	115-1,278
Other	3	658	651-980

CUP: Cancer of unknown primary origin. Cancer *versus* no cancer, $p=0.001$.

Discussion

It has been suggested that CA 19.9 may be a tumor marker in different malignancies, mainly in digestive tract tumors, such as pancreatic or biliary tract tumors. The sensitivity of CA 19.9 is high, especially in advanced stages, and is frequently used in helping to achieve a diagnosis. However, CA 19.9 cannot be used in patients with a Lewis antigen-negative phenotype because the techniques currently available are not able to detect this antigen in these patients. In summary, the use of CA 19.9 in Lewis antigen-negative patients may be misinterpreted.

Delanghe *et al*. reported a peak in the β2 fraction in protein electrophoresis (27) which was not due to paraprotein interference (28). Likewise, with the use of high-pressure gel permeation chromatography their group reported that CA 19.9 presents electrophoretic mobility in this area. Finally, the same group reported a reduction of the β2 fraction when treatment was administered and CA 19.9 levels decreased from 2×10^6 to 5.5×10^4 U/ml. These data suggest that serum electrophoresis may be used to detect the antigen, providing a new tool for the diagnosis and follow-up of Lewis antigen-negative patients.

Our results show that an abnormal β2 fraction may frequently be found in patients with high serum CA 19.9 level, suggesting that this antigen may migrate to this zone. However, we did not find any relationship between serum CA 19.9 levels and the presence of a peak in this area. It is of note that a patient with an extremely high serum CA 19.9 level had a normal β2 fraction (Figure 2). These data suggest that the electrophoretic pattern is not useful in determining

Table II. *Serum carbohydrate antigen 19.9 level according to the β2 fraction in the serum electrophoretic pattern.*

Diagnosis	β2 Fraction intensity	Patients (n)	Median (U/ml)	95th Percentile (U/ml)
No cancer	Normal	8	797	1,098
	Positive	3	633	-
Active cancer	Normal	29	1,531	509,463
	Weakly positive	15	1,044	9,690
	Positive	20	2,621	40,022

Table III. *Serum C-reactive protein (CRP) and carbohydrate antigen 19.9 (CA 19.9) levels according to diagnosis and β2 fraction in the serum electrophoretic pattern.*

		CRP (mg/dl)			CA 19.9 (U/ml)		
	Patients (n)	Median	95th Percentile	*p*-Value	Median	95th Percentile	*p*-Value
Diagnosis							
Benign	11	0.5	0.9	0.0001[a]	658	1,098	$p<0.001$
Cancer	64	6.0	29.0		1,822	37,719	
B2 Fraction							
Normal	29	1.6	9.7	0.006[b]	1,531	509,463	
Weakly positive	15	4.0	27.4		1,044	9,690	
Positive	20	13.2	26.7		2,621	40,022	

[a]*Versus* benign; [b]*versus* positive.

Table IV. *Serum C-reactive protein (CRP) and carbohydrate antigen 19.9 (CA 19.9) levels according to inflammatory profile.*

		CRP (mg/dl)			CA 19.9 (U/ml)		
Diagnosis	Patients (n)	Median	95th Percentile	*p*-Value[a]	Median	95th Percentile	*p*-Value
Total							
No inflammation	36	0.95	17.3	0.0001	957	40,692	n.s.s
Total							
Inflammation	39	10.2	35.0		2,077	29,083	
Normal β2							
No inflammation	25	0.6	2.9	0.04	979	36,372	n.s.s
Inflammation	12	7.35	9.7		1,739	14,354	
Abnormal β2							
No inflammation	11	2.1	5.9	0.006	650	2,909	n.s.s
Inflammation	27	11.4	37.0		2,256	25,887	

[a]*Versus* no inflammation. n.s.s, No significance.

the possibility of high CA 19.9 protein concentration in patients, regardless of Lewis antigen status.

