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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prognostic Value of Angiopoietin-2 for Death Risk Stratification in Patients with Metastatic Colorectal Carcinoma

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Abstract

Background: Baseline prognostic biomarkers stratifying treatment strategies in first-line metastatic colorectal cancer (mCRC) are lacking. Angiopoietin-2 (Ang-2) is proposed as a potential biomarker in several cancers. We therefore decided to establish the additional prognostic value of Ang-2 for overall survival (OS) in patients with first-line mCRC.

Methods: We enrolled 177 patients treated with a bevacizumab containing chemotherapy in two prospective phase II clinical trials. Patient plasma samples were collected at baseline. ELISAs were used to measure Ang-2.

Results: The multivariable Cox model identified increased lactate dehydrogenase [HR, 1.60; 95% confidence interval (CI), 1.04–2.45; $P = 0.03$] and Ang-2 log-transformation level (HR, 1.59; 95% CI, 1.14–2.21; $P = 0.0065$) as two significant independent OS prognostic factors. It exhibited good calibration ($P = 0.8$) and discrimination (C-index: 0.64; 95% CI, 0.58–0.68). Ang-2 parameter inclusion in the GERCOR reference model significantly and strongly improved its discriminative ability because the C-statistic increased significantly from 0.61 to 0.63 (bootstrap mean difference = 0.07; 95% CI, 0.069–0.077). Interestingly, the addition of Ang-2 binary information with a 5 ng/mL cutoff value to the GERCOR model allowed the reclassification of intermediate-risk profile patients (41%) into two subsets of low and high risks.

Conclusions: Our study provides robust evidence in favor of baseline Ang-2 prognostic value for OS adding to the conventional factors. Its assessment appears to be useful for the improvement in risk stratification for patients with intermediate-risk profile.

Impact: Ang-2 ability to predict OS at diagnosis could be of interest in the selection of patients eligible for intermittent or sequential therapeutic strategies dedicated to the optimization of patients' quality of life and chemotherapy cost-effectiveness.

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Introduction

Remarkable improvement in the survival of patients with colorectal cancer was reported in last years, mainly due to the increasing indications of metastatic surgery and the availability of a growing number of chemotherapies and biotherapies during the course of the disease (1). Several medical options are currently available to treat patients with metastatic colorectal cancer (mCRC) in the first-line setting ranging from chemotherapy intensification using FOLFOXIRI ± biotherapies (2, 3) and step-up strategies based on a first prescription of 5-fluorouracile monotherapy ± bevacizumab (4–7). Therefore, the identification of biomarkers at diagnosis contributing to the prediction of individual mCRC patient's prognosis will be a critical step to better individualize and stratify mCRC treatments.

Formation of new blood vessels is a major process allowing cancer progression and tumor spread. Several evidence showed that angiogenic molecular regulation is linked to the multistep oncogenesis leading to activation of an increasing number of angiogenic-related growth factors during the course of the disease (8). The impact of bevacizumab, an anti-VEGFA, on mCRC patient's survival, confirmed the role of VEGF-dependent neoangiogenesis in this disease. In addition, the bevacizumab lower efficacy in advanced disease (beyond the second line of therapy) pointed out that the regulation of advanced mCRC angiogenesis might involve other angiogenic growth factors.

Several investigations were performed to determine the role of cancer-related angiogenesis in mCRC prognosis. Over the last

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decade, many seric potential prognostic factors were investigated in patients with mCRC without any positive association with overall survival (OS) at baseline (9, 10).

The presence of alternative angiogenesis pathways promoting cancer progression was firstly suggested by the lack of efficacy of VEGF blockade in some tumor models in mice (11). Further studies demonstrated that Angiopoietin-2 (Ang-2), a ligand of Tie2 receptor (12), was able to induce an anarchical blood vessel organization during cancer progression (13, 14). Preclinical studies confirmed that VEGFR and Tie2 signaling were two independent mechanisms promoting tumor angiogenesis and cancer progression (15). Moreover, VEGF and Ang-2 were independent biomarkers at baseline to predict survival in patients with advanced hepatocarcinoma treated by sorafenib in the SHARP study (16).

In first-line mCRC, Goede and colleagues (17) proposed Ang-2 as a possible prognostic biomarker for OS at diagnosis, based on a pioneering study performed in 34 patients treated with bevacizumab and chemotherapy. In a cohort of 51 patients with mCRC treated by FOLFIRI-3 and bevacizumab, we have also recently observed an association between baseline Ang-2 plasmatic levels, OS and progression-free survival (PFS; ref. 18). Other exploratory studies pointed out its potential prognostic value by the description of an association between Ang-2 and OS or PFS, in small cohorts of patients (19, 20). However, the independent and additional Ang-2 prognostic value for OS, among the conventional prognostic factors and prognostic scores used in clinical practice, is not yet established.

