Fluoropyrimidine-induced cardiotoxicity in colorectal cancer patients: a prospective observational trial (CHECKPOINT)

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Abstract. Fluoropyrimidines (FP) are the backbone chemotherapy in colorectal cancer (CRC) treatment; however, their use is associated with cardiotoxicity, which is underreported. In the present study, it was aimed to prospectively determine the incidence rates and related risk factors of FP-induced cardiotoxicity (FIC) in CRC patients and at identifying predictive biomarkers. A total of 129 consecutive previously untreated CRC patients underwent active cardiological monitoring, including 5-items simplified questionnaire on symptoms, electrocardiogram (ECG) and plasma sample collection during FP chemotherapy. FIC was defined as the presence of ECG alterations and/or the arising of at least one symptom of chest pain, dyspnoea, palpitations or syncope. The primary objective was the evaluation of FIC incidence. Secondary objectives were the correlation of FIC with well-known cardiological risk factors and the identification of circulating biomarkers (serum levels of troponin I, pro hormone BNP; miRNA analysis) as predictors of FIC. A total of 20 out of 129 (15.5%) patients experienced FIC. The most common symptoms were dyspnoea (60%) and chest pain (40%), while only 15% of patients presented ECG alterations, including one acute myocardial infarction. Retreatment with FP was attempted in 90% of patients with a favourable outcome. Despite 48% of patients having cardiological comorbidities, an increased FIC was not observed in this subgroup. Only the subgroup of females with the habit of alcohol consumption showed an increased risk of FIC. None of the circulating biomarkers evaluated demonstrated a clinical utility as FIC predictors. FIC can be an unexpected, life-threatening adverse event that can limit the subsequent treatment choices in patients with CRC. In this prospective study, well-known cardiological comorbidities were not related to higher FIC risk and circulating biomarkers predictive of toxicity could not be found. With careful monitoring, mainly based on symptoms, almost all patients completed the FP treatment.

Introduction

Fluoropyrimidines (FP), namely 5-fluorouracil (5-FU) and capecitabine, are widely used to treat solid malignancies, including those arising from gastrointestinal tract. In addition, FP are frequently used concurrently with radiotherapy, due to their radio-sensitizing properties. 5-FU and capecitabine are generally well tolerated, being myelosuppression, gastrointestinal, and skin toxicity (hand-foot syndrome) the most common adverse events, which vary according to dose and schedule (1).

Cardiotoxicity is a feared, uncommon toxicity of FP; although the most common manifestation of FP-induced cardiotoxicity (FIC) is unstable angina (2), other severe adverse cardiac events, including cardiomyopathy, myocarditis, and sudden cardiac death, have been reported (3). FIC typically occurs during the first few treatment cycles, which usually results in the discontinuation of treatment (4). As a consequence, FIC represents an important issue particularly

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in colorectal cancer (CRC) treatment, due to the central role of FP in the management of this disease, both in adjuvant and palliative settings. Patients with CRC that experienced FIC have a considerable reduction in their therapeutic options with a negative influence on prognosis (5).

Proposed mechanisms that underlie FIC include coronary artery vasospasms, vascular endothelial dysfunction, direct toxicity on the myocardium, and thrombogenicity due to altered blood rheology (6).

Coronary artery vasospasms are considered to be the main pathogenic mechanism (7), and it has been suggested that N-terminal (NT)-pro hormone BNP (proBNP) may act as a biomarker of FIC (8). The compound that may be responsible for these effects is fluoroacetate. 5-FU and capecitabine are catabolised by dehydro-pyrimidine dehydrogenase (DPYD) and in several steps degraded into α-fluoro-β-alanine, and then into fluoroacetate, a highly cardiotoxic and neurotoxic compound (9).

The reported incidence of cardiac symptoms and events varies greatly in the existing literature, with a range from 0-34.6% of patients (10). Serious cardiac events, including myocardial infarction, cardiogenic shock and cardiac arrest, occurred in 0-2% (11). No significant differences were observed in the incidence of cardiac events between capecitabine and 5-FU (11).

The identification of patients at risk for adverse cardiac events remains the major challenge, either due to the limited data on baseline cardiac risk factors or the lack of predictive biomarkers (10). Indeed, the correlation between cardiovascular (CV) risk factors, CV comorbidities and FIC remains unclear. Circulating biomarkers, such as proBNP and the cardiac structural proteins troponin I and T (TnI and TnT), useful for the diagnosis and prognosis of patients suffering from heart disease, have a limited role in the identification of FIC (10).

Other suitable and reliable plasma biomarkers could be represented by microRNAs (miRNA), small molecules of 20-22 nucleotides of non-coding RNA with a post-transcriptional regulation function. Different miRNAs are involved in CV diseases, including myocardial infarction, heart failure and cardiac hypertrophy. Certain of them, including miR-1, miR-133, miR-145, miR-208 and miR-499, abundantly expressed in the myocardium, have a diagnostic and prognostic role in cardiac damage (12,13). Consistent results were obtained for doxorubicin-treated patients with breast cancer: in particular, miR-1 is significantly overexpressed in the case of cardiotoxicity and has higher sensitivity compared with the classical TnI dosage (14). However, the ability of circulating miRNAs expression to predict FIC has not been explored.

