The prognostic role of baseline CEA and CA 19-9 values and their time-dependent variations in advanced colorectal cancer patients submitted to first-line therapy.

THE PROGNOSTIC ROLE OF BASELINE CEA AND CA 19-9 VALUES AND THEIR TIME-DEPENDENT VARIATIONS IN ADVANCED COLORECTAL CANCER PATIENTS SUBMITTED TO FIRST-LINE THERAPY

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INTRODUCTION

Colorectal cancer is a frequent and lethal disease globally representing the third most commonly diagnosed cancer in males and the second in females[1]. Multidisciplinary management of metastatic patients has been demonstrated to prolong overall survival; thus, while there is a general consensus to administer aggressive regimens when tumor reduction could lead an unresectable metastasis to a possible resection, there is not unanimity on the management of patients with advanced disease not suitable for surgery. In fact, in this setting of patients, anti-cancer drugs may be administered singly or may be delayed in a “continuum of care” in patients with a slow-growing tumor to maximize

outcome. In this sense, an easy available, low-cost predictive and prognostic tool could be useful to rationalize clinical resources.

CEA is a complex glycoprotein produced by 90% of colorectal cancers. It can be measured in serum quantitatively, and its level in plasma can be useful as a marker of disease[2]. CEA has been extensively studied in metastatic colorectal cancer patients since the '70s and a considerable amount of literature has been published. In summary, CEA has been demonstrated to predict response to chemotherapy and overall survival[3,4,5]. All these studies, however, have a series of limitations: they enrolled a relatively low number of patients; the results of those studies reporting data from clinical trials comparing different chemotherapies can be hardly referred to the daily oncological routine as marker evaluation is a secondary aim and enrolled patients are selected by strict including and excluding criteria; patients with CEA levels below the upper normality threshold have never been studied as marker levels were considered as false negative. Finally, clinical response to chemotherapy is determined by the radiological workout normally performed at 3 and at 6 months after chemotherapy onset. Some authors described a solid correlation between marker variations and radiological tumor response[6]; considering that responding patients are those with a better prognosis, it is questionable whether a marker which simply reflects tumor burden could add something to the clinical management of these patients.

Carbohydrate antigen 19-9 (CA 19-9) is a Lewis blood group oligosaccharide that is generated by exocrine epithelial cells. It has been extensively studied in pancreatic cancer patients and in those suffering from gastrointestinal neoplasm. In colorectal cancer patients its role has been questioned as it presents a lower sensitivity than CEA[7].

We then explored and compared the predictive and prognostic role of baseline CEA and CA 19-9 levels and their variation during first-line chemotherapy in a large database of patients with advanced colorectal disease observed from the time of first metastasis appearance. In particular we

described whether CEA and CA 19-9 levels simply parallel tumor burden variations or were independent marker of prognosis, helping oncologist to discriminate within responding patients those destined to have a worse prognosis, and within progressing patients those with a more favorable disease.

PATIENTS AND METHODS

Patients and study design

Clinical databases of two Italian Institutes (University of Torino, Oncology Unit, San Luigi Gonzaga Hospital [center 1]; and University of Eastern Piedmont, Maggiore della Carità Hospital, Novara [center 2]) were investigated. Records related to those patients with metastatic colorectal cancer disease treated from 1998 up to the end of 2011 were extracted and constitute the basis of this study. Patients characteristics and characteristics of the primary tumor, disease free-interval, site of recurrence, type of chemotherapy, response to therapy, progression free-survival (PFS) and overall survival (OS) together with CEA and CA 19-9 determinations before, after 3 and 6 months of first-line chemotherapy were put into a database generated for the purpose of this study. We analyzed the predictive and prognostic role of CEA and CA 19-9 and their variations during first-line therapy in patients in whom basal and at least one measurement at 3 or at 6 months was present in the database. Finally, multivariate analysis in which CEA and CA 19-9 were considered as time-dependent covariates was performed including only patients with marker values available for the three time points (see CONSORT Diagram).

CEA and Ca 19-9 measurement.

For each patient, CEA and Ca 19-9 were measured locally by mean of an automatic chemiluminescent microparticle immunoassay (CMIA). The upper limits of the reference range for

CEA and CA 19-9 were defined—according to the manufacturer's instructions— with 5 ng/ml and 37 U/mL, respectively.

Four subgroups of patients for each marker were identified: group 0 (G0) including patients with marker levels always beneath the upper limit of normality for the entire observation period; group 1 (G1) including patients with >50% reduction of marker levels or marker positive that became negative during chemotherapy; group 2 (G2) patients with no significant variations of marker values; group 3 (G3) patients with >50% increase of marker levels or marker negative that became positive during chemotherapy. Patients with marker decrease >50% at 3 months that increased >50% or remained stable at 6 months were included in G1; patients with marker increase >50% at 3 months that decreased >50% from baseline at 6 months were included in G1; finally, patients with marker increase >50% at 3 months that remained stable at 6 months were included in G3.

