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Research Article

Biological Aging and Chemotoxicity in Patients with Colorectal Cancer: A Secondary Data Analysis Using EHR Data

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Abstract: Background. Biological aging influences cancer outcomes, but its changes during chemotherapy and impact on chemotoxicity in colorectal cancer (CRC) remain underinvestigated. We examined (1) trajectories of biological aging (using Levine Phenotypic Age) during six months of chemotherapy, (2) sociodemographic and clinical risk factors for biological aging, and (3) links between biological aging and chemotoxicity. **Methods.** Using electronic health records data (2013–2019) from 1,129 adult CRC patients, we computed Levine Phenotypic Age and age acceleration (Levine Phenotypic Age–chronological age) from routine blood tests (e.g., complete blood counts, hepatorenal/inflammatory markers). Chemotoxicity was identified primarily via International Classification of Diseases (ICD-9/10) codes. **Results.** Chemotherapy accelerated biological aging. Biological aging (i.e., Levine Phenotypic Age and its age acceleration) at baseline and changes over time, predicted chemotoxicity. However, changes in biological aging over time showed stronger associations than baseline biological aging. **Advanced cancer stages, higher comorbidity burden, and socioeconomic disadvantage (especially area-level deprivation) were associated with accelerated biological aging at baseline and over time.** Biological aging occurred across both young and older adults. **Conclusions.** Levine Phenotypic Age, computed from routine blood tests in EHRs, offers a feasible clinical tool for aging-related chemotoxicity risk stratification. Validation in diverse cohorts and the development of predictive models are needed.

Keywords: biological aging; age acceleration; risk factors; chemotoxicity; colorectal cancer; Levine Phenotypic Age; electronic health records.

1. Introduction

Colorectal cancer (CRC) remains a significant public health burden in the United States, being the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths [1]. While advancements in screening, advanced treatment modalities, and systemic therapies have improved survival rates, leading to a growing population of CRC survivors, these individuals often face substantial long-term challenges due to chemotoxicity and related side effects [1]. Over the past two decades, the 5-year survival rate for CRC has increased, with approximately 65% of patients surviving beyond this milestone [2]. This rise in survivorship is attributed to earlier detection through colonoscopy screening and more effective treatment regimens, including the first-line chemotherapy (i.e., 5-Fluorouracil [5-FU]-based chemotherapy) [3]. However, a critical consequence of improved survival is the increasing number of patients experiencing persistent chemotoxicity and adverse symptoms following treatment.

A major challenge for CRC survivors is the substantial burden of chemotherapy-induced side effects, with approximately 50% of patients reporting such effects. These toxicities typically emerge during the first six months of first-line treatment and commonly include gastrointestinal (GI) complications and hematological disorders [4]. These adverse effects not only impair quality of life but may also result in early treatment discontinuation, emergency room visits, hospitalizations, dose reductions, cancer-related mortality, disease recurrence, and long-term functional impairments [4-6]. Current evidence indicates that between 40% and 70% of CRC survivors continue to experience persistent chemotherapy-related toxicity even after treatment completion, significantly compromising their daily functioning and overall well-being [6].

The growing population of CRC survivors and the high prevalence of treatment-related morbidity underscore the urgent need to further understand the mechanisms underlying chemotoxicity and develop targeted interventions to alleviate these complications [7,8]. Sensitive biomarkers of chemotoxicity remain limited due to inconclusive findings (e.g., variations by cancer stage), non-modifiable factors (e.g., race), or factors difficult to modify at the individual level (e.g., socioeconomic status, geographic disparities) [9]. However, emerging evidence suggests that biological aging processes may contribute to chemotherapy-induced toxicity in cancer patients [9]. Specifically, several modifiable (e.g., lifestyles, stress, and pro-inflammatory status) and non-modifiable (e.g., race, area-level socioeconomic status) factors are shown to be associated with chemotoxicity in cancer patients [4,10]. These risk factors are closely associated with measures of biological aging; thus, biological aging markers may potentially be used as a sensitive risk factor for chemotoxicity.