In the present study, it was aimed to prospectively evaluate incidence, clinical manifestations, risk factors and predictive biomarkers of FIC in FP-treated patients with CRC.

Materials and methods

A monocentric prospective study linked to a real-world intervention in patients with either localized or advanced stage CRC who were eligible for treatment with FP-based therapy (CHECKPOINT trial, NCT02665312), was conducted. Patients were enrolled at Candiolo Cancer Institute, Italy, between January 2016 and February 2020. An accrual of 200 patients was originally planned, however, due to the COVID-19 pandemic and the restrictions applied to reduce not necessary accesses, the enrolment was suspended and the study was prematurely closed. The study was approved (approval no. 251/2015; October, 22nd 2015) by the local review board and by the institutional Ethical Committee of Candiolo Cancer Institute (Turin, Italy).

Inclusion criteria for the study were: lack of any prior treatment with FP, age ≥18 years; histologically confirmed diagnosis of localized or metastatic CRC; a clinical indication to receive a 5-FU or capecitabine-based chemotherapy regimen, according to the current literature and national guidelines (15,16). A personal history of cardiac diseases was not an exclusion criterion for the study. Written informed consents were obtained from all patients before their participation. None of these patients were examined for DPYD variant. Although accumulating data now suggest a correlation between DPYD polymorphisms and FP toxicities, at the time of the present study, such preliminary analyses were not routinely recommended by regulatory authorities (15).

5-FU and capecitabine treatments. The choice of the chemotherapy regimen was not dictated by the study protocol, but was left to clinical judgement according to the treatment setting. 5-FU-based chemotherapy was administered according to the mFOLFOX6, FOLFOX6, FOLFIRI, FOLFOXIRI and DeGramont regimens. Specifically, the doses were given in 2-week cycles. Capecitabine-based chemotherapy (capecitabine monotherapy or CAPOX) was given in 21-day cycles as a 2-week-on/1-week-off regimen (chemotherapy regimens are described in detail at Appendix S1).

Study procedures and FIC assessment. All enrolled patients were evaluated for potential CV risk factors (including physical measurements and collection of physiological, family and past medical history) at the screening visit and divided into two groups, low and high CV risk. Patients were considered at high CV risk in case of i) history of coronary artery disease; ii) uncontrolled hypertension; iii) cerebrovascular accident/stroke within six months prior to the enrolment; iv) chronic heart failure of NYHA Grade II or higher; v) cardiac arrhythmia; vi) significant electrocardiogram (ECG) abnormalities; vii) symptoms considered potentially related to relevant cardiovascular diseases. Patients without any of these conditions were considered at low CV risk. All patients deemed with high CV risk at the screening visit received a cardiological evaluation and cardiac ultrasonography. In this evaluation, the cardiologist could optimize therapy before the start of chemotherapy, if necessary.

In the present study, patients were actively monitored from day 1 (D1) of the first cycle (C1) to day 3 (D3) of the third cycle (C3). During this period, patients underwent complete physical examination (D1, C1-3), blood samples collection for cell blood count and serum biochemical analysis, proBNP dosage, (D1, C1-3), ECG and blood samples for high-sensitivity cardiac TnI levels and miRNAs expression analysis (D1, C1-3 + D3, C1-3), and they filled the patient-reported outcomes (PROs) with a 5-items simplified questionnaire on symptoms and