Outcome evaluation

Response evaluation was performed under the standard assessment criteria used at each institution for the considered timeframe. Up to 2001, treatment response was classified according to International Union Against Cancer (UICC) criteria[8], wherein complete response was defined as the complete disappearance of all detectable malignant disease, partial response as a decrease >50% in the sum of the products of the two longest perpendicular diameters of all measurable lesions, and progressive disease as an increase of at least 25% in the size of measurable lesions and the development of new lesions. After 2001, centers were invited to classify responses according to the RECIST criteria[9], wherein response was defined as a decrease >30% in the sum of the longest diameters of target lesions, and a progressive disease as an increase >20% of this sum. Only the best tumor response was recorded. The two methods have been demonstrated to be comparable as agreement between the unidimensional and bidimensional criteria was generally found to be good and acceptable[9].

PFS and OS were estimated from first-line treatment onset till progression or death from any cause or date of the last follow-up. The cut-off date for the collection of data was December 31st, 2011. Patients not progressing or alive or lost to follow-up at the time of the cut-off date were censored at the time of the last follow-up examination.

Statistical analyses

The predefined end point of this study was to show a correlation of baseline CEA and CA 19-9, and their kinetics during first-line therapy with clinical response, PFS and OS. The predictive role of baseline marker values and their variations during therapy to identify patients responding to first-line chemotherapy was explored using the chi-square test with Yates correction, when necessary. Differences between groups of non parametric unpaired variables were validated by the Mann-Whitney U test when comparing two groups or the Kruskal-Wallis Analysis of Variance when analyzing multiple groups. Correlations between marker values (baseline and their variations) and PFS or OS were represented by survival curves plotted according to the Kaplan-Meier method and validated using the log-rank test. Multivariate survival analysis was performed according to the Cox proportional-hazards model with backward elimination. CEA, CA 19-9 and age were considered as continuous variable. Categorical variables were managed as follows: values of dichotomous variables were 1 and 0. When a cut-off limit was applied, 1 represented those values above the limit. As far as gender is concerned, male was coded as 1 and female as 0. Ordinal variables, such as stage of the primitive or Performance Status, were ranked according to their prognostic role with lower value assigned to the best option (i.e. stage A=1, stage D=4). The effect of the serially measured CEA and CA 19-9 values (CEA and CA 19-9 kinetics) was also modeled by using the Cox model where the logarithmic transformation of CEA and CA 19-9 values were treated as a time-varying covariate with a constant value between two measurements, according to the method proposed by Boek et al[10]. For the multivariate analysis of CEA and CA 19-9 kinetics, the three

marker determinations during chemotherapy had to be available. All statistical computations were performed using SPSS for Windows Ver 16.0 and STATISTICA for Windows Ver. 6.0 software.

RESULTS

We extracted data regarding 937 patients with metastatic colorectal cancer, of whom 892, 644 from Center 1 and 248 from Center 2, met the eligibility criteria and entered the study (See CONSORT Diagram). Their characteristics are depicted on Table 1.

CEA and CA 19-9 measurements

Data on CEA were recorded in 888 patients at baseline, in 790 at 3 months, and in 755 at 6 months. The corresponding figures for CA 19-9 were: 883, 786, and 748, respectively. Median marker values (range) at baseline, after 3 months, and after 6 months of front-line therapy were: 15.6 ng/ml (0.1-32,380), 8.5 ng/ml (0.4-50,000), 8 ng/ml (0.1-68,076) for CEA; and 29 U/ml (0.1-56,000), 19.9 U/ml (0.1-42,817), 20 U/ml (0.1-63,630) for CA 19-9, respectively (Table 1). At baseline, 602/888 patients presented with CEA values greater than 5 ng/ml, whereas we recorded in the same patient setting circulating levels of CA 19-9 above 37 U/ml in 405/883 subjects. Thus, marker sensitivities resulted to be 67.8% and 45.9%, respectively. Forty-five patients were CEA negative and CA 19-9 positive, with a combined sensitivity of 73.6% (647 positive patients out of 879 patients in whom the two markers were both determined).

Basal and at least one CEA and CA 19-9 determination at 3 or 6 months were available for 824 and 816 patients, respectively. According to study design, patients were grouped as summarized on Table 2. Median marker variations (lower - upper quartile) at 3 and 6 months were: -22.8% (-70.4% - 48.9%) and 0% (-70.4% - 100.0%) for CEA and -7.4% (-65.9% - 32.6%) and 0% (-62.6% - 93.3%) for CA 19-9, respectively.