Cancer patients frequently exhibit accelerated biological aging, wherein their physiological state appears significantly older than their chronological age would suggest, compared to healthy individuals [11-13]. This phenomenon is more evident in cancer patients than healthy individuals, who may maintain robust physical and cognitive function well into older age [11,14]. The accelerated aging observed in cancer patients manifests through multiple clinical dimensions, including premature frailty, increased vulnerability to age-related diseases, and functional decline in daily activities [15,16]. These effects stem largely from the unintended consequences of cancer treatments, which can trigger fundamental aging processes at the cellular and molecular level [11,17,18]. For example, therapy-induced DNA damage and epigenetic alterations, such as aberrant DNA methylation patterns, further contribute to the accelerated aging phenotype by disrupting normal cellular function and gene regulation [19,20]. Epidemiological studies demonstrate substantially higher rates of frailty among cancer survivors compared to age-matched controls [21], with hematopoietic cell transplant recipients showing particularly pronounced effects [22]. The risk of developing secondary malignancies increases dramatically in survivors, with childhood cancer patients facing a 20% cumulative incidence of subsequent neoplasms over 30 years [23]. Cardiovascular disease, cognitive decline, and other age-related conditions also emerge earlier in cancer survivors [24,25]. To our knowledge, only a few studies in other cancer types [26-28] showed

potential links between biological aging markers and overall chemotoxicity incidences and cancer survival. The link between biological aging markers and chemotoxicity in CRC is unknown.

Biological age represents an individual's functional and physiological condition compared to their actual chronological age, serving as an indicator of the biological aging processes [29]. Recent advances in aging research have introduced multiple biomarker-based approaches to quantify biological aging more precisely. Among these, epigenetic clocks such as the Horvath, Hannum, and GrimAge models analyze DNA methylation patterns and exhibit robust correlations with lifespan and age-related health outcomes [30]. However, these epigenetic techniques typically involve complex laboratory analyses and substantial costs, making them impractical for widespread epidemiological research [31]. As an alternative, Levine Phenotypic Age offers a more practical solution by combining routine peripheral blood tests – including albumin, creatinine, glucose, hepatic renal functions, and C-reactive protein levels using routine circulatory blood samples – to evaluate biological aging comprehensively, without requiring further blood assays [32]. This approach proves particularly valuable for large-scale studies due to its cost-effectiveness and accessibility. Levine Phenotypic Age acceleration quantifies the difference between an individual's biological and chronological ages, where positive values suggest age acceleration [32]. Existing research has established significant relationships between Phenotypic Age acceleration and mortality risks in various chronic conditions, including cardiovascular disease and diabetes [24,25]. However, the utility of Levine Phenotypic Age for measuring longitudinal changes in biological aging (both absolute values and age acceleration) during chemotherapy remains unexplored, along with the associated risk factors and their impact on chemotoxicity in CRC patients receiving 5-FU-based chemotherapy.

To address this knowledge gap, this study utilized electronic health record (EHR) data from The Ohio State University Comprehensive Cancer Center to investigate: (1) the change of biological aging as measured by Levine Phenotypic Age (without requiring further blood assays) over six months of 5-FU-based chemotherapy, (2) potential risk factors for accelerated aging, and (3) the relationship between biological aging and chemotoxicity in patients with CRC. We hypothesized that elevated biological age and age acceleration at baseline (i.e., <30 days before chemotherapy initiation) and the relative increase in these measures during 6-month chemotherapy would be associated with increased chemotoxicity. These findings could improve our understanding of aging dynamics in CRC patients undergoing chemotherapy and inform the development of personalized tools for screening chemotoxicity risk and optimizing long-term patient management strategies.

2. Materials and Methods

2.1. Data Collection

Our study employed a retrospective cohort design using de-identified EHR data from The Ohio State University Comprehensive Cancer Center, a major academic medical institution serving Ohio and surrounding Midwestern regions. Data spanned January 2013 through December 2019, with patient identification and data extraction performed by the institution's Honest Broker Operations Committee in full compliance with HIPAA regulations. The analysis focused on 1,129 adult patients (age ≥ 18 years) with stage II-III CRC who completed 8-12 cycles of 5-FU-based chemotherapy (including 5-FU monotherapy, Xeloda, or FOLFOX regimens) following tumor resection without stoma. Inclusion criteria included patients with a single primary CRC diagnosis, available routine blood test results at baseline (< 30 days before starting chemotherapy) and 6-month post-treatment initiation, documented sociodemographic characteristics, and residential zip code information. Exclusion criteria included patients with active ostomies, chronic GI conditions, steroid/immunosuppressant use, a history of neoadjuvant therapy, and/or concurrent radiation/immunotherapy, pregnancy status, or incomplete medical records at either baseline or 6-month follow-up.