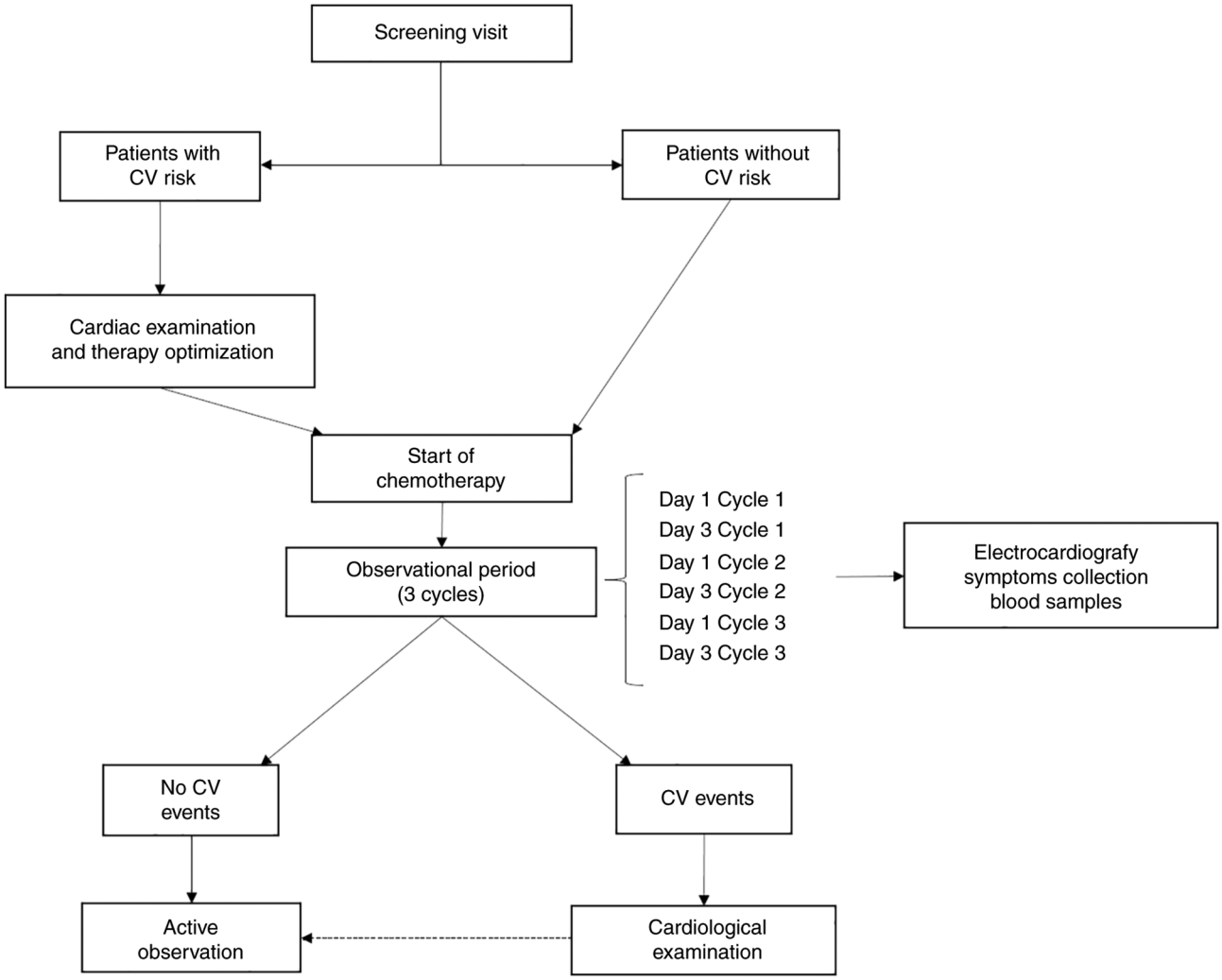

Figure 1. Checkpoint protocol scheme. Enrolled patients were evaluated for CV risk factors and, if resulted at high CV risk, they were submitted to cardiac examination and therapy optimization before starting fluoropyrimidine-based chemotherapy. Patients were evaluated with electrocardiogram and blood sample for TnI and proBNP before start chemotherapy and on day 3 of chemotherapy during the first, second and third cycle. Patients who developed CV events were submitted to TnI, N-terminal-proBNP and cardiac examination. CV, cardiovascular; TnI, troponin I; proBNP, pro hormone BNP.

concomitant medications (D1, C1-3 + D3, C1-3). After the first three cycles, patients continued active observation with PROs collection.

If patients reported symptoms potentially related to cardiotoxicity on other days than D1 or D3, or during the observation period, they were referred for an unscheduled visit with the same aforementioned procedures, including biochemical analyses, ECG and cardiac examination, if needed. The scheme of the trial is presented in Fig. 1.

Cardiovascular toxicities. CV toxicity was defined as the occurrence of any signs, symptoms or ECG changes potentially related to impaired cardiac function. Cardiotoxicity was recorded and graded according to the Common Toxicity Criteria version 4.0, using recorded signs, PROs questionnaires and ECG changes at predefined points (D1, C1-3 + D3, C1-3 and during follow-up). CV events were defined as: significant ECG changes (ST deviation, T inversion/variation, ventricular arrhythmia, new-onset bundle branch block) with or without signs or symptoms; typical chest pain, with or without ECG changes; syncope, with or without ECG changes; dyspnoea or palpitations (of new and sudden onset), with or without ECG changes.

Patients developing CV events were referred within 24 h to cardiological examination, cardiac ultrasonography, blood samples and further investigations and treatments, if considered appropriate by the cardiologist (Fig. 1). Patients developing CV events were treated by the cardiologist and treatment with FP was discontinued or resumed upon clinical judgement.

Cardiac biomarker evaluation. Plasma proBNP and TnI levels were determined at predefined points (D1C1-3 + D3C1-3 and during follow-up). Levels of BNP were determined with the Triage BNP test (Beckman Coulter, Inc.) using the Beckman Access DxI 800 platform. The upper limit of normal was 100 pg/ml. Levels of high-sensitivity TnI were determined by the Access AccuTnI immunometric assay (Beckman Coulter, Inc.) using the Beckman DxI 800 platform. The upper limit of normal was 0.05 ng/ml. Determinations were carried out according to the manufacturer's instructions.

Collection of samples for circulating biomarkers. Two tubes of 7 ml blood samples were drawn at D1 and D3 of the first three cycles of therapy to collect serum (in heparin) and plasma (in EDTA). Briefly, both tubes were centrifuged at room temperature for 10 min at 1,800 x g without brake. Serum was directly collected, while supernatant of EDTA tubes was collected and transferred in a 15 ml tube and centrifuged for further 10 min at 2,200 x g with brake. Both serum and plasma samples were aliquoted in 1.5 ml collection tubes and stored at -80˚C.

miRNA profiling. Total circulating RNA was extracted from 200 μl of plasma using the Mirneasy serum/plasma advanced kit (Qiagen GmbH) following the manufacturer's instructions. To improve RNA extraction, MS2 carrier (Merck Life Science S.r.l.) was added to each sample before lysis step. RNA elution was performed in 30 μl of nuclease-free water.