Markers and tumor response to chemotherapy

Data on tumor response after first-line chemotherapy were available in 840 patients. Clinical response was evident in 312 patients (37.2%), whereas 318 (37.8%) obtained a stabilization of the disease and 210 (25.0%) progressed. Figure 1 summarizes marker variations according to clinical response. A good concordance between marker reduction (G1) and response to chemotherapy was more evident for CEA (50.2%) than for CA 19-9 (34.4%). The corresponding figures between G3 and marker increase were 46,4% and 35,3% for CEA and CA 19-9, respectively. On the other hand, 9,8% and 5,3% of those patients who obtained a clinical response had an increase in CEA and CA 19-9 levels (G3), whereas in 14,6% and 9,4% of those who progressed to chemotherapy we recorded a decrease of CEA and CA 19-9 levels (G3), respectively. If we considered the 45 patients with normal CEA and abnormal CA 19-9 values at baseline, in 12 out of 16 (75%) who responded to therapy a marker decrease was recorded, whereas we found the opposite concordance in 6/10 patients (60%) who progressed. Finally, CEA or CA 19-9 levels remained beneath the upper normality limit in a not negligible proportion of patients, with a higher proportion in those patients who obtained a disease response (26,9% for CEA and 52% for CA 19-9) than in those who progressed (13% and 32,1%, respectively. $p<0.0001$ for both markers).

Markers and survivals

At the time of data computation 730 (81.8%) patients had progressed. Median follow-up time (range) for those not progressing was 9 (0.7-173) months. A longer median PFS was recorded in those patients who presented normal CEA levels at baseline (15.1 vs 10.5 months; $p<0.0001$). A similar pattern was evident for those patients with CA 19-9 levels below the normality threshold at baseline (13.6 vs 10.2 months; $p<0.0001$). Median progression free-survivals for G0, G1, G2, and G3 were: 16.1, 12.1, 9.2, and 7.1 months for CEA ($p<0.0001$); 13.8, 11.9, 8.7, and 5.4 months for Ca 19-9 ($p<0.0001$), respectively. In the 45 patients with low CEA and high CA 19-9 at baseline, median PFS was 11.4 months.

At 31st December 2011, 610 patients (68.4%) had died. Median follow-up time (range) for the survivors was 19.6 (0.7-200.4) months. Survival curves are plotted on Figure 2. Patients with CEA levels beneath the normality limit at baseline survived longer than those with abnormal levels (32 vs 22.3 months; $p<0.0001$). A similar figure was evident when analyzing those patients with normal vs those with abnormal CA 19-9 levels at baseline (30.5 vs 20.1 months; $p<0.0001$).

Survival curves for each group are plotted on Figure 3 (CEA) and 4 (CA 19-9). Median survival times for G0, G1, G2, and G3 were: 36.3, 25.5, 17.8, and 17.0 months for CEA ($p<0.0001$); 31.7, 23.9, 18.6, and 13.8 months for CA 19-9 ($p<0.0001$), respectively. In the 45 patients with low CEA and high CA 19-9 at baseline, median OS was 23.2 months.

Multivariate analyses

Multivariate Cox analyses on the impact on TTP and OS of baseline CEA and CA 19-9 values and their variation during first-line chemotherapy are summarized on Table 2.

Stage of the primary, baseline haemoglobin levels >12 g/dl, performance status, and marker variation as ordinal variable (groups) confirmed their independent role on TTP. Markers as continuous variables, age, grading, and the number of metastatic sites failed to enter the model.

Variables that demonstrated an independent impact on OS were: number of metastatic sites, baseline haemoglobin levels >12 g/dl, Performance status, baseline CA 19-9 values >37 U/ml, clinical response to first-line chemotherapy, CEA variation as ordinal variable (groups), and liver or lung surgery. Marker as continuous variable, age, and grading failed to demonstrate a prognostic role.

When marker values were analyzed as time-dependent covariates, CEA and CA 19-9 confirmed their independent impact on TTP and OS.

DISCUSSION

In our study we retrospectively collected data from patients prospectively followed, demonstrating that serum values of CEA and CA 19-9 may give prognostic indications independently from other

well established prognostic factors. Thus, they can be considered as easy-to-obtain, low-cost, dynamic clinical tools that may help clinicians to discriminate patients with aggressive tumors. Several new biomolecular markers have been proposed: EGFR gene copy number, NRAS, PIK3CA mutations, loss of PTEN expression, the presence of Insulin-like growth factor 2 messenger RNA binding protein 3 in cancer cells[11-14] The determination of these factors is costly and some of them are experimental and then reserved to specialized centers. Moreover, all these markers are not time-dependent, and thus they could not reflect a change in tumor biology over time.

The findings of our study confirmed the recommendation of the guidelines as a good correlation between marker change and response to therapy have been documented both for CEA and CA 19-9 (Figure 1). In addition, we showed that patients with higher values of the markers at the time of first metastasis appearance had a shorter survival (Figure 2), as elsewhere described in a selected and limited subgroup of patients[15]. We observed patients with discordancy between marker variation and response to therapy (marker increase in case of response and marker decrease in case of tumor progression) and patients with marker values always beneath the upper normality threshold for the entire observation period (see Figure 1). Patients with marker increase during first-line treatment were those who had a shorter progression-free and overall survival independently from tumor response. When considering only responding patients, in fact, those who reported a CEA or CA 19-9 increase were those with a shorter PFS and OS. On the other hand, within progressing patients, those with marker stabilization or decrease were those with a better PFS and OS (data not shown). Moreover, patients with CEA or CA 19-9 values always negative had the longest PFS and OS (Figure 3 and 4). These groups have never been described before as patients with marker levels always beneath the upper normality threshold have always been excluded as false negative. These patients had the longest PFS and OS. A possible explanation for this observation may be the relative lower biological aggression of tumor cells which may lead to a lower marker production and a slower tumor growth. These data were also confirmed by multivariate analyses in which marker values together with stage of the primitive, PS and haemoglobin levels at the time of chemotherapy

