Tumour Markers in the Differential Diagnosis of Patients With Isolated Involuntary Weight Loss

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Abstract. *Background/Aim:* Paraneoplastic syndrome symptoms include isolated involuntary weight loss (IIWL). The differential diagnosis of cancer from other diseases may require a significant number of tests. Tumour markers (TMs) can be used for the diagnosis and stratification of patients according to cancer risk. *Patients and Methods:* This study included 606 patients (48% females) seen at the rapid diagnostic unit for IIWL. We determined the levels of TMs carcinoembryonic antigen, carbohydrate antigen 19-9, soluble fragments of cytokeratin 19, carbohydrate antigen 15-3, carbohydrate antigen 125, neuron specific enolase, alpha-fetoprotein, prostatic specific antigen using the multiparametric analyser COBAS 601. Two cut-off points were established, the upper reference limit described by the manufacturer and a high cut-off point suggested by Molina et al., to stratify patients according to cancer risk. *Results:* Patients were classified according to TM levels as follows: I) all TMs below the upper reference limit; II) highest number of TMs between the two cut-offs; III) at least one TM above the higher cut-off. The odds ratio for malignancy was

4.3 for group II and 248 for group III. These results indicate that when at least one TM is above the higher cut-off, neoplasia is highly probable. *Conclusion:* TM determination allowed to establish cancer risk in patients with IIWL.

Many cancer patients do not present specific symptoms or signs when they consult medical services (1, 2). These symptoms, including cachexia, weight loss, and muscle wasting arise from tumour secretion of hormones, peptides or cytokines (3, 4) or in case of autoimmune neurological disorders, from immune cross reactivity between malignant and normal host tissues (5, 6). Other disorders are due to tumour invasion or compression in surrounding or distant tissues.

In some cases, paraneoplastic syndromes are presented before cancer diagnosis and may be used to select patients for more exhaustive tests, in order to perform differential diagnosis with benign diseases.

In our country, specialised hospital units have been created to avoid unnecessary delays for patients with these symptoms. The rapid diagnostic unit (RDU) is an ambulatory resource for the study of severe pathologies without the need for hospital admission. It refers to patients with guide-symptoms derived from other hospital services or primary care. Some of the frequent signs that require referral to RDU are constitutional syndrome, wasting syndrome, or isolated involuntary weight loss (IIWL). Between 10 and 30% of patients who arrive at these units present involuntary weight loss as the only sign. In the RDU, they are being prioritized to undergo endoscopies, image tests, blood tests and biopsies in order to reach a diagnosis and start treatment when possible within one month (7-9).

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Key Words: Weight loss, tumour markers, differential diagnosis, cancer, CEA, CYFRA 21-1.

Figure 1. Flow chart.

As these signs or symptoms are not specific to cancer, in patients with constitutional syndrome, wasting syndrome or IIWL, a differential diagnosis must be made between different types of cancers such as lung cancer, lymphoma, renal cancer, and benign diseases such as tuberculosis, toxoplasmosis, pneumonia, and chronic intestinal infections (1).

TM measurement can aid diagnosis and serve as a complement to expensive imaging techniques and invasive testing (10-15). However, increased plasma levels of TMs may also be observed in patients without cancer due to a variety of processes, and thus give rise to false positives (16).

The aim of our study was to determine the diagnostic accuracy of TMs in the differential diagnosis of patients with IIWL and their ability to predict the risk of cancer.

Patients and Methods

This is an observational study including patients seen for IIWL at the RDU between January 2005 and December 2013. The follow-up of the last patient was completed in December 2014.