We evaluated the relationship of an abnormal β2 fraction with the inflammatory pattern to explain the abnormal β2 fractions observed in our study population. Our results clearly showed that there is a relationship between the β2 fraction with an inflammatory pattern and CRP, a parameter frequently used to help in the diagnosis and therapeutic monitoring of inflammation (29, 30). In contrast, no relationship was found between serum CA 19.9 levels and CRP or the inflammatory pattern.

Conclusion

Serum CA 19.9 levels are not related to the intensity of the β2 fraction in protein electrophoresis. Rather, modifications in this

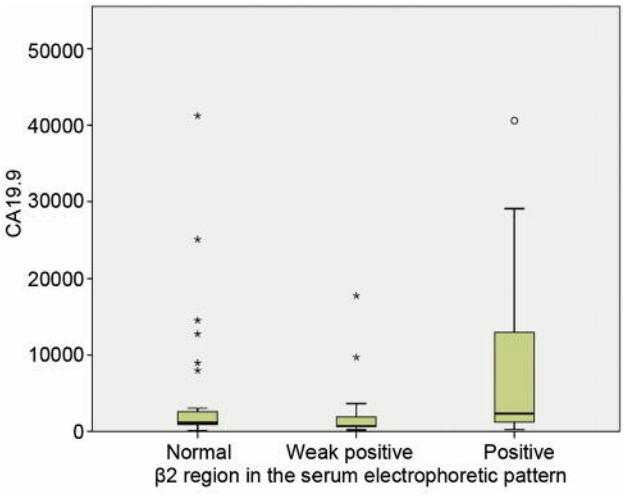

Figure 1. *Serum CA 19.9 levels subdivided according to the β2 intensity in the protein electrophoretic pattern.*

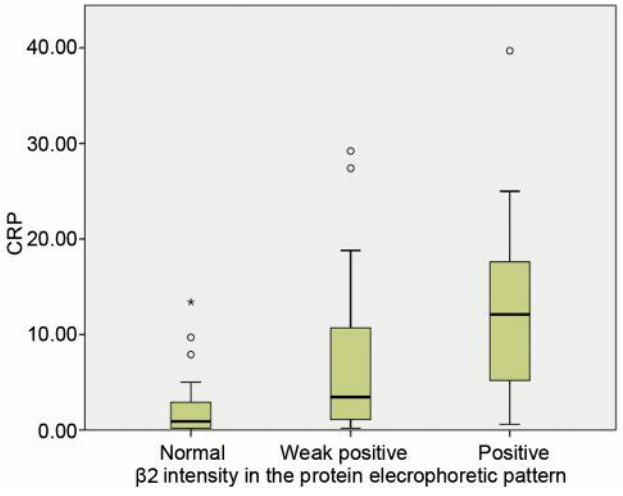

Figure 3. *Serum CRP levels according to β2 intensity in the serum electrophoretic pattern.*

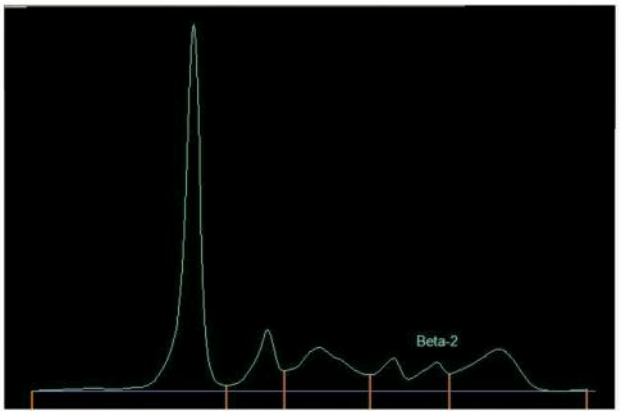

Figure 2. *Serum protein electrophoretic pattern in a patient with serum CA 19.9 level of 977,700 U/ml.*

area in patients with cancer or other diseases with abnormal serum CA 19.9 levels appear to be related to inflammation.

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