onset were identified as independent prognostic factors. Curiously, both marker kinetics expressed as ordinal variables (groups) and not their basal values were independent factors for TTP, reflecting the predictive role of these markers in indicating those tumors that were responding to chemotherapy. As far as OS is concerned, only CEA and not CA 19-9 kinetic entered the statistical model. This could be explained by the low CA 19-9 sensitivity. In fact, only basal CA 19-9 levels above the upper limit of normality resulted to be a prognostic factors, probably indicating that those patients had a more aggressive and/or a bulky disease at diagnosis. We further analysed data according to the model proposed by Boek and coll. In their statistical model, CEA and CA 19-9 values were entered as time-varying covariates and thus analysing them as continuous variables and not as categorized variables, which is a widely used but criticizable approach[16]. We performed this more accurate statistical analyses in order to confirm the results in terms of prognostic role of CEA and CA 19-9 as categorization of marker variation during time permits an easier application in the practical daily routine. To summarize, CEA and CA 19-9 determinations demonstrated to provide basal prognostic information contributing with the above mentioned biochemical markers in helping clinicians to discriminate aggressive tumors. Differently from those markers, CEA and CA 19-9 showed to be time-dependent indicators, probably reflecting not only tumor load, but also biological variations of cancer cells.

Our findings computed from a large database drawn from two different institutions are in complete accordance to what reported by Strimpakos and coll. in an analysis from a single institution which focused primarily to the prognostic role of CEA flare and its kinetic in the same patient setting[17]. Authors affirmed that it might be possible that higher serum CEA levels promote disease progression and metastasis rather than simply reflect a tumour burden increase. We agree with this fascinating hypothesis. However, in our study we observed the same independent prognostic role for CA 19-9, a glycoprotein expressed in several neoplastic disease especially gastrointestinal[18,19], and in inflammatory processes such as Hashimoto thyroiditis or viral hepatitis[20,21]. To our knowledge, CA 19-9 has never been associated to cell adhesion and/or tumor growth and

metastatization. Thus, as reported above, we may hypothesize that beside other possible biological properties of the markers, CEA or CA 19-9 levels might simply reflect either the tumor burden and the metabolic activity of cancer cells, as we already shown for CA 15-3 in advanced breast cancer patients[22].

We did not find any difference in the prognostic or predictive information of a marker rather than the other. CA 19-9 sensitivity at baseline was lower than CEA and a higher number of patients had CA 19-9 values always beneath the upper limit threshold. The combination of the two markers increased sensitivity from 63,8% to 73,6%. We believe that this 5,8% increase do not worth the costs of dosing the two markers in this patient setting, confirming guideline recommendations. Thus, our data prompt us to recommend to follow patients with serial dosages of CEA and not of CA 19-9. Evaluation of a possible role of basal CA 19-9 dosage in all patients and CA 19-9 serial determinations in those few patients in whom baseline CEA is negative and CA19-9 is positive could be proposed.

The main limitations of this investigation arise from its retrospective nature. However, data were extracted from clinical database of two institution in which patients were followed prospectively. Moreover, in our study we did not included only patients enrolled in experimental clinical trials with strict inclusion and exclusion criteria, better reflecting the daily routine. In fact, response rate to chemotherapy resulted to be 37.2%, lower than those normally reported for phase II or III studies which included selected patients. Thus, our finding could be generalized and applied even outside experimental trials.

In conclusion, baseline and serial evaluations of CEA and CA 19-9 levels in patients with advanced colorectal cancer submitted to first-line chemotherapy permitted to obtain prognostic information independently from known prognostic factors. CA 19-9 did not add any supplementary information and then its dosage is not recommended. A possible role of basal CA 19-9 in all patients and its serial determination in those patients with CEA levels below the upper normality limit at baseline could worth further evaluation.

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Figure 1. Correlation between marker variation (Groups) and response to first-line chemotherapy (columns). Numbers in the boxes are the percentages of each Group in the respective response group (column).

Figure 2. Overall Survival for patients stratified according to marker levels. Differences in OS between higher and lower baseline CEA or Ca 19-9 were both statistically significant ($p<0.0001$)

Figure 3. Overall survival of patients stratified according to CEA variation during first-line chemotherapy. Groups were defined as G0 (always negative); G1 (decrease); G2 (stable); and G3 (increase).

Figure 4. Overall survival of patients stratified according to CA 19-9 variation during first-line chemotherapy. Groups were defined as G0 (always negative); G1 (decrease); G2 (stable); and G3 (increase).

CONSORT Diagram of the Study.