At the discretion of the attending physician, analytical parameters including TMs were obtained from patients with IIWL referred from primary or emergency care to the RDU. All patients presenting documented idiopathic IIWL of at least 5% over the past 12 months were included in the study. IWL was considered isolated when it was not accompanied by symptoms or signs specific of an organ or system. When weight loss was not documented, the criteria of Marton *et al.* were used (17), according to which patients were eligible if they met at least two of the following criteria: evidence of change in clothes size, confirmation of weight loss by a friend or relative, and ability to give a numerical estimate of weight loss. Exclusion criteria were as follows: (i) specific symptoms (jaundice, ascites, dysphagia for at least 6 months, diarrhoea for at least 6

months, rectorrhagia, serious rhythm deposition changes, rectal tenesmus and/or suspicious rectal examination, subocclusive crises, change in cough for at least one month, haemoptysis of unknown origin, dysphonia for at least one month, palpable breast mass, nipple discharge, macroscopic haematuria, significant increase in lymph nodes volume, (>1 cm) and malignant hepatomegaly); (ii) intentional weight loss; (iii) start of diuretic treatment three months before start of IWL; (iv) weight loss of <5% or no weight loss observed during first visit at RDU; (v) refusal to participate in follow-up assessments and/or undergo further complementary tests; (vi) non-compliance with study criteria for RDU; and (vii) death during the diagnostic process.

The RDU database was used to identify patients. Demographic data, clinical and follow-up history were obtained from patients' clinical records. The study protocol was approved on March 6, 2015 by an independent clinical research ethics committee (Comitè d'Ètica d'Investigació Clínica de la Fundació Unió Catalana d'Hospitals) (Ethics Committee number CEIC 15/16).

All TMs were measured on the day of the first visit: carcinoembryonic antigen (CEA), carbohydrate antigen CA19-9 (CA19-9), soluble fragments of cytokeratin 19 (CYFRA 21-1), carbohydrate antigen CA15-3 (CA15-3), carbohydrate antigen 125 (CA125), neuron specific enolase (NSE), alpha-fetoprotein (AFP), prostatic specific antigen (PSA) in a multiparametric analyser COBAS 601 by an electrochemiluminescent assay (Roche Diagnostics, Sant Cugat, Barcelona, Spain). Upper reference limit was that described by the manufacturer for CEA, CA19-9, CYFRA 21-1, CA15-3, CA125, NSE, AFP and PSA were 5 µg/l, 37 U/ml, 3.3 µg/l, 30 U/ml, 35 U/ml, 17 µg/l, 10 µg/l and 4 µg/l respectively. Samples in which NSE determinations had been requested were visually inspected and those that showed any orange coloration suspected of minimal haemolysis were excluded. When NSE values were above the upper reference limit, another visual verification of haemolysis was carried out, and in the case of minimum orange coloration the result was invalidated. The cut-off values described

by Molina *et al.* (18) were used for patients without hepatopathy and renal failure: 15 µg/l, 7.8 µg/l, 200 U/ml, 45 µg/l, 40 µg/l, 30 µg/l, and 100 U/ml for CEA, CYFRA 21-1, CA19-9, NSE, AFP, PSA, and CA15-3 respectively. Patients were classified into three groups, group I: those with all TMs below the reference limit; group II: those with at least one TM between the upper reference limit and below the cut-off proposed by Molina *et al.* (18); and group III: those with at least one TM above the cut-off proposed by Molina *et al.* (18). To obtain a definitive diagnosis, serological testing, and culture and image analysis were required. The diagnosis of cancer was established by histological study of biopsies obtained by gastroscopy, colonoscopy, bronchoscopy, laparoscopy, thoracoscopy or direct study of the tumour obtained by tumorectomy.

Statistical analysis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were calculated for each TM and for the combination of all TMs using the two cut-off points. The parameters of diagnostic accuracy are shown together with their 95% confidence interval (95%CI).

A two-sided 5% significance level was assumed. All statistical analyses were performed using IBM® SPSS® Statistics for Windows v.22 (IBM Corporation®, Armonk, NY, USA) and Stata® v.14 (StataCorp LP®, College Station, TX, USA).