For miRNA profiling, 4 μl of total RNA of each sample were used and individually processed with the miRNA Complete Labeling and Hybridization kit (Agilent Technologies S.p.A.) and hybridized on 8x60 K oligonucleotides arrays miRbase V21 (Agilent Technologies Italia S.p.A.). After hybridization, washing and scanning, by Agilent G2505C scanner, images were analyzed using Feature Extraction software v10.7 (Agilent Technologies S.p.A). Raw data were processed by means of the limma R library, applying background correction (normexp) and quantile normalization, and averaging miRNA levels when they are represented with the same probe on the array.

Statistical analysis. The primary objective of the present study was to assess the incidence of FIC during FP-based chemotherapy. Secondary objectives were: i) to assess the relationship between FIC and CV risk factors, ii) to investigate the role of known circulating biomarkers of cardiac damage (including TnI, proBNP), iii) to explore the possible role of circulating miRNAs as predictive biomarkers of a CV event.

Two class comparison was then applied to detect differentially expressed miRNAs between patients with and without a CV event, between high and low CV risk patients, and between patients with CV event and low CV risk patients without an event, using the limma R package version 3.52.4 (https://bioconductor.org/packages/release/bioc/html/limma.html). Associations between CV events and patient characteristics were studied by using the Chi-Square test (dichotomous variables). Two-tailed paired t-test was applied to evaluate proBNP changes between C1 and C3 of the first three cycles in both CV event and no CV event patients and according to the chemotherapy administered (5-FU-based vs. capecitabine-based). Two-tailed unpaired t-test was applied to evaluate proBNP changes between CV event and no CV event patients within the same time point. $P<0.05$ was considered to indicate a statistically significant difference. Chi-square and t-tests were performed using the GraphPad Prism software, version 9.0 (GraphPad Software, Inc.).

Univariate logistic regression was used to predict the correlation with the occurrence of a CV event, [95% confidence intervals (CI) for odds ratios, $P<0.05$ considered as statistically significant]. Since none of the single variables provided a significant prediction value, a logistic stepwise analysis was used to identify the variables to retain in a multivariable analysis. These analyses were carried out using the glmnet R package version 4.1-4 (https://cran.r-project.org/web/packages/glmnet/index.html).

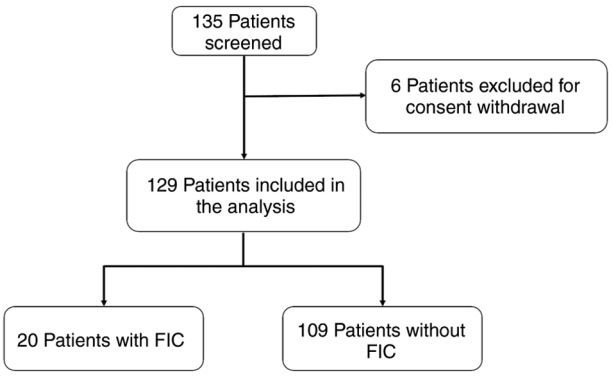

Figure 2. Consort diagram showing the enrolment of patients and their allocation to the analysis.

Results

Study population. A total of 135 patients with CRC were enrolled in the study after the informed consent was signed. A total of 6 patients were excluded from this analysis owing to subsequent consent withdrawal.

A total of 129 consecutive patients with CRC were thus eligible for analysis (Fig. 2). Patient characteristics are listed in Tables I and SI. The median age was 69 years (range 27-83 years), 121 patients (93.8%) initially received 100% dose, while 8 patients (6.2%) were treated with 75% dose. A total of 62 patients (48%) resulted at high CV risk prior to treatment initiation and were referred to the cardiologist for a baseline assessment. Of these, 7 (5.4%) had a history of acute myocardial infarction, while 21 (16.3%) had other types of cardiac disease, including coronary artery disease (n=3), atrial fibrillation (n=5), other cardiac arrhythmias or previous syncopal episodes (n=7), heart failure history (n=2), and heart valve disease (n=1).

Cardiotoxicity. A total of 20 out of 129 patients (15.5%) experienced one or more symptoms of FIC. The most common symptoms were dyspnoea (12 of 20 patients, 60%), chest pain (8 patients, 40%) and palpitations (8 patients, 40%) (Table SII). A total of 3 patients (15%) had clinically relevant ECG changes. One patient had new-onset supraventricular paroxysmal tachycardia, one had a new-onset left bundle branch block, and one had an ST deviation. The latter case showed also elevated TnI levels and was diagnosed as acute myocardial infarction. No patient had a lethal outcome. The first occurrence of FIC was at the first cycle for 7 of 20 patients (35%), at the second for 4 patients (20%) and at the third for 7 patients (25%). A total of 3 other patients (15%) experienced FIC after the third cycle.

A cardiological treatment was initiated in 6 of these patients. FP-based chemotherapy was resumed in 18 cases: 14 patients were retreated with the same FP dose without initiation of cardiac therapy, and 4 were retreated with the same FP dose but received concomitant cardiac therapy with angiotensin-converting enzyme inhibitors, antiarrhythmics or

Table I. Patient characteristics and pre-existing disease of patients at baseline.