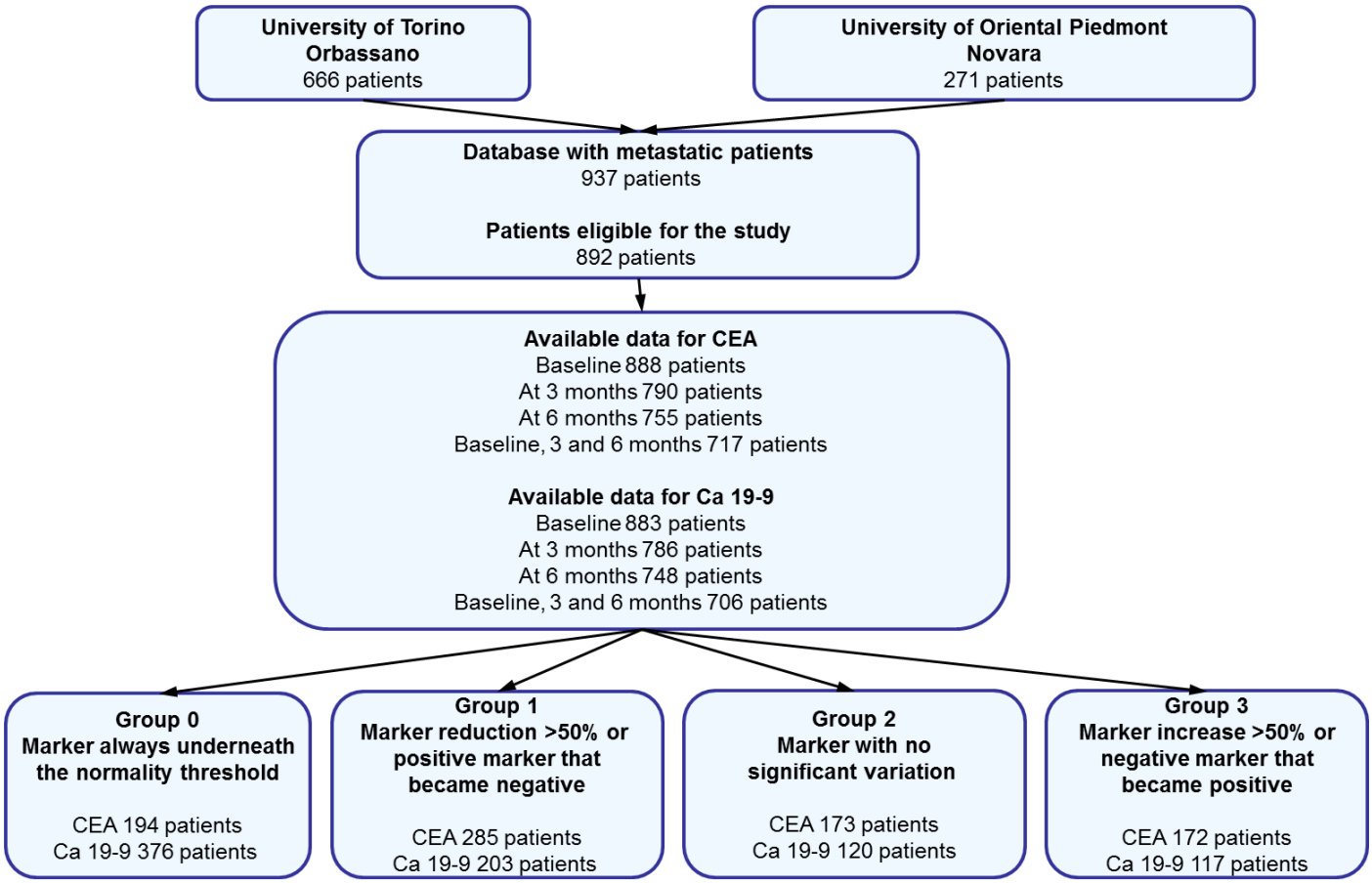
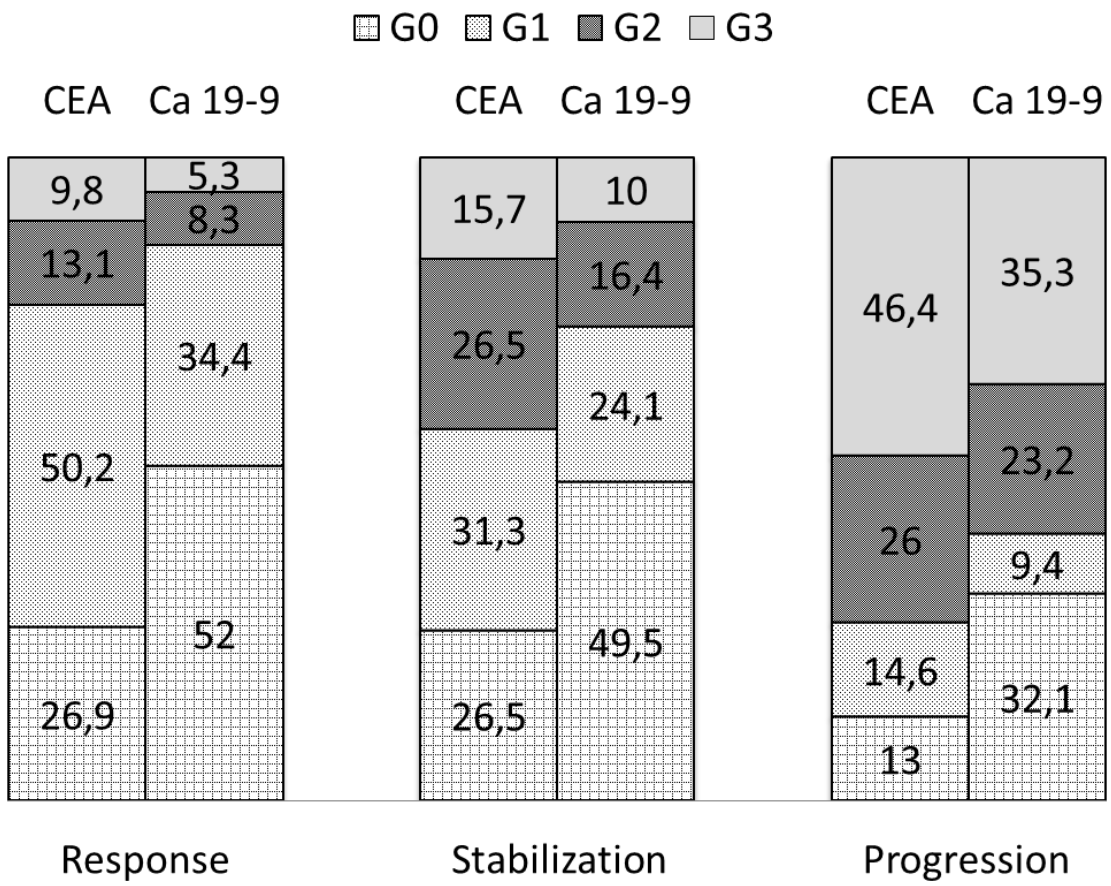