Some prognostic scores have been proposed in patients with mCRC, based on clinical, biologic, and radiologic parameters. The clinical risk score of Fong (from Memorial Sloan Kettering Cancer Center, New York, NY), or the Nordlinger score (21, 22), is used in daily practice to determine the prognosis of mCRC patients candidate for metastatic surgery. Another score usually chosen is the Kohne score, based on the performance status (PS), white blood cell count, number of metastatic sites, and alkaline phosphatase level (23). More recently, the simplified score of the *Groupe Coopérateur Multidisciplinaire en Oncologie* GERCOR offered a convenient fashion to investigate mCRC patient's prognosis, by monitoring PS and lactate dehydrogenase (LDH) status and exhibited a better discrimination ability (24). Using these two parameters, the GERCOR score could identify three distinct groups of patients with mCRC according to their risk of death: A low-risk group (median OS = 29.8 months), an intermediate-risk group (median OS = 19.5 months), and a high-risk group (median OS = 13.9 months). Kohne and GERCOR scores are mainly used to estimate the prognosis of unresectable mCRC. To date, such scores are necessary tools in unresectable mCRC for the management of aggressiveness of the treatment's strategy, its personalization, and the design optimization for future clinical trials. Nowadays, staging systems still remain rare and have to be improved.

Consequently, we decided to perform a validation study to assess the prognostic value of Ang-2 in mCRC. For this purpose, Ang-2 plasmatic levels were monitored in 177 patients with mCRC enrolled in two prospective clinical trials, to confirm the potential prognostic value of Ang-2 as a biomarker of interest for the prediction of OS. We also investigate the added value of Ang-2 at baseline among conventional parameters.

Materials and Methods

Population

Individual patient data were collected from two previous prospective cohorts, both containing bevacizumab treatment in patients with first-line mCRC.

The "cohort set-1," was a pilot, single-arm, multicenter, phase II trial conducted to characterize the response rate and toxicity profile of a FOLFIRI-3/bevacizumab association as initial treatment for mCRC. Sixty-one patients were enrolled between October 2007 and 2009, and levels of plasma Ang-2 were measured in 51 patients by ELISA at baseline. Tumor responses were assessed every 8 weeks by spiral CT until progression. As the main objective was to assess the tumor response rate, surgery of metastases was allowed after 6 cycles of treatment, and the precise timing left at discretion of investigators (18). This phase II study was funded by the University Hospital of Besançon (Besançon, France), and registered on ClinicalTrials.gov (study NCT00544011, approved by the French Ethical Committee on February 1, 2007).

The "cohort set-2" was conducted from 2007 to 2010 to evaluate the role of contrast-enhanced ultrasound with gas-encapsulated microbubbles, to assess antiangiogenic efficacy of bevacizumab in first-line mCRCs with unresectable liver mCRC metastases (25). Of note, all patients had unresectable liver metastases. Tumor responses were assessed every 12 weeks or less until progression. This study was funded by the French National Institute of Cancer and registered on ClinicalTrials.gov (study NCT00489697, approved by the French Ethical Committee on November 11, 2006).

In both studies, all patients gave written informed consent, and were followed until death. Data were anonymized. Population selection during the study is summarized in the flow chart (Fig. 1).

Data extraction

The following data were collected for each patient in the two cohorts: center and patient identification, age, sex, PS, primary tumor site (colon, rectum), site of metastases (liver-limited, liver and other, other), time of metastasis diagnostic (synchronous and metachronous), primary tumor resection, LDH level at baseline (normal value was considered if LDH were below 350 UI/L), lymphocyte and leucocyte counts, survival status, and date of last news or death. Of note, ALP level was not available in "cohort set-2."

Ang-2 plasma sample measurement procedure is precisely described in Supplementary Methods.

Statistical analysis

We provided the mean (SD) values and frequency (percentages) for the description of continuous and categorical variables, respectively. The means and the proportion were compared using the Student t test and the χ^2 test (or Fisher exact test, if appropriate), respectively. Because of the skewed Ang-2 distribution, we used for its description the median and the interquartile range for the dispersion measurement, as recommended (26). The Wilcoxon rank-sum test was performed for Ang-2 distribution comparison among the cohort set.

OS was calculated from the date of study enrolment to the date of death from any cause. Alive patients were censored at the last follow-up. OS was estimated using the Kaplan–Meier method and described using median or rate at specific time points with 95% confidence interval. Follow-up was calculated using reverse

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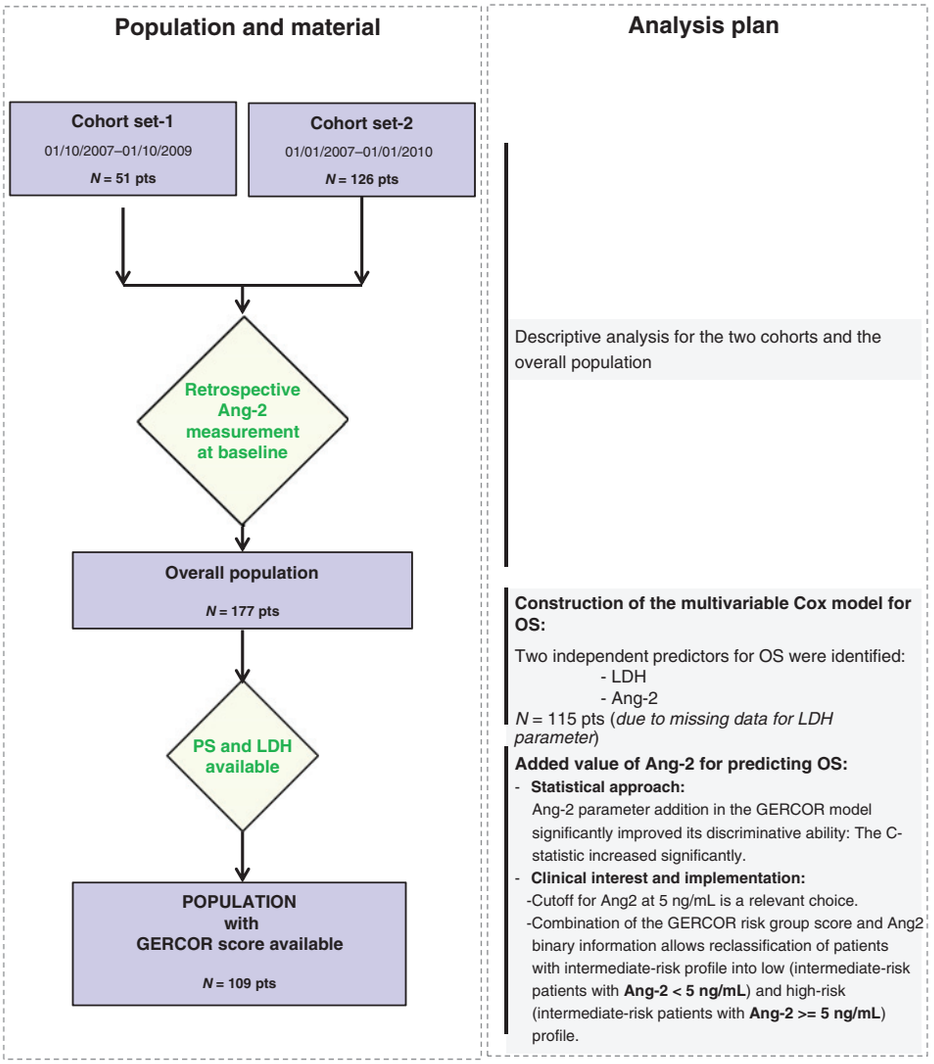