2.2. Measurements

Patient Characteristics

The dataset incorporated comprehensive demographic (race, ethnicity, age, sex, marital status) and clinical variables (cancer sites, types of chemotherapy, history of other cancer treatments, cancer stages, body mass index, comorbidities, and lifestyle factors).

Biological Age Assessment

Levine's Phenotypic Age formula [32] was implemented using nine routinely measured biomarkers: albumin, alkaline phosphatase, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean cell volume, red cell distribution width, and white blood cell count at baseline (<30 days before starting a chemotherapy) and 6 months after chemotherapy initiation.

The calculation formula for Levine Phenotypic Age

$$\text{Levine Pheno Age} = 141.50 + \frac{\text{Ln}[-0.00553 \times \text{Ln}(\exp(\frac{-1.51714 \times \exp(xb)}{0.0076927}))]}{0.09165}$$

where $xb = -19.907 - 0.0336 \times \text{Albumin (g/L)} + 0.0095 \times \text{Creatinine } (\mu\text{mol/L}) + 0.1953 \times \text{Glucose (mmol/L)} + 0.0954 \times \text{LnCRP (mg/dL)} - 0.0120 \times \text{Lymphocyte Percent (\%)} + 0.0268 \times \text{Mean Cell Volume (fL)} + 0.3306 \times \text{Red Cell Distribution Width (\%)} + 0.00188 \times \text{Alkaline Phosphatase (U/L)} + 0.0554 \times \text{White Blood Cell Count (1000 cells/ } \mu\text{L)} + 0.0804 \times \text{chronological age (years)}$.

Biological Age Acceleration. We computed the biological age acceleration as Levine Phenotypic Age – Chronological Age at the time of biomarker collection [32].

Social Determinants of Health (SDOH)

We included available SDOH in the EHR data, including insurance type, marital status and employment information. Geographic disparities were quantified using the Area Deprivation Index (ADI) [33,34], a composite metric (scale 1-100) incorporating 17 neighborhood-level socioeconomic indicators. Patient zip codes were matched to 2015 ADI data, with scores categorized into tertiles denoting relative disadvantages.

Chemotherapy Toxicity Evaluation

Global, GI, and Hematological toxicities. Treatment-related adverse events were tracked for six months post-initiation, combining incidences reported as: (1) clinician-reported Common Terminology Criteria for Adverse Events (CTCAE), v5.0 graded events (mild to life-threatening), and (2) relevant ICD-9/10 codes. In cancer clinical studies, adverse events (AEs) are documented and categorized according to the U.S. National Cancer Institute's (NCI) CTCAE. It is validated in cancer patients (reliability = 0.95, and high validity [35]). The positive toxicity cases required at least one positive documentation for 6 months of chemotherapy [35].

2.3. Statistical Methods

Descriptive statistics characterized the study population. We utilized IBM SPSS Statistics (version 28.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were computed for all variables, including means ± standard deviations or standard errors for continuous variables and frequencies (percentages) for categorical variables. First, we examined changes in biological aging (“original values of biological age” and “age acceleration: biological age-chronological age”) from baseline to 6 months after chemotherapy initiation, using the paired t-tests. Second, we examined the associations of potential risk factors with biological aging at baseline, 6 months after chemotherapy initiation, and changes in biological aging over time for 6 months, and also examined the associations of biological aging measures with chemotoxicity using a one-way analysis of variance (ANOVA). All ANOVA models met the assumptions of normality and homogeneity of variance. Lastly, we performed both unadjusted and adjusted logistic regression analyses (with Odds Ratio [OR] and 95% Confidence Interval [CI]) to examine the impact of biological aging measures on chemotoxicity outcomes. Adjusted models controlled for covariates and other significant risk factors of chemotoxicity. In our study, we identified covariates based on the literature and Table 2 showing some relationships with biological aging and chemotoxicity outcomes, including age groups, employment status, cancer

stages, comorbidities, marital status, employment status, and ADI measured at baseline. The variance inflation factor (VIF) among independent variables ranged from 1.1 to 3.7, indicating no multicollinearity in our analyses. Analyses were conducted using SPSS Version 28 (Chicago, IL) with a two-sided significance level of 0.05. Multiple comparison corrections were not applied as our study was a hypothesis-driven study [36].