Figure 1


G0
G1
G2
G3
CEA
Ca 19-9
CEA
Ca 19-9
CEA
Ca 19-9
9,8
13,1
50,2
26,9
5,3
8,3
34,4
52
15,7
26,5
31,3
26,5
10
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35,3
23,2
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32,1
Response
Stabilization
Progression

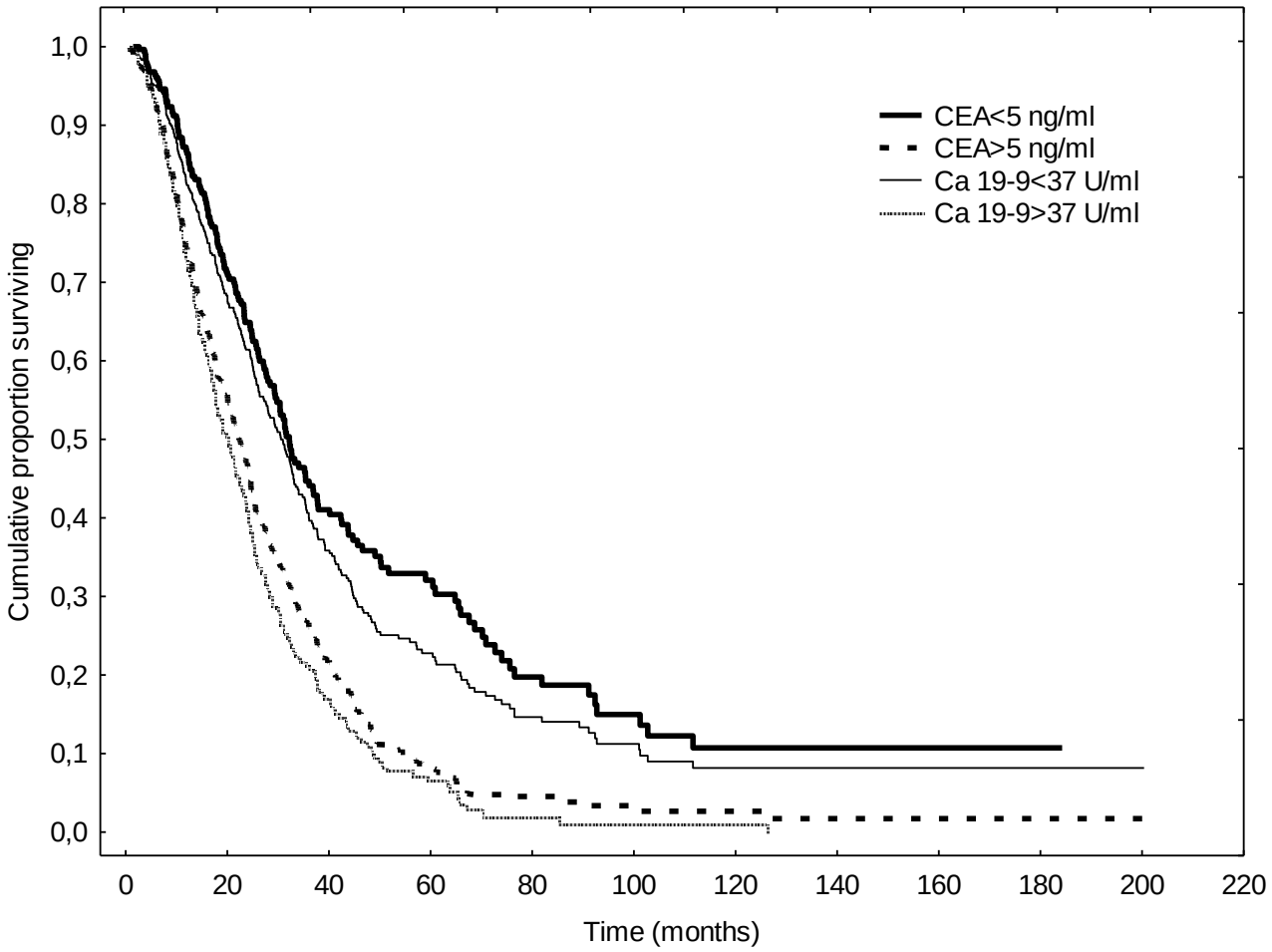
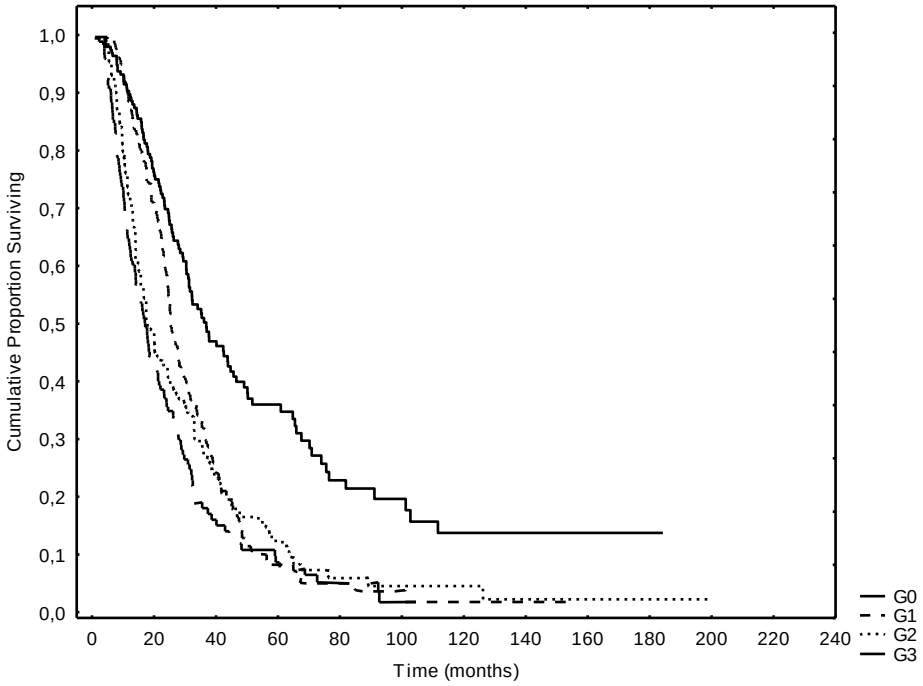

CEA<5 ng/ml