Figure 1.
Flow chart and analysis plan of the study. Population selection during the study is summarized in the flow chart. In the left, the population selection during the study is described. In the right, the derived analysis and their synthetic results are summarized.

Kaplan–Meier estimation (27). A Cox proportional hazard model was performed to estimate the HR and 95% confidence intervals for the factors associated with OS.

The association of parameters at enrolment with OS was first assessed using univariate Cox analysis and then included (for those with $P < 0.05$) in a final multivariate Cox regression model with stepwise backward elimination. When used in continuous in the Cox modelization, Ang-2 variable had to be normalized by logarithmic transformation, considering its skewed distribution. Hazard proportionality was checked by plotting log-minus-log survival curves.

Accuracy of the model was checked regarding two parameters: discrimination and calibration (28). The predictive value and the discrimination ability of the model were evaluated with the Harrell Concordance (*C*)-index. One thousand random samples of the population were used to derive 95% confidence interval for the Harrell *C*-statistic. Calibration and goodness of fit of the model were assessed by using the extension of the Hosmer–Lemeshow test for survival analysis, and a *P* value greater than 0.1 was considered as an indicator for acceptable agreement.

Internal validity of the model was assessed by a bootstrap sample procedure. Several approaches have been proposed to assess the performance in samples of the same population (internal validation; ref. 29).

Sensitivity analyses were performed for univariate and multivariate Cox models with a stratified approach on the cohort set parameter that allowed to consider the two cohort heterogeneities in the Cox modelization.

We further focused on the improvement of one reference prognostic model for OS (GERCOR model) performance after the inclusion of Ang-2 measurement, comparing two sets of predictions for death: one based on a Cox proportional hazard model without Ang-2 parameter and one based on a model with Ang-2 parameter. The discrimination ability and incremental value of Ang-2 level to the GERCOR score were evaluated with the use of *C*-statistic. This analysis was repeated 1,000 times using bootstrap samples to derive 95% confidence intervals for the difference in the *C*-statistic between models. We also used net continuous reclassification improvement (NRI) and integrated discrimination improvement (IDI) to quantify the performance and the net benefit of the addition

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of Ang-2 to the reference model for the prediction of 48 months death probability (30–32). Continuous NRI has several limitations, but would give a consistent message, and is therefore a descriptive marker (33). Of note, cNRI does not consider the magnitude of the change, but only the direction. This is done by the IDI. When significantly greater than 0, IDI and cNRI are in favor of a net benefit of the addition of the marker of interest to the reference model considered.

We finally investigated the possibility to provide a simple implementation of Ang-2 monitoring in clinical practice with the determination of a cutoff value by contrasting Ang-2 level distributions in boxplot among healthy volunteers and patients with mCRC.

The analyses were conducted using SAS 9.2 (Statistical Analysis System) and R 3.0.2 (R foundation for Statistical Computing). All statistical tests were two-sided, and probability values <0.05 were regarded as significant.

Results

Population

The characteristics of the 177 eligible patients are summarized in Table 1. Fifty-one samples were available from "cohort set-1," and 126 from "cohort set-2." Patient characteristics of these two cohorts are similar, except for sex and liver metastatic involvement. Ang-2 values were also different in the two cohorts. Of note, the statistical unbalance in metastatic sites between the two cohorts was awaited because liver metastasis was an inclusion criterion in the "cohort set-2." The rate of PS 2 was 2% in the "cohort set-1," instead of 5% in the "cohort set-2," we consequently differentiated the patients PS 0 from the patients PS > 0. There were 23 (45%), and 45 (36%) patients with liver exclusive metastatic sites. Surgery of the primary tumor was performed in 28 (57%) and 82 (65%) patients, in the cohorts set-1 and set-2, respectively. LDH values at baseline were available in 33 and 82 patients and increased upper limit normal values in 13 (39%) and 46 (56%) patients of cohorts set-1 and set-2, respectively.