Results

A total of 606 patients with IIWL were analysed, aged between 23 and 95 years, mean 69 years (SD 14.1), and 194 (48%) were women. Figure 1 shows the flow chart of the study. Table I shows the final diagnosis: 23.6% had cancer (42.7% digestive, 17.5% lung, and 11.9% non-epithelial), 30.9% psychiatric disorders (mainly depressive syndrome), 42.2% non-neoplastic organic disease and 3.3% idiopathic IIWL. Ninety-two cancer patients (64.3%) had metastatic disease and 37 (25.9%) had stage IIIA or IIIB. Table II shows tumour marker concentrations according to disease type in the final diagnosis.

Table III and Table IV show the sensitivity, specificity, PPV, NPV, PLR and NLR ratios for TM at two different cut-offs for CEA, CA125, CYFRA 21-1, CA15-3, CA19-9, NSE, AFP and PSA. Sensitivity and NPV were 91.6% and 95.7%, respectively (Table III) when the upper reference limit was used as cut-off. A higher specificity (98.3%) and PPV (92.0%) were obtained at the cut-off proposed by Molina *et al.* (18) (Table IV). Figure 2 shows tumour prevalence, stage (metastatic vs. no metastatic), epithelial vs. non-epithelial, type of benign disease, and odds ratio for cancer according to each group.

Discussion

Our study evaluated the performance of TMs to differentiate between neoplasia and benign disease in 606 patients with involuntary isolated weight loss as the only sign suggestive of cancer. Regarding diagnostic performance, using the high

Table I. Final diagnosis of patients with unintentional weight loss included in the study.

Aetiology	Percent	Age (years)	% Women
Neoplasia	143 (23.6%)	71.2±11.5	31.2
Epithelial	126 (20.8%)		
Lung cancer	25 (4.1%)		
Stomach	22 (3.6%)		
Colorectal	21 (3.5%)		
Pancreas	18 (3.0%)		
Cancer of unknown primary	11 (1.8%)		
Kidney and urinary tract	10 (1.7%)		
Liver and bile ducts	6 (1.0%)		
Prostate	3 (0.5%)		
Breast	3 (0.5%)		
Others	7 (1.2%)		
Non epithelial	17 (2.8%)		
Lymphoma	6 (1.0%)		
Multiple myeloma	5 (0.8%)		
Leukaemia	4 (0.7%)		
Leiomyosarcoma	2 (0.3%)		
Myelodysplasia syndrome	1 (0.2%)		
Organic no neoplastic	256 (42.2%)	69.3±14.7	47.9
Digestive system	100 (16.5%)		
Peptic disorders	35 (5.8%)		
Hiatus hernia	15 (2.8%)		
Chronic alcohol induced liver disease	13 (2.1%)		
Gallstones	7 (1.2%)		
Functional disorders	5 (0.8%)		
Barrett's esophagus	3 (0.5%)		
Chronic alcohol induced pancreatitis	3 (0.5%)		
Chronic Hepatitis C	2 (0.3%)		
Benign colon polyps	2 (0.3%)		
Other disorders	15 (2.8%)		
Pharmacological causes	37 (6.1%)		
Digoxin	8 (1.3%)		
Metformin	7 (1.2%)		
Opioids	5 (0.8%)		
Non-steroidal anti-inflammatories	4 (0.7%)		
Levothyroxine	4 (0.7%)		
Others	9 (1.5%)		
Infections	23 (3.8%)		
Rheumatism	20 (3.3%)		
Neurological disorders	20 (3.3%)		
Endocrine disorders	11 (1.8%)		
Lung disorders	10 (1.7%)		
Musculoskeletal disorders	10 (1.7%)		
Heart disorders	8 (1.3%)		
Psychosocial circumstances	7 (1.2%)		
Active alcoholism	3 (0.5%)		
Others	7 (1.2%)		
Psychiatric disorders	187 (30.9%)	67.6±15.8	64.5
Depression	16 (26.7%)		
Anxiety	15 (2.5%)		
Eating disorders	3 (0.5%)		
Others	7 (1.2%)		
Unknown origin	20 (3.3%)	62.1±20.5	45.0

Table II. Serum concentrations of tumours markers according to final diagnosis.