Patients	Number	Percentage of patients (%)
Sex		
Female	51	39.5
Male	78	60.5
Primary site		
Colon	106	82.2
Rectum	23	17.8
Age		
<70 years	66	51.2
≥70 years	63	48.8
FP		
Capecitabine	60	46.5
5-fluoropyrimidine	69	53.5
Setting		
Adjuvant	68	52.7
Metastatic	61	47.3
Cardiological risk		
Low	67	51.9
High	62	48.1
Body mass index		
<25	55	42.6
≥25	74	57.4
Smoke		
Current or former	68	52.7
Never	61	47.3
Alcohol intake (< or ≥10 g per day)		
Yes	72	55.8
Never	57	44.2
Sedentary (< or ≥30 min aerobic activity/3 times per week)		
No	53	41.1
Yes	75	58.1
NA	1	0.8
Hypertension		
Yes	71	55
No	58	45
Diabetes		
Yes	24	18.6
No	105	81.4
Dyslipidaemia		
Yes	39	30.2
No	88	68.2
NA	2	1.6
Myocardial infarction		
Yes	7	5.4
No	121	93.8
NA	1	0.8
Stroke		
Yes	4	3.1
No	124	96.1
NA	1	0.8
Heart disease		
Yes	21	16.3
No	107	82.9
NA	1	0.8

beta-blockers. In these patients, neither further interventions nor dose reductions were needed.

Only one of the 14 patients treated with full dose FP-based chemotherapy and who had not received new cardiological therapy had recurrent symptoms at retreatment. This patient had chest pain, palpitation and dyspnoea during C2 of adjuvant mFOLFOX6. A complete cardiological assessment was performed, including coronary angiography, without evidence of cardiac disease; thus, FP treatment was resumed under strict cardiac monitoring. Nevertheless, during C6, he presented a symptomatic recurrence with chest pain, dyspnoea, elevated cardiac enzymes and ST deviation that required hospitalization. She had a complete recovery from this acute event, but FP treatment was discontinued.

The patient with new onset of left branch block had his FP-based chemotherapy discontinued with the resolution of the aberrant ECG pattern. The patient with supraventricular paroxysmal tachycardia was treated with antiarrhythmics with a symptomatic resolution, but FP treatment was discontinued by cardiologist's decision. Nine events of FIC occurred in the subgroup of patients with cardiac comorbidity (n=62) (14.5%), a similar rate (P=0.77) to the one observed among patients without overt cardiac comorbidities (n=67) (11 events, 16.4%).

Correlation between CV event and clinical pathological characteristics. Firstly, we investigated a potential association of the occurrence of a CV event with CV risk factors, tumor type, and FP-based chemotherapy regimen. As shown in Table II, only sex was minimally associated with CV events, with female patients displaying a higher risk (30%, vs. male 11.6%; P=0.05). A trend was observed for higher body mass index (BMI) and capecitabine-based regimens (P=0.07 for both).

Univariate logistic regression was also applied to examine any association of CV events with age, single risk factors (smoke, alcohol intake, BMI, diabetes, hypertension, sedentary habits, and cholesterol), type of chemotherapy (5-FU/capecitabine), or inflammatory indexes (i.e. neutrophils/lymphocytes ratio or platelets). None of the variables tested was able to predict the occurrence of a CV event. Low infusion volume displayed the lowest P-value (0.06), showing a modest protective effect (Table III).

Stepwise logistic regression selected sex and alcohol intake (defined as < or ≥10 g per day) as variables to perform multiple logistic regression. As revealed in Table IV, the chance

Table II. Contingency table (2x2) analyzed by χ-square test (P<0.05 as statistically significant).

	FIC	No FIC	P-value (Chi square test)
Primary site			0.32
Colon	18	88	
Rectum	2	21	
Tumor stage			0.09
Non-metastatic	14	54	
Metastatic	6	55	
Sex			0.05
Male	8	69	
Female	12	40	
Age at diagnosis			0.4
≤70 years	13	60	
>70 years	7	49	
Fluoropyrimidine based regimen			0.07
5-FU-based	7	62	
Capecitabine-based	13	47	
Cardiovascular risk at screening			0.76
Low	11	56	
High	9	53	
Smoke			0.45
Yes	9	59	
No	11	50	
Alcohol			0.16
Yes	14	58	
No	6	51	
Hypertension			0.62
Yes	10	61	
No	10	48	
Concomitant heart disease			0.26
Yes	5	16	
No	15	92	
Diabetes			0.28
Yes	2	22	
No	18	86	
Body mass index			0.07
≤25 kg/m^2	15	58	
>25 kg/m^2	5	51	

FIC, fluoropyrimidine-induced cardiotoxicity.