Ang-2 plasma level biomarker and prediction of OS

The association of clinical, biologic, and radiologic parameters with risk of death in univariate and multivariate Cox regression analysis with stepwise backward elimination was performed and results are shown in Table 2. We identified six variables as prognostic factors for OS in the univariate analysis: Ang-2 log-value (HR, 1.91; 95% CI, 1.492–2.448; $P < 0.0001$), metastatic localizations (liver and other: HR, 1.82; 95% CI, 1.305–2.527; other: HR, 0.69; 95% CI: 0.326–1.442; $P = 0.0003$; liver alone as the reference), absence of resection of the primary site (HR, 1.43; 95% CI, 1.035–1.984; $P = 0.0304$), synchronous metastases (HR, 1.46; 95% CI, 1.035–2.054; $P = 0.0308$), high LDH level (HR, 2.03; 95% CI, 1.373–2.988; $P = 0.0004$), and leucocyte count (HR, 1.12; 95% CI, 1.056–1.188; $P = 0.0002$; Table 2).

Two independent predictors of OS were identified by the multivariate analysis: LDH high level (HR, 1.60; 95% CI, 1.04–2.45; $P = 0.03$) and log Ang-2 high level (HR, 1.59; 95% CI, 1.14–2.21; $P = 0.0065$; Table 2).

Table 1. Baseline characteristics of patients, according to the cohort set

	Overall population ($N = 177$)		Cohort set-1 ($N = 51$)		Cohort set-2 ($N = 126$)		
Characteristics	***N***		***N***		***N***		***P*[c]**
Age, y[d]			51		126		
≤65	177	93 (53%)		27 (53%)		66 (52%)	
>65		84 (47%)		24 (47%)		60 (48%)	0.9461
Patient male sex, *n* (%)[d]	177	74 (42%)	51	28 (55%)	126	46 (37%)	**0.0246**
PS OMS[d]	167						
0, *n* (%)		91 (55%)	48	27 (56%)	119	64 (54%)	
>0, *n* (%)		76 (45%)		21 (44%)		55 (46%)	0.7719
Primary tumor site[d]	177		51		126		
Colon, *n* (%)		120 (68%)		32 (63%)		88 (70%)	
Rectum, *n* (%)		57 (32%)		19 (37%)		38 (30%)	0.3602
Metastases localization[d]	177		51		126		
Liver and other, *n* (%)		98 (55%)		17 (33%)		81 (64%)	
Liver alone, *n* (%)		68 (39%)		23 (45%)		45 (36%)	
Other alone, *n* (%)		11 (6%)		11 (22%)		0 (0%)	**0.0001**
Timing of metastases[d]	173		48		125		
Synchrone, *n* (%)		119 (69%)		30 (63%)		89 (71%)	
Metachrone, *n* (%)		54 (31%)		18 (37%)		36 (29%)	0.2688
Surgery of the primary tumor, *n* (%)[d]	175	110 (63%)	49	28 (57%)	126	82 (65%)	0.3293
Leucocyte ($\times 10^6$/mL)[a]	137	8.1 ± 3.2	49	8.1 ± 3.5	88	8.2 ± 3.0	0.8926
Lymphocyte ($\times 10^6$/mL)[a]	132	1.5 ± 0.7	45	1.7 ± 0.7	87	1.4 ± 0.6	0.0550
LDH[d]	115		33		82		
≤350 (ULN)		56 (49%)		20 (61%)		36 (44%)	
>350 (ULN)		59 (51%)		13 (39%)		46 (56%)	0.1050
Ang-2 (ng/mL)[b]	177	4.249 (2.683–7.153)	51	2.793 (2.103–4.330)	126	4.728 (3.298–7.798)	**0.0001**
Death event[d]	177		51	36 (71%)	126	126 (100%)	**0.0001**
Median follow-up time in months 95% CI All patients were follow-up until death				57.9 (53.1–60.3)		Max time observed = 64.1	

NOTE: Bold characters represent significative results.

Abbreviation: ULN, upper limit of normal.

[a]Plus–minus values are means ± SD, and the unpaired *t* test was used for the comparison of variable among groups.

[b]Median and interquartile range are described, and the Wilcoxon rank-sum test was used for the comparison of variable among groups.

[c]*P* values are for the comparison between cohort set-1 and cohort set-2 populations.

[d]χ^2 or Fisher's exact tests were used for the comparison of categorical variables.

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Table 2. Univariate and multivariate analyses

	Number of patients	Number of deaths	Univariate analysis		Multivariate analysis		Multivariate analysis with bootstrap procedure
			HR (95% CI)	*P*	HR (95% CI)	*P*	95% percentile CI
Age, y							
≤65	93	84	1 (—)	—			
>65	84	78	1.243 (0.912–1.694)	0.1689			
Sex							
Male	74	65	1				
Female	103	97	1.310 (0.955–1.798)	0.0941			
PS OMS							
0	91	80	1				
>0	76	73	1.274 (0.927–1.751)	0.1355			
Primary tumor site							
Colon	120	113	1				
Rectum	57	49	0.855 (0.610–1.196)	0.3597			
Timing to metastasis							
Metachronous	119	111	1				
Synchronous	54	47	1.458 (1.035–2.054)	**0.0308**			
Metastases localization							
Liver alone	68	59	1				
Liver and other	98	95	1.816 (1.305–2.527)				
Other alone	11	8	0.686 (0.326–1.442)	**0.0003**			
Surgery of the primary tumor							
Yes	110	101	1				
No	65	59	1.433 (1.035–1.984)	**0.0304**			
Leucocyte ($\times 10^6$/mL)	137	122	1.120 (1.056–1.188)	**0.0002**			
Lymphocyte ($\times 10^6$/mL)	132	120	0.795 (0.589–1.073)	0.1339			
LDH							
≤350 (ULN)	56	49	1		1		
>350 (ULN)	59	58	2.026 (1.373–2.988)	**0.0004**	1.598 (1.040–2.454)	**0.0323**	(1.039–2.628)
Log_Ang-2 (pg/mL)	177	162	1.911 (1.492–2.448)	<**0.0001**	1.587 (1.138–2.213)	**0.0065**	(1.161–2.263)