		Neoplasia			Benign diseases		
		All (n=143)	Neoplasia epithelial (n=126)	Neoplasia non-epithelial (n=17)	Organic no neoplastic (n=256)	Psychiatric (n=187)	Unknown origin (n=20)
CEA (µg/l)	Mean (SD)	94.6 (355.6)	107 (377)	2.1 (1.1)	3.1 (2.7)	2.7 (2.2)	2.1 (1.95)
	Median (IQR)	5 (20.9) ^a	6.6 (30.0) ^{b,c}	1.8 (2.0)	2.2(2.4)	2.2 (1.7)	1.9 (1.1)
CYFRA21-1 (µg/l)	Mean (SD)	14.2 (26.0)	16.0 (27.4)	1.7 (0.8)	2.3 (2.3)	1.8 (1.1)	1.8 (0.9)
	Median (IQR)	4.2 (9.9) ^a	5.5 (14.7) ^{b,c}	1.6 (0.9)	1.7 (1.4)	1.1 (1.1)	1.5 (1.3)
CA 19-9 (KU/l)	Mean (SD)	2,286 (14,497)	2,622 (15,511)	15.3 (17.4)	19.9 (25.8)	14.7 (20.0)	12.4 (2.8)
	Median (IQR)	33.5 (160) ^a	53 (214) ^{b,c}	7.0 (21.0)	11.0 (16.0)	10.0 (10.0)	8.0 (12.0)
CA 15-3 (KU/l)	Mean (SD)	50.4 (106)	53.9 (113.1)	26.9 (15.4)	21.0 (11.1)	19.2 (9.4)	18.6 (7.6)
	Median (IQR)	24.0 (21.5) ^a	25.4 (20.9) ^b	20.5 (48.9)	19.1 (12.1)	17.2 (11.7)	18.7 (9.9)
CA 125 (KU/l)	Mean (SD)	155 (649.1)	172 (695)	41.0 (50.3)	24.0 (33.9)	16.4 (14.8)	12.8 (7.7)
	Median (IQR)	24.5 (48.1) ^a	25.5 (57.1) ^b	20.2 (40.9) ^e	14.8 (17.9) ^{d,f}	12.2 (9.5)	9.7 (8.9)
AFP (µg/l)	Mean (SD)	921 (7310)	1,062 (7847)	2.2 (2.0)	3.1 (9.3)	2.6 (2.0)	2.3 (1.3)
	Median (IQR)	2.0 (2.0)	2.1 (2.1)	1.7 (1.0)	2.1 (1.9)	2.1 (1.7)	2.1 (2.2)
NSE (µg/l)	Mean (SD)	32.0 (76.1)	34.6 (83.6)	19.9 (17.6)	11.1 (3.8)	11.0 (4.3)	10.3 (1.6)
	Median (IQR)	12.8 (15.3) ^a	12.8 (16.1) ^b	13.5 (14.3) ^e	10.5 (3.5)	10.1 (4.2)	10.4 (2.5)
PSA (µg/l)	Mean (SD)	29.7 (170.5)	33.1 (180)	1.2 (0.9)	2.6 (3.4)	2.2 (3.0)	1.8 (1.6)
	Median (IQR)	1.4 (2.3)	1.4 (2.6)	1.5 (1.7)	1.3 (2.7)	1.2 (1.9)	1.4 (1.9)

^aSignificant difference ($p<0.02$) between neoplasia and benign disease; ^bSignificant difference ($p<0.05$) between epithelial neoplasia and benign disease; ^cSignificant difference ($p<0.001$) between neoplasia epithelial and no epithelial; ^dSignificant difference ($p<0.05$) between organic no neoplastic and psychiatric; ^eSignificant difference ($p<0.03$) between neoplasia non-epithelial and benign disease; ^fSignificant difference ($p<0.021$) between organic no neoplastic and unknown origin.