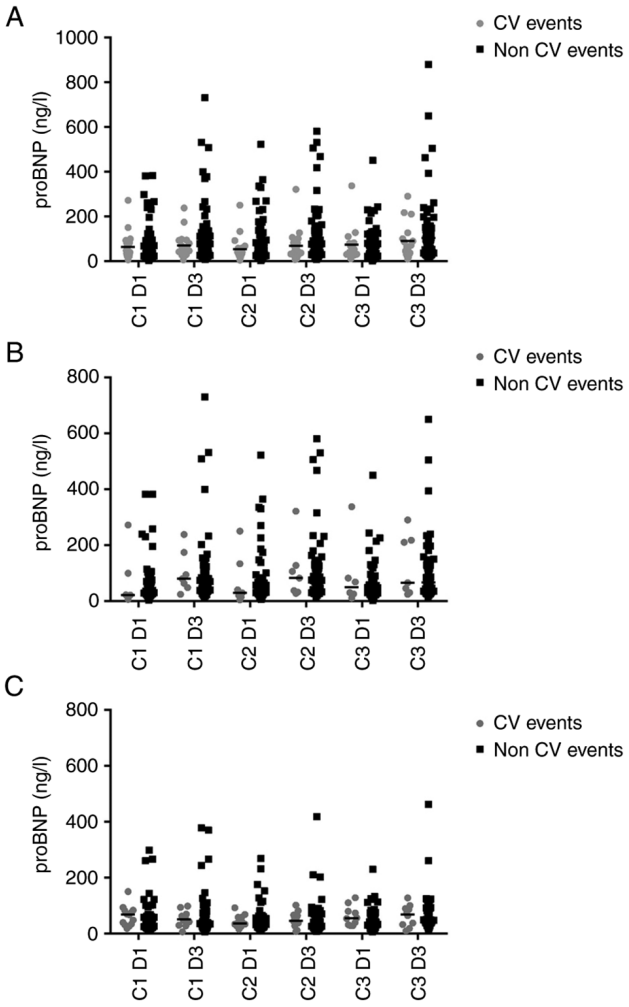

Figure 3. proBNP values modifications in CV event and no CV event patients at D1 and D3 of the first three cycles of therapy. (A) proBNP values independently from the therapy received. (B) proBNP values in patients treated with 5-fluorouracil. (C) proBNP values in patients treated with capecitabine. proBNP, pro hormone BNP; CV, cardiovascular.

of having a CV event in males who drank less than 10 g of alcohol per day was very low [exp(b_0)=0.04]. The chance of CV among females who drank less than 10 g per day is exp(b_1) times the chance of the reference group and among males who drink more than 10 g per day is exp(b_2) times the chance of the reference group, which gives 0.17 for both groups and remains very low. However, among women who drank more than 10 g per day, the chance of a CV event increased to 0.7 [exp(b_1+b_2) times exp(b_0)].

Circulating biomarkers of FIC. Plasma TnI increased to 0.07 g/l in the patient experiencing acute myocardial infarction during 5-FU treatment, while remaining below the lower limit of detection in all other patients. For this reason, the statistical analysis for this variable was not performed. The influence of FP-based chemotherapy on proBNP levels is demonstrated in Fig. 3A; proBNP values changes considering 5-FU and capecitabine separately are presented in Fig. 3B and C.

No significant differences were observed in proBNP levels, measured at the same time point, between patients who experienced FIC and those who did not (Table SIII).

The proBNP levels registered in the observational period increased after drug administration in patients without FIC, but they did not significantly change in patients with FIC (Table SIV). In particular, in patients without FIC, there was an increase of proBNP in patients treated with 5-FU.

Table III. Association between clinical-pathological characteristics and occurrence of CV event analyzed by univariate logistic regression.

	Univariate analysis	
Characteristics of patients	Odds ratio (95% confidence interval)	P-value
Age (≤70 vs. >70)	0.971 (0.934-1.012)	0.15
Sex (female vs. male)	2.18 (0.805-5.654)	0.13
Therapy (5-FU vs. capecitabine)	0.620 (0.232-1.618)	0.33
Type of infusion (low vs. high volume)	0.377 (0.126-1.014)	0.06
Risk factor		
Body mass index (≤25 vs. >25 kg/m^2)	0.949 (0.859-1.045)	0.29
Smoke (yes vs. no)	0.544 (0.199-1.421)	0.22
Alcohol (yes vs. no)	2.052 (0.762-6.155)	0.17
Hypertension (yes vs. no)	0.786 (0.299-2.066)	0.62
Diabetes mellitus (yes vs. no)	0.439 (0.067-1.682)	0.29
Sedentary (yes vs. no)	1.071 (0.409-2.935)	0.89
Previous Heart diseases (yes vs. no)	1.917 (0.562-5.766)	0.26
Basal CV risk (high vs. low)	0.679 (0.248-1.772)	0.43
Hematochemical indexes		
Lactate dehydrogenase (≤ vs. >300 U/l)	0.100 (0.996-1.001)	0.5
Haemoglobin (≤10 vs. >10 g/dl)	0.935 (0.714-1.122)	0.58
Platelets (≤400 vs. >400 10^3/mm^3)	0.100 (0.993-1.004)	0.78
Neutrophil/Lymphocyte ratio (≤5 vs. >5)	1.063 (0.825-1.310)	0.59

CV, cardiovascular.

Table IV. Association between sex, alcohol intake and occurrence of cardiovascular event, analyzed by multivariable logistic regression.