CEA>5 ng/ml
Ca 19-9<37 U/ml
Ca 19-9>37 U/ml
Cumulative proportion surviving
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
0
20
40
60
80
100
120
140
160
180
200
220
Time (months)

Figure 2

Figure 3


Cumulative Proportion Surviving
1,0
0,9
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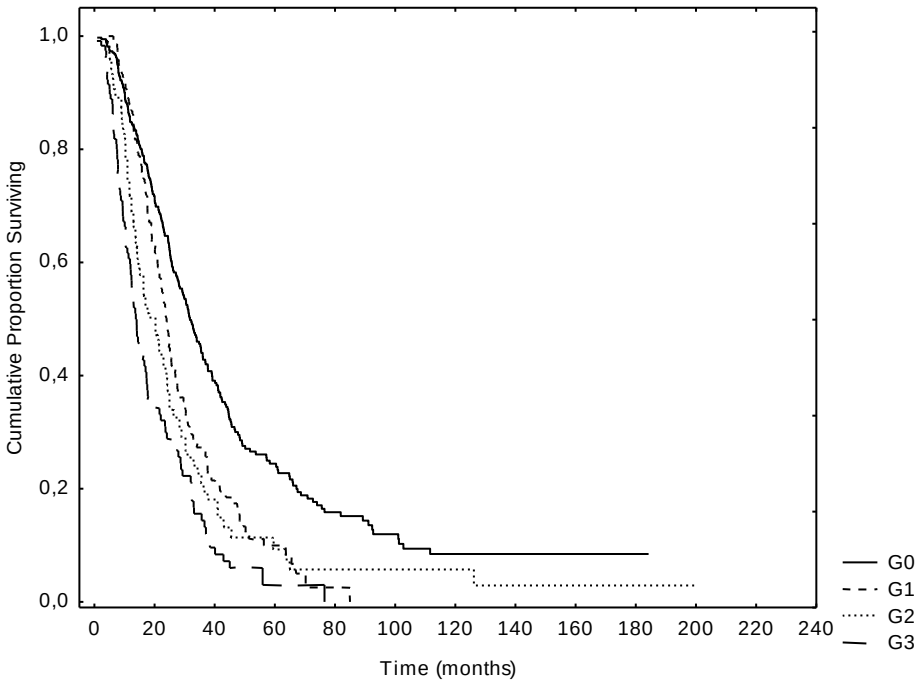
220
240
Time (months)
G0
G1
G2
G3