Table III. Diagnostic accuracy of tumour markers based on the cut-off of upper reference interval.

Tumour markers	Sensitivity (95%CI)	Specificity (95%CI)	NPV (95%CI)	PPV (95%CI)	NLR (95%CI)	PLR (95%CI)	Accuracy (95%CI)
CEA (5 µg/l) (n=600)	50.7% (42.3-59.1)	86.5% (82.9-89.4)	85.0% (81.4-88.1)	53.7% (44.9-62.3)	0.6 (0.5-0.7)	3.7 (2.8-5.0)	78.0% (74.4-81.2)
CYFRA 21-1 (3.3 µg/l) (n=567)	55.0% (46.0-63.6)	87.4% (83.8-90.2)	86.6% (83.0-89.5)	56.7% (46.0-63.5)	0.5 (0.4-0.6)	4.4 (3.3-5.8)	79.9% (76.3-83.1)
CA19-9 (37 U/ml) (n=572)	46.9% (38.3-55.8)	90.2% (87.0-92.8)	85.0% (81.4-88.1)	59.0% (49.0-68.4)	0.59 (0.5-0.7)	4.81 (3.4-6.7)	80.2% (76.6-83.5)
NSE (17 µg/l) (n=404)	29.2% (20.6-39.5)	96.7% (93.8-98.3)	73.7% (56.6-86.0)	81.1% (76.6-84.9)	0.73 (0.6-0.8)	8.78 (4.4-17.4)	80.3% (76.0-84.1)
CA15-3 (30 U/ml) (n=560)	38.3% (30.0-47.3)	89.3% (85.9-92.0)	77.4% (73.6-80.8)	52.1% (41.6-62.4)	0.7 (0.6-0.8)	3.6 (2.5-5.1)	77.4% (73.6-80.8)
CA125 (35 U/ml) (n=549)	43.2% (34.7-52.1)	83.0% (79.1-86.3)	85.7% (82.0-88.8)	43.2% (34.7-52.1)	0.62 (0.5-0.7)	2.85 (2.2-3.8)	75.7% (71.8-79.1)
AFP (10 µg/l) (n=547)	6.7% (3.2-13.1)	99.1% (97.4-99.7)	79.1% (75.3-82.4)	66.7% (35.4-82.4)	0.94 (0.90-0.99)	7.12 (2.2-23.2)	78.8% (75.1-82.1)
PSA (4 µg/l) (n=254)	14.6% (8.1-24.6)	82.6% (75.9-87.7)	67.0% (60.1-73.2)	28.6% (16.2-44.8)	1.03 (0.9-1.1)	0.84 (0.45-1.55)	60.6% (54.3-66.6)
At least one TM>cut-off (n=606)	91.6% (85.5-95.4)	55.9% (51.3-60.5)	95.7% (92.2-97.6)	39.1% (33.9-44.6)	0.15 (0.09-0.26)	2.1 (1.9-2.3)	64.4% (60.4-68.2)

95%CI: 95% Confidence interval; NPV: negative predictive value; PPV: positive predictive value; NLR: negative likelihood ratio; PLR: positive likelihood ratio.

cut-off point, a sensitivity of 64.3%, a specificity of 98.3%, a PPV of 92%, and a PNV of 89.9% were obtained. In order to assess the cancer risk, we used two cut-off points (upper

reference limit and the high cut-off point) which allowed us to classify patients into three groups with low, moderate (OR=4.3) and very high (OR=245) cancer risk.

Table IV. Diagnostic accuracy of tumour markers based on the high cut-off proposed by Molina *et al.* (18).