	Coefficients	SE	95% CI	Odds ratio (95% CI)	P-value
Intercept	b_0=-3,239	0,6674	-4,649; -2,029		
Sex (female vs. male)	b_1=1,438	0,5855	0,3108; 2,626	4.12 (1.364-13.81)	0.01
Alcohol (yes vs. no)	b_2=1,441	0,6211	0,2741; 2,731	4.225 (1.315-15.35)	0.02

CI, confidence interval.

Baseline circulating miRNA profiling in the plasma of 8 patients who experienced a CV event was compared with that of 16 patients without CV event. In Table SV, clinical and pathological characteristics of this subset of patients are reported.

No significant differences in terms of miRNA expression were found comparing patients with and without a CV event. Similarly, a lack of difference was observed when comparing high CV risk with low CV risk patients. Since the number of CV events was slightly higher in low CV risk patients, miRNA profiling of patients experiencing CV events was compared with control patients, selecting among them only those at low CV risk. In this analysis, three downregulated miRNAs were identified in CV-events patients: hsa-miR-6089, which regulates the expression of genes involved in inflammation processes and is already described as a biomarker of stroke and vascular damage (17); hsa-miR-4459, which stimulates endothelial cells, promotes autophagy and regulates atherosclerosis through a miRNA-long non coding RNA loop (18) and has already been described as a putative biomarker of cardiovascular risk; hsa-miR-4505, which did not have any known role in CV risk or disease.

Discussion

In this prospective observational study, incidence and clinical manifestations of FIC were evaluated in patients with CRC. The combination of female sex and high alcohol intake was identified as risk factor for FIC development.

The observed incidence of FIC, 15.5%, is in stark contrast with the general notion that this is an infrequent complication. The existing literature displays a moderate-high variability in the delineation of this value, to which different factors can contribute: retrospective nature, selection bias, differences in CV risk profiles of the analysed samples, and, importantly, the lack of a shared and widely accepted standard for a practical definition of FIC.

In the present study, a collection of PROs together with an objective assessment of the cardiac function was proposed. This led to the individuation and inclusion in our cohort of asymptomatic and mildly symptomatic patients with cardiovascular disease, potentially excluded from other prospective or retrospective analyses. Suspected FIC cases were subject to a cardiological verification.

In the present trial, 40% of patients with FIC reported chest pain, but only 15% presented an ECG alteration. Most of the patients with chest pain and normal ECG had also normal coronary enzymes, whereas severe events, such as acute myocardial infarction, were rare. This pattern is in accordance with other studies. Both the sudden onset of chest pain and the rarity of the occurrence of life-threatening complications support the pathogenetic hypothesis of a FP-induced transient vasospastic mechanism. In our experience, this was further corroborated by the subsequent angiographic finding of normal coronary arteries in the patient who had experienced FP-related acute myocardial infarction.

In line with this hypothesis, only a minority of patients displayed objective signs (e.g. ECG changes) of cardiotoxicity, and FIC was initially suspected on the clinical relevance of subjective symptoms. In this context, the physician's assessment of the patient's symptoms takes on a relevant role, passing from a passive registration of symptomatology to active discrimination of cardiac events.

Compared with other case series, the symptoms reported were prospectively and actively analyzed according to a global patient evaluation. This led to the discrimination between non-specific symptoms and clinically relevant cardiological events, showing the actual FIC incidence in the real-world CRC population. As an example, patient 13 was correctly identified at C2 as a CV event and a consequent cardiological assessment was performed. Nevertheless, due to the completely negative cardiological work-up, retreatment with FP was performed with strict monitoring and patient experienced an acute CV event at C6.

Except for this single event, retreatment with FP after the occurrence of cardiotoxicity was attempted in all other patients without any FIC recurrence. Overall, out of 18 patients experiencing FIC, 4 started a cardiological protective therapy without recurrence, whilst among 14 patients who were not prescribed any further cardiological therapy, only one experienced a FIC recurrence. The real value of cardiological therapy and FP dose reduction is questionable. A retrospective study on 668 patients and a prospective study on 644 patients treated with 5-FU or capecitabine reported a benefit from initiation of a prophylactic cardiological therapy that prevented symptoms at retreatment in 9 out of 12 patients and 12 out of 15 patients, respectively (19,20). By contrast, a small and non-randomised study could not demonstrate a prophylactic effect of calcium channel blockers on the occurrence of cardiotoxicity (21). With the limitation of the small numbers, the complex of these data suggested that resumption of FP-based chemotherapy is a generally feasible strategy with the following caveats: i) however carefully conducted, selection of patients to be subject to retreatment is rendered difficult by the proteiform manifestations of FIC; ii) rather than pharmacological secondary prevention, close cardiac monitoring is crucial; iii) retreatment requires considerable precaution given the unpredictable time-cardiotoxicity correlation. As more general considerations, the added value offered in this context by the collaboration between the oncologist and the cardiologist/cardioncologist should be noted, as well as the need for a frank discussion with the patient, in which duty of the oncologist is to carefully review the risk/benefit balance of resuming the treatment.

The multivariate logistic regression, applied to sex and alcohol consumption, revealed that both males and females are more protected from CV events if they do not consume alcohol; the subpopulation of females drinking more than 10 g of alcohol per day are the most at risk to FIC development and thus deserve particular attention.