NOTE: Bold characters represent significative results.
Abbreviation: ULN, upper limit of normal.

Final multivariate model performance assessment

Accuracy of the model was checked regarding two parameters: discrimination and calibration, which measure the ability to separate patients with different prognosis, and to provide unbiased survival predictions in groups of similar patients, respectively.

Our final multivariable Cox model exhibited good calibration (Hosmer–Lemeshow with deciles $P = 0.8$) and acceptable discrimination (*C*-statistic 0.64; 95% CI, 0.58–0.68).

Internal validation of the final model

With the replicated datasets ($n = 1{,}000$) derived from the bootstrap sample procedure, uncertainties around HR estimates can be measured.

Bootstrapping results for the internal validation reflect the robustness of the final model [HR 95% CI percentile for LDH: 1.039–2.628 and HR 95% CI percentile for Ang-2 (log value): 1.161–2.263].

Sensitivity analysis

Considering the differences observed between the two cohorts (Table 1), we performed a sensitivity analysis to validate the robustness of our final model with a stratified approach in the Cox modelization.

With this approach, similar results were obtained for the univariate analysis except for timing to metastases parameters, which were reported to be nonstatistically significant (Supplementary Table S1). The multivariate analysis confirmed that Ang-2 and LDH were two independent predictors for OS (HR, 1.800; 95% CI, 1.081–2.998; $P = 0.0239$ and HR, 1.548; 95% CI, 1.028–2.331; $P = 0.0365$, respectively).

Added value of the Ang-2 parameter for predicting OS

Then, we decided to investigate the performance improvement in OS discrimination after the inclusion of Ang-2 measurement in a reference prognostic model.

Currently, in our situation, two main staging systems are available: Kohne and GERCOR scores. In our population, the Kohne model cannot be calculated. However, the GERCOR score displayed a better discriminative ability ($C = 0.64$) than the Kohne model ($C = 0.55$; ref. 24). We then used the GERCOR model as the reference score.

Ang-2 was first identified as a factor independently associated with OS (HR, 1.568; 95% CI, 1.111–2.213; $P = 0.0104$). Then, the inclusion of the Ang-2 parameter in the GERCOR reference model significantly improved its discriminative ability because the *C*-statistic increased significantly from 0.61 to 0.63 (bootstrap mean difference = 0.07; 95% CI, 0.069–0.077).

The IDI was 0.03 ($P = 0.07$). Similarly, the addition of Ang-2 measurement to the reference model showed a favorable trend to adequately reclassified patients at lower risk for death and those at higher risk, as reported by a continuous NRI of 0.26 (95% CI, −0.23 to 0.75). Indeed for patients without death at 48 months, Ang-2 measurement moved risk prediction in the correct (downward) direction in 11 of 18 = 61%. Conversely, patients with

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death indicate a correct, upward, change in risk assessment when using the Ang-2 measurement (47/91 = 52%). The risk reclassification analysis (IDI and NRI) results are greater than 0 and statistically border line reflecting a favorable tendency for Ang-2 parameter.

Proposal for an implementation of Ang-2 monitoring in clinical practice

After the statistical investigation and determination of the baseline Ang-2 added value for predicting OS among the conventional factors, we investigated the possibility to provide a simple implementation of Ang-2 monitoring in clinical practice.

Cutoff value of Ang-2 fixed at 5 ng/mL. Simple implementation of Ang-2 monitoring in clinical practice is first guided by the determination for a relevant cutoff value to categorize patients into groups with low and high Ang-2 level at baseline.

A preliminary set of experiments was done by dosing the Ang-2 value in 41 healthy volunteers, blood donors in the *Etablissement Français du Sang* (EFS, Bourgogne Franche-Comté). These volunteers were major (more than 55-years-old), having signed an informed consent and were randomly chosen. We hypothesized that this population have no active angiogenesis. Among these healthy donors, 40 (98%) had mean values lower than 5 ng/mL. This observation was in agreement with the results previously published by Goede and colleagues (17), in which levels of Ang-2 were inferior to 5 ng/mL in the 33 healthy volunteers.

In our study population, the median value of Ang-2 was 4.045 ng/mL for the 109 patients included in the final analysis. Of note, similar results were observed when the median value was used as a cutoff value. A value of 5 ng/mL was then chosen considering results provided by previous publications (17, 20) regarding that 98% of healthy volunteers have baseline plasmatic levels of Ang-2 below 5 ng/mL. In consequence, to investigate a cutoff value for clinical use, a level of 5 ng/mL seemed to be a relevant choice (Fig. 2).