Tumour markers	Sensitivity (95%CI)	Specificity (95%CI)	NPV (95%CI)	PPV (95%CI)	NLR (95%CI)	PLR (95%CI)	Accuracy (95%CI)
CEA (15 µg/l) (n=600)	31.0% (23.6-39.4)	99.6% (98.3-99.9)	82.3% (78.8-85.4)	95.7% (83.4-99.2)	0.69 (0.62-0.77)	71.1 (17.5-289.7)	83.3% (80.0-86.2)
CYFRA 21-1 (7.5 µg/l) (n=567)	38.9% (30.7-47.9)	99.1% (97.5-99.7)	84.4% (80.9-87.3)	92.7% (81.6-97.6)	0.62 (0.54-0.71)	42.4 (15.6-115.2)	85.2% (81.9-88.0)
CA19-9 (200 U/ml) (n=572)	22.7% (16.1-31.0)	100% (98.9-100)	81.2% (77.6-84.3)	100% (85.9-100)	0.77 (0.70-0.85)	>9,999	82.1% (78.7-85.1)
NSE (45 µg/l) (n=404)	14.6% (8.5-23.6)	100% (98.4-100)	78.6% (74.1-82.5)	100% (98.4-100)	0.85 (0.79-0.93)	>9,999	79.4% (75.0-83.2)
CA125 (350 U/ml) (n=549)	7.0% (3.5-13.3)	100% (98.9-100)	77.9% (74.1-81.3)	100% (62.3-100)	0.93 (0.89-0.98)	>9,999	78.6% (75.0-81.9)
CA15-3 (100 U/ml) (n=560)	6.1% (2.85-12.0)	99.8% (98.5-99.9)	77.5% (73.7-80.9)	88.9% (50.7-99.4)	0.94 (0.9-0.98)	25.9 (3.3-205.5)	77.7% (74.0-81.0)
AFP (40 µg/l) (n=547)	5.0% (2.0-11.0)	99.7% (97.9-99.9)	72.5% (67.9-76.7)	85.7% (42.0-99.2)	0.95 (0.91-0.99)	15.1 (1.8-124.1)	79.0% (75.3-82.3)
PSA (30 µg/l) (n=254)	3.7% (1.0-11.1)	100% (97.3-100)	68.5% (62.5-74.3)	100% (31.0-100)	0.96 (0.92-1.0)	>9,999	69.0% (62.9-74.6)
At least one TM>cut-off (n=606)	64.3% (55.8-72.0)	98.3% (96.5-99.2)	89.9% (86.9-92.4)	92.0% (84.4-96.3)	0.36 (0.29-0.45)	37.3 (18.6-75.0)	90.3% (87.6-92.4)

95%CI: 95% Confidence interval; NPV: negative predictive value; PPV: positive predictive value; NLR: negative likelihood ratio; PLR: positive likelihood ratio.

*Only Epithelial tumors

Figure 2. Distribution of type of disease according to the group of tumour markers.

Although some investigators advocate the measurement of TMs for cancer diagnosis, it is not currently included in the guidelines produced by scientific societies. Kilpatrick (19) reported falsely elevated results of certain markers in patients with cirrhosis and ascites, a situation that required the performance of other tests to rule out the suspicion of cancer. These authors also warned that results within the reference limit give a false sense of security, dispelling the suspicion of cancer and delaying the diagnosis. Sturgeon (20) also argued against the use of TM panels because of the limited benefit they provide in individual patients. In recent years, however, some studies have shown that in some types of cancer, a test for a combination of TMs can improve individual assessment. In 2016, Molina (21) created a panel of six TMs in patients with suspected lung cancer, which had a higher diagnostic accuracy than the same TMs considered individually. This panel was able to distinguish small cell lung cancer from non-small cell lung cancer based on the NSE and ProGRP markers. Blanco-Prieto (22) using two conventional TMs and other biological markers (CEA, CYFRA 21-1, sCD26, calprotectin, MMP1, MMP7 and MMP9 EGF) improved the clinical model, obtaining a sensitivity of 95% and a specificity of 43.6%.