Cumulative Proportion Surviving
1,0
0,8
0,6
0,4
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20
40
60
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140
160
180
200
220
240
Time (months)
G0
G1
G2
G3

Figure 4

Table 1

	# Patients (%)
No. of patients	892
Center 1	644 (72.2)
Center 2	248 (27.8)
Gender	
Male	549 (61.5)
Female	343 (38.5)
Age	
Median (range) years	64 (28.9-87.4)
Stage of primary	
A	5 (0.5)
B	112 (12.6)
C	240 (26.9)
D	535 (60.0)
Grading	
1	23 (3.2)
2	493 (68.4)
3	205 (28.4)
Unknown	171 (-)

Disease free interval	
Median (range) months	0.5 (0-218)
Site of recurrence	
Liver	620 (69.5)
Lung	263 (29.5)
Other	308 (34.5)
Number of sites of recurrence	
1	640 (71.7)
2	205 (23.0)
>2	47 (5.3)
Performance Status	
0-1	721 (92.0)
2-4	63 (8.0)
Unknown	105 (-)
First-line chemotherapy	
Oxaliplatin-containing	587 (65.8)
Irinotecan-containing	75 (8.4)
Triplet	2 (0.2)
5FU-based	228 (25.6)
With Bevacizumab	42
With anti-EGFR (experimental)	12

Median CEA values (range) ng/ml	
Baseline	15.6 (0.1-32,380)
3 months	8.5 (0.4-50,000)
6 months	8 (0.1-68,076)
Median Ca 19-9 values (range) U/ml	
Baseline	29 (0.1-56,000)
3 months	19.9 (0.1-42,817)
6 months	20 (0.1-63,630)

Table 2. Multivariate Cox analyses on the impact of baseline CEA and Ca 19-9 values and their variation during first-line chemotherapy on TTP and OS.

Variable	HR (95% CI)	p
TTP		
Stage of the primitive*	1.22 (1.16-1.28)	<0.001
Hemoglobin levels > 12 g/dl	0.74 (0.65-0.83)	<0.001
Performance Status (0-4)	1.29 (1.23-1.35)	<0.0001
CEA Group (0-3)§	1.22 (0.18-1.26)	<0.0001
Ca 19-9 Group (0-3)§	1.22 (0.18-1.26)	<0.0001
OS		
Number of metastatic sites	1.19 (1.13-1.25)	0.005
Response to chemotherapy (0-2)^	0.59 (0.52-0.63)	<0.0001
Hemoglobin levels > 12 g/dl	0.76 (0.66-0.86)	0.004
Performance Status (0-4)	1.34 (1.27-1.41)	<0.0001
Liver surgery (1 yes; 0 no)	0.56 (0.42-0.70)	<0.0001
Lung surgery (1 yes; 0 no)	0.22 (0-0.49)	<0.0001
CEA Group (0-3)§	1.18 (1.13-1.23)	<0.001
Baseline Ca 19-9 >37 U/ml	1.79 (1.69-1.89)	<0.0001
Marker as time-dependent covariate		
TTP		

CEA	1.02 (1.01-1.03)	0.05
Ca 19-9	1.03 (1.02-1.04)	0.007
Hemoglobin levels > 12 g/dl	0.75 (0.62-0.88)	0.03
Performance Status (0-4)	1.22 (1.13-1.31)	0.03
OS		
CEA	1.08 (1.06-1.10)	<0.0001
Ca 19-9	1.03 (1.00-1.06)	0.05
Number of metastatic sites	1.15 (1.09-1.21)	0.01
Hemoglobin levels > 12 g/dl	0.74 (0.66-0.82)	<0.001
Performance Status (0-4)	1.22 (1.16-1.28)	0.001
Response to chemotherapy (0-2)^	0.57 (0.51-0.63)	<0.0001
Liver surgery (1 yes; 0 no)	0.54 (0.42 – 0.66)	<0.0001
Lung surgery (1 yes; 0 no)	0.23 (0.0-0.47)	<0.0001

* stage A=0; B=1; C=2; D=3

§ categorized as per study design: G0=0; G1=1; G2=2; G3=3

^ progression=0; disease stabilization=1; response=2