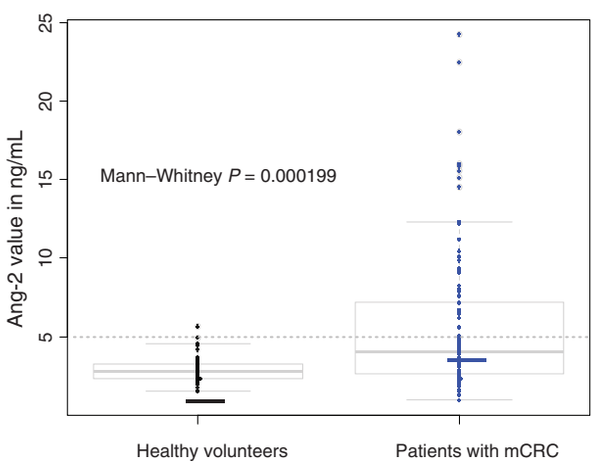

Figure 2.
Distribution of Ang-2 measurement value among healthy volunteers ($n = 41$) and patients with mCRC ($n = 109$) involved in the final analysis. In our study population, the median value of Ang-2 was 4.045 ng/mL for the 109 patients included in the final analysis. In consequence, to investigate a cutoff value for clinical use, a level of 5 ng/mL seemed to be a relevant choice.

Phenotypic characterization of patients with high and low Ang-2 levels. The determination of 5 ng/mL as a cutoff value allowed us to classify patients into two groups: low (<5 ng/mL) and high (≥5 ng/mL) Ang-2 level groups at baseline. Among the 109 patients involved in the final analysis, 40 (36.7%) had levels of Ang-2 above 5 ng/mL. The clinicobiologic characteristics according to the Ang-2 value are summarized in Table 3. Increased Ang-2 level was associated with enhanced LDH levels (77%, $P < 0.0001$), metastatic sites (63% liver and other, $P = 0.04515$), and synchronous metastases (92%, $P < 0.0001$). Patients with high Ang-2 levels exhibited more surgery of the primary tumor, increased leucocytes count, and lymphopenia.

As expected, we noted a significant difference for the GERCOR prognostic score distribution among groups of patients with low and high Ang-2 levels (42%–38%–20% vs. 7%–48%–45%; $P = 0.0002$ for low-, intermediate-, and high-risk GERCOR groups, respectively).

Kaplan–Meier curves for OS by combining Ang-2 binary information and conventional score. Considering the added value of Ang-2 measurement for OS risk stratification among conventional factors, previously described, we investigated the interest for a combination of Ang-2 simple binary information and the GERCOR prognostic score in clinical practice.

In our population, 30 (59%) and 79 (63%) patients are eligible for the GERCOR score calculation among the 51 and 126 patients in the cohorts set-1 and 2, respectively (Fisher exact test, $P = 0.7332$). In total, data were available to assess the GERCOR prognostic score in 109 patients. This risk model identified 32 patients at "low-risk," 45 patients at "intermediate-risk," and 32 patients at "high-risk" levels. In our study, the median OS was 27.1 months 95% CI, 20.2–38.4 for the low-risk, 24.3 months 95% CI, 19.3–29.5 for the intermediate-risk, and 19.7 months 95% CI, 12.3–23.8 for the high-risk groups (Fig. 3A, global P log-rank = 0.02).

According to the Ang-2 value, the median OS was significantly better in patients with low levels of Ang-2 than in patients with high levels of Ang-2 (median OS = 26.2 months; 95% CI, 23.2–34.5 vs. 17.8 months; 95% CI, 10.1–24.1, respectively; HR, 2.06; 95% CI, 1.37–3.09; $P < 0.0001$; Fig. 3B).

Finally, the Ang-2 binary status was combined with the GERCOR score. In low and high-risk patients, the Ang-2 value only triggers a trend in better discrimination of prognosis, without statistical significance ($P = 0.27$ and 0.45, respectively). However, in the intermediate-risk GERCOR group ($n = 45$; 41%), the consideration of Ang-2 binary status allowed us to split the population in two subsets of patients. Indeed, patients with low Ang-2 level had a significant better prognosis than those with high Ang-2 level (26.3; 95% CI, 22.2–38.2 vs. 17.6 months; 95% CI, 7.6–29.5; $P = 0.02$; HR, 2.07; 95% CI, 1.11–3.86; Fig. 3C).

Interestingly, patients with intermediate risk and Ang-2 <5 ng/mL ($n = 26$, 58%) had a similar risk profile than the GERCOR low-risk patients (OS = 26.3 and 27.1 months, respectively). Similarly, those with Ang-2 >5 ng/mL ($n = 19$; 42%) had a similar risk profile than the GERCOR high-risk patients (OS = 17.6 and 19.7 months, respectively, Fig. 3D).