In 2015, using three TMs (CEA, CYFRA 21-1, and CA19-9), our group (23) studied 234 patients with symptoms and signs of cancer using two cut-off points, one for patients without liver and kidney involvement and a higher one for those who had either renal involvement (creatinine >1.5 mg/dl) and/or liver involvement (bilirubin >1.5 mg/dl), obtaining a sensitivity of 54% and a specificity of 100%.

Molina *et al.* 2012 (18) evaluated the diagnostic capacity of 10 TMs (CEA, CYFRA 21-1, CA15-3, CA19-9, CA72-4, CA125, NSE, SCC, AFP and PSA) in the differential diagnosis between benign and malignant disease, in 2,711 patients with signs or symptoms of cancer. These authors applied two cut-off points: one above the upper reference limit (the one used in the present study) to increase the specificity, and another for patients with clinicopathological alterations that raised TM concentration well above the upper reference limit, such as renal failure, jaundice, effusions and dermatological diseases, and obtained a sensitivity of 67% and a specificity of 98%. In 2021, the same group (24) added ProGRP to the panel and with a higher case volume (4,776 patients) obtained a sensitivity of 72.2% and a specificity of 98%.

The present study focused on outpatients with IIWL as the only sign of cancer. The same cut-off point as in studies of Molina (2012) and Bosch (2021) (24) was used because none of the patients had serous effusions, dermatological disease, kidney failure or jaundice. The sensitivity was similar to that obtained in previous studies; the higher the number of TMs, the greater the sensitivity, with a specificity around 98%.

The presence of IIWL may raise the suspicion of cancer, but it is by no means definitive. Some authors have searched

for predictive models of cancer risk on the basis of laboratory tests. When only biochemical and hematologic parameters are used, the capacity for predicting cancer is moderate. Baicus *et al.* (25) found ORs for age>60 years, albumin>3.5 g/dl and alkaline phosphatase <104 U/l of 5.1 (1.88-15.02), 2.44 (1.14-5.23), and 2.67 (1.23-5.8) respectively, Casarrubias-Ramírez *et al.* (26) found ORs of 3.9 (1.7-8.9) for albumin>3.5 g/dl, and of 4.7 (1.3-17.5) for alkaline phosphatase>300U/l. The use of TMs has obtained better classifications: Torné-Cachot (27) found TMs to be the only factors that maintained their power for predicting cancer in a multivariate analysis, with ORs of 2.38 (95%CI=1.17-4.8) for a one TM above cut-off and of 6.51 (95%CI=2.62-16.13) for two TMs above cut-off. In our study, we found an OR of 4.3 (95%CI=2.2-8.4) for at least one marker between the upper reference limit and the high cut-off, and one of 245 (95%CI=98-626) for patients with at least one marker above the high cut-off. Considering our classification into three groups, the rates of tumors were 4.4% (n=12) in the low-risk group, 16.6% (n=39) in the moderate-risk group and 92% (n=92) in the high-risk group. In group I the NPV was higher than 95%, whereas in group III the PPV was 92%, which allows an accurate evaluation of the cancer risk and the selection of the most appropriate additional tests in order to obtain a prompt diagnosis.

Although the markers were determined before obtaining the final diagnosis, the main limitation of this study was that it was performed at a single centre. The results should now be validated in multicentre studies involving laboratory medicine specialists and clinicians and using feasible end points, such as less invasive and less expensive diagnostic procedures.

Conclusion

The determination of TMs for the classification of patients with IIWL according to cancer risk allows for a better selection of complementary tests to obtain the final diagnosis.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Designed research/study: JT, JA, JO, AS-J, DR. Performed research: JT, JA, JO, MV. Collected data: MB, AAb, MS, CF, CG-F, MV, LM, OE-B, RO, EG-G. Analyzed data: Aar. Wrote the paper: JT, JA.

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Received July 15, 2021

Revised August 7, 2021

Accepted August 11, 2021