Pursuing the objective of a real-world study, 44.6% of patients with high CV risk factors (such as myocardial infarction history) were included in the present cohort. Nevertheless, a predictive role for FIC was not demonstrated for pre-existing cardiac disease. High-risk subgroups cannot be identified on these bases, and, since FIC can be lethal in patients without a history of cardiac disease, attention must be paid in all FP-treated patients indiscriminately.

Although previous studies suggested that capecitabine is more likely to induce cardiotoxicity compared with 5-FU chemotherapy (20,22), results from the literature are contradictory (11,19). In our cohort, a comparable incidence of FIC between the oral pro-drug and the infusion regimens was observed.

Based on these results, careful monitoring of possible FIC throughout in all patients is recommended regardless of CV comorbidities, FP chemotherapy regimen, through all treatment cycles and, particularly, upon the occurrence of mildly symptomatic FIC. An effective work-up should be applied (e.g. ECG monitoring or use of cardiac therapy) but, in relation to its unpredictable nature, strict monitoring must be performed to avoid lethal events, particularly in the adjuvant setting, in which FP have a curative role and the risk-benefits should be carefully considered.

Albeit routinely used in the clinical management of cardiac disease, TnI and proBNP cannot be efficiently used for FIC prediction. Particularly, neither significantly changed their expression in CV-event patients at any cycle of treatment, except for patient 17 in which a TnI elevation was observed at the recurrence. No variation in proBNP expression between FIC and no FIC patients was found. A statistically significant increase of proBNP levels between D3 and D1 of all the first three cycles was observed only in patients treated with 5-FU; a potential explanation could be the cardiac parietal stress exerted by the transient mild hypervolemia due to the fluids infused during the 5-FU-based chemotherapy administration.

The exploratory analysis of miRNA profiling conducted on a small subset of patients who experienced FIC (n=8) and 16 control patients, at baseline, revealed no significant

differences in terms of miRNA expression. A comparison between patients who experienced FIC and low risk controls revealed three differentially expressed microRNAs, two of which had been previously associated with cardiovascular diseases. Exploration of miRNA expression, either as a baseline regulators of enzymes activities or as modulators induced by treatment with chemotherapy and targeted therapy, is a promising field (23-25). Nevertheless, this was not pursued as the present study aimed to identify potential predictive biomarkers in FP-naïve patients; for this reason, plasma samples collected after the completion of FP treatment were not analyzed.

Certain limitations to the present study need to be discussed. Due to the early closure of accrual, a smaller sample size than planned was obtained, resulting in a low number of events (20 CV events) leading to wide CIs, low statistical power and increased risk of type II statistical errors. This, moreover, reduces the number of risk factors which could be could confidently examined in our multivariate model. The low number of events and multiple testing (increasing the risk of false-positive results) are weaknesses of most studies analysing risk factors and render the conclusions that can be drawn from these studies less valid and may conceal clinically significant differences between events and no event.

Lastly, the DPYD status was not available. While DPYD polymorphisms exert a well-known influence on enzymatic activity, FP catabolism and toxicity, the impact of the enzyme deficiency on cardiotoxicity remains unclear but appears to be limited.

In a large prospective study, 487 oncologic patients treated with a 5-FU-based chemotherapy regimen were characterized for single nucleotide polymorphisms (SNPs) of DPYD. Although 187 patients had SNPs, only 4 of these patients (2%) developed severe (grade 3 or 4) cardiotoxicity (26).

Nevertheless, DPYD polymorphisms are not the only factors that could affect DPDYD enzyme activities. The miR-27 level expression has been linked to the function of the DPD enzyme in the liver cells, and p53 status plays an important role in controlling pyrimidine catabolism through repression of DPYD expression (25,27). In addition, even abnormalities of thymidylate synthase, the main target of FP actions, appear to predict the risk of severe toxicity and the combination of DPYD and TYMS genotyping could identify ≥50% of patients at the greatest risk of adverse events (28).

However, differently from most other large studies on this topic, the present study is a monocentric, rigorous, prospective trial. This may result in a minor inter-variability in symptomatology assessment and cardiological evaluation, but it may not reflect the characteristics of the general population and practice patterns.

In conclusion, cardiotoxicity can be a life-threatening, unexpected complication of FP therapy that limits the appropriate treatment of a highly morbid and aggressive cancer. Despite females with an alcohol consumption habit being more prone to experience FIC at our institution, FIC was not clearly associated with classical CV comorbidities. Consequently, optimal information about the risk and close monitoring represent the best option to prevent serious events and lethal outcome in all patient subgroups, regardless of common risk factors.

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Availability of data and materials

Raw and processed data or miRNA analysis are available on Gene Expression Omnibus (GEO) database (accession no. GSE217768).

Authors' contributions

ID, AB and FL conceived and designed the present study. PA, GA, AB, ID, EF, RF, VQ and MM collected the data. PA, GA, CPN, MB, GCa, LG, CC, MA, PO and GCh analyzed and interpreted the results. PL, GA, CPN, RF and FL prepared the draft of the manuscript. All authors reviewed the results, read and approved the final version of the manuscript. CPN and GCh confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 251/2015; October, 22nd 2015) by the Ethics Committee of Candiolo Cancer Institute (Turin, Italy). Written informed consent was provided by all patients before the start of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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