Discussion

The present results, consistent with previous studies, confirm that Ang-2 is a biomarker of interest for OS prediction in non–

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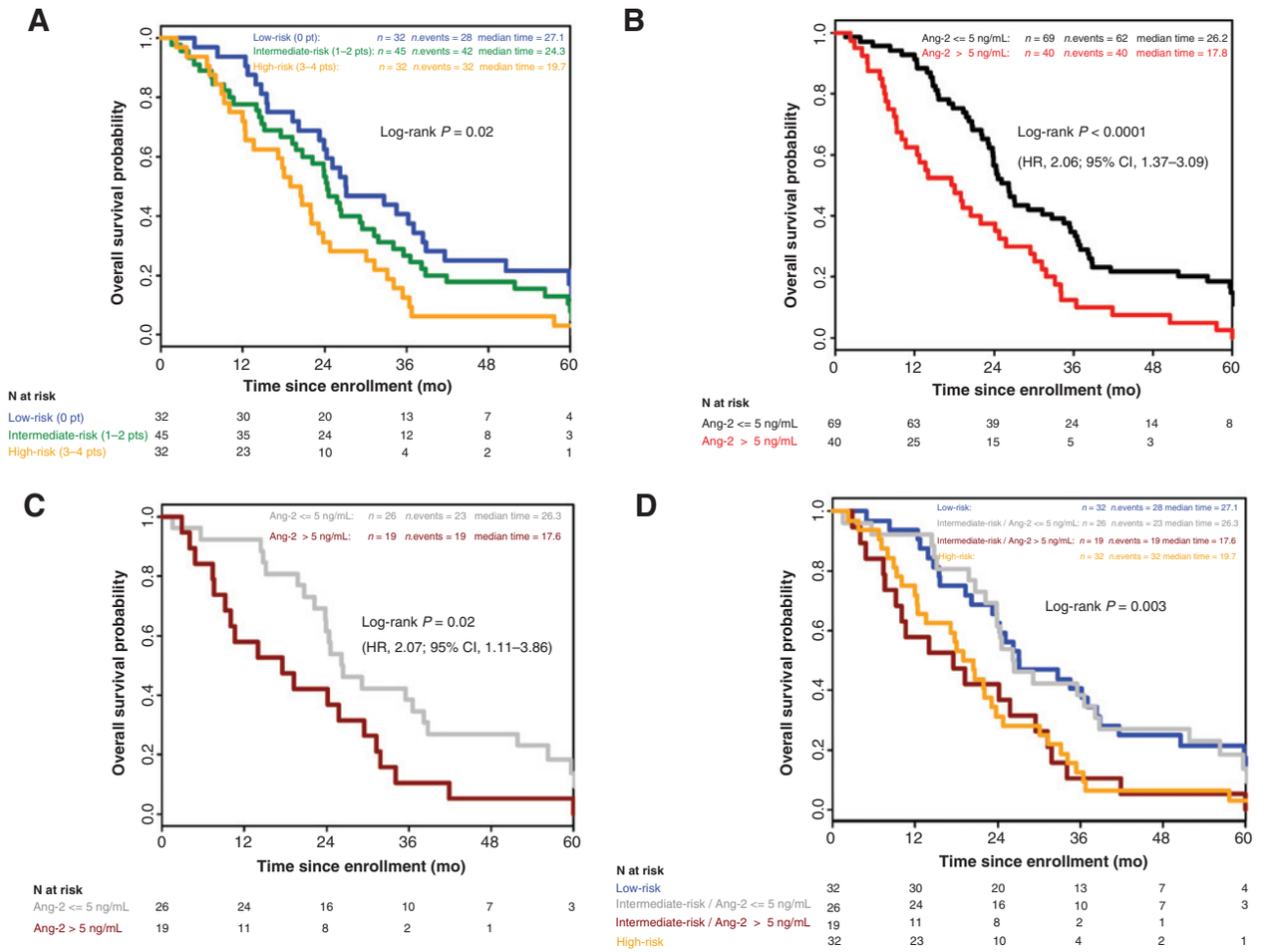

Figure 3.
Kaplan–Meier curves for OS. A, the classic approach based on the GERCOR score in which LDH and PS are key parameters. B, the stratification according to the level of Ang-2. C, the stratification according to the level of Ang-2 in the 45 (41%) patients classified in intermediate risk with the GERCOR score. D, the determination of Ang-2 in the intermediate-risk patients with the GERCOR group ($n = 45$) reclassified these patients in two groups with similar profiles than low- and high-risk GERCOR groups.

previously treated patients with mCRC (17, 18). The main interest of Ang-2 as a biomarker is its ability to predict OS when measured at diagnosis, before treatment initiation. Low levels of Ang-2 identify a significant population ($n = 69$; 63%) displaying an encouraging median OS of 26.2 months (HR, 2.06; 95% CI, 1.37–3.09; $P < 0.0001$).

Identification of patients with a favorable prognosis at baseline might be of clinical interest to better individualize cancer therapies. Such prognostic biomarkers might enable the appropriate selection of patients eligible to intermittent chemotherapy or sequential therapeutic strategies dedicated to the optimization of patient's quality of life and chemotherapy cost-effectiveness (5, 6, 34).

In our cohort, the GERCOR model allows the identification of 32 (29%) patients of good prognosis, with a median OS of 27.1 months. When considering the population exhibiting both low Ang-2 level and low GERCOR risk, no difference was established in OS, as well as for elevation of Ang-2 level and high GERCOR risk.

However, considering the intermediate GERCOR subgroup risk ($n = 45$; 41% of patients in the present study), the binary Ang-2 status succeeded in reclassifying the prognostic risk into a low ($n = 26$; 58%) and a high risk ($n = 19$; 42%), corresponding to those of the GERCOR model. In this case, the soluble Ang-2 value appears to be of particular interest because (i) the intermediate subgroup represents a consequent number of patients, and (ii) the relevance of an intermediate group in clinical practice is not clear leading to considerable confusion for their management.

There are some limitations in our study. First, all patients of these cohorts were treated with bevacizumab and chemotherapy, excluding the possibility to analyze the predictive value of Ang-2. This question was recently addressed by Llovet and colleagues (16) in the SHARP study. Ang-2 was monitored in 491 patients with hepatocellular carcinoma treated by sorafenib or placebo. Baseline plasma Ang-2 was correlated with OS both in sorafenib and placebo groups, ruling out a predictive value of Ang-2 in the context of this antiangiogenic therapy. More recently, the predictive impact of Ang-2 has also been assessed in pancreatic cancer. In that study, three seric factors have been proposed as predictive for efficacy in 328 patients treated by gemcitabine with bevacizumab

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Table 3. Baseline characteristics of the patients involved in the multivariate analysis according to the Ang-2 level ($n = 109$)

	n	Ang-2 < 5 ng/mL ($n = 69$)	Ang-2 ≥ 5 ng/mL ($n = 40$)	*P*[b]
Age, y[c]				
≤65	56	31 (45)	25 (63)	
>65	53	38 (55)	15(37)	0.11
Sex[c]				
Male	45	39 (57)	25 (63)	
Female	64	30 (43)	15 (37)	0.54
PS OMS[c]				
0	57	41 (59)	16 (40)	
>0	52	28 (41)	24 (60)	0.07
Primary tumor site[c]				
Colon	73	46 (67)	27 (68)	
Rectum	36	23 (33)	13 (32)	0.93
Timing to metastasis[c]				
Synchronous	74	39 (57)	35 (92)	
Metachronous	32	29 (43)	3 (8)	**<0.0001**
Metastases localization[c]				
Liver alone	43	28 (41)	15 (37)	
Liver and other	58	33 (48)	25 (63)	
Other alone	8	8 (12)	0 (0)	**0.04515**
Surgery of the primary tumor[c]				
No	73	16 (24)	18 (46)	
Yes	34	52 (76)	21 (54)	**0.0189**
Leucocyte ($\times 10^6$/mL)[a]	89	7.3713 ± 2.3972	9.4180 ± 3.8269	**0.0026**
Lymphocyte ($\times 10^6$/mL)[a]	85	1.6589 ± 0.6355	1.2187 ± 0.5816	**0.0017**
LDH[c]				
≤350 (ULN)	52	43 (62)	9 (23)	
>350 (ULN)	57	26 (38)	31 (77)	**<0.0001**
GERCOR score[c]				
0 Low-risk	32	29 (42)	3 (7)	
1 Intermediate-risk	45	26 (38)	19 (48)	
2 High-risk	32	14 (20)	18 (45)	**0.0002**

NOTE: Bold characters represent significative results.

[a]Plus–minus values are means ± SD, and the unpaired *t* test was used for the comparison of variable among groups.

[b]*P* values are for the comparison between cohort set-1 and cohort set-2 populations.

[c]χ^2 or Fisher's exact tests were used for the comparison of categorical variables.

or placebo, including Ang-2, stomal cell–derived factor-1, and VEGF-D (35). Another limitation of our study is the absence of PAL recording in the "cohort set-2." Nevertheless, we did not observe any association between PAL and Ang-2 in the "cohort set-1" Moreover, the GERCOR analysis, performed on 803 patients mCRC treated by FOLFOX or FOLFIRI in the first-line setting demonstrated that LDH, number of metastatic sites, and PS were the only independent prognostic factors (24).

From a statistical point of view, the assessment of models performance measures such as discrimination, calibration, and internal validation strengthen the present investigation. Although the model developed here has good calibration, discrimination, and robust internal validation (reproducibility), these results have to be replicated and confirmed in a prospectively recruited validation cohort, to ensure wider transportability and generalizability of our results.

One scheming observation is the association between Ang-2 level and presence of the primary tumor. Surgery of the primary tumor was linked to a decreased probability to observe plasmatic Ang-2 in patients with mCRC (76% Ang-2 <5 ng/mL vs. 54% Ang-2 >5 ng/mL). The prognostic value of primary tumor resection still remains a matter of debates. It appears to be significantly associated with OS in our univariate analysis, but not in the multivariate one. Many clinical and biologic investigations support a potential role of the presence of the primary carcinoma in mCRC prognosis (36–39). Data reported in two phase III clinical trials CAIRO and CAIRO II, and a recent meta-analysis suggested a prognostic association of primary tumor removal with OS in patients with mCRC (38, 40–42). The potential association of primary colorectal cancer on Ang-2 production might account for its potential detrimental effect on prognosis. Prospective studies should monitor Ang-2 production before and following primary tumor resection.

The biologic basis underlying the adverse role of Ang-2 also deserves further investigations. There are some evidences that VEGF is early expressed during cancer progression (43) Ang-2 could be overexpressed in latter stages of the disease, as suggested by Abajo and colleagues (44). In line with this hypothesis, the production of Ang-2 within the tumor microenvironment could depend on VEGF (44). Moreover, Ang-2 expression was shown to be correlated with colorectal cancer stages and progression (44, 45). Nevertheless the precise role of Ang-2 cancer progression needs to be further clarified.

In conclusion, the assessment of the Ang-2 value at baseline could guide clinicians in stratifying risk for death in patients with first-line mCRC, and in providing a basis for early and adapted therapeutic interventions. The determination of Ang-2 at baseline should allow death risk stratification that could also be useful in the design optimization for future clinical trials.

Disclosure of Potential Conflicts of Interest

T. Lecomte has received speakers bureau honoraria from Roche. O. Bouche is a consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other authors.

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Prognostic Value of Angiopoietin-2 for Death Risk Stratification in Patients with Metastatic Colorectal Carcinoma

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