3. Results

3.1. Participants Characteristics

Table 1 provides a summary of study participant characteristics, biological aging and chemotoxicity data in our study. The study population consisted of 1,129 CRC patients with a mean chronological age of 57.3 years (standard deviation 13.7, range 18-89). The cohort comprised 53.4% female participants, with racial/ethnic distribution showing 68.1% Non-Hispanic White, 29.0% Non-Hispanic Black, and 2.9% other racial/ethnic groups. No Hispanic patients were identified in our dataset. 55.8% of patients had colon cancer only, 34.5% had rectal cancer only, and 9.7% had cancer involving both sites. The majority (62.2%) were stage II, while 37.8% were stage III. Nearly 60% (59.7%) had a modified comorbidity index score above 2, indicating frequent comorbidities. The majority received GI surgery (79.7%), radiation (17.2%), and immunotherapy (5.1%). Health behaviors included 17.1% current smokers, 21.6% heavy alcohol users, and 42.9% reporting regular physical activity. Insurance coverage showed 61.5% with private insurance and 38.5% with Medicare/Medicaid. 56.0% of patients were married/partnered, and 44.0% were divorced/widowed/single. The majority of participants (42.0%) lived in moderately disadvantaged areas (ADI 34–66), while 33.0% resided in the least disadvantaged neighborhoods (ADI 0–33) and 25.0% in the most disadvantaged (ADI 67–100). Nearly half (47.1%) were currently employed, 27.6% were unemployed, and a quarter (25.4%) were retired.

Biological age. The biological age, as measured by Levine Phenotypic Age, showed an average increase from 59.1 years before chemotherapy to 61.8 years after six months of treatment, representing a mean increase of 2.7 years (Table 1). The age acceleration (i.e., difference between biological and chronological age) widened from 1.2 years at baseline to 2.8 years post-treatment, indicating accelerated aging worsened during chemotherapy.

Chemotoxicity. Chemotoxicity was frequently observed, with 56.3% experiencing global chemotoxicity, 40.8% GI toxicity, and 22.8% hematological toxicity. These findings collectively demonstrate significant biological aging effects and substantial treatment-related morbidity in this colorectal cancer population undergoing chemotherapy.

Table 1. Characteristics of participants.

		Total samples (N = 1,129)
Demographic Factors		
Age (years), mean ± SD (range)		57.3 ± 13.7 (18-89)
Female (n, %)		603 (53.4)
Race/Ethnicity (n, %):	Non-Hispanic White	769 (68.1)
	Non-Hispanic Black	327 (29.0)
	Non-Hispanic Other	33 (2.9)
Clinical Factors (n, %)		
Cancer Site:	Colon only	630 (55.8)
	Rectal only	389 (34.5)
	Colon and Rectal	110 (9.7)
Cancer stages	II	702 (62.2)
	III	427 (37.8)
5-FU (fluorouracil)-based chemotherapy regimens with an average of 2,600mg/m ² and 8 cycles		

FOLFOX (infusion)	459 (40.6)
FOLFIRI (infusion)	325 (28.8)
CAPEOX (oral)	199 (17.6)
Single-Agent 5-FU (infusion)	146 (13.0)
Body mass index (BMI)	Obese (≥30)
226 (20.0)	
	Overweight (25≤ <30)
452 (40.0)	
	Normal (21≤ <25)
395 (35.0)	
	Underweight (<21)
56 (5.0)	
Modified Comorbidity Index (≥2)	1,035 (59.7)
Previous cancer treatment history	
Radiation	172 (17.2)
Immunotherapy	58 (5.1)
GI surgery	881 (79.7)
Cancer Health Behaviors (Yes)	
Current Smoking Status	193 (17.1)
Current Heavy Alcohol Use	244 (21.6)
Regular Physical Activity	482 (42.9)
Social Determinants of Health (SDOH) (n, %)	
Primary Insurance Types: Private	694 (61.5)
Medicare/Medicaid	435 (38.5)
Marital status: Married/Partnered	632 (56.0)
Divorced/Widowed/Single	497 (44.0)
Area Deprivation Index (ADI), Tertile: 0-33	372 (33.0)
34-66	470 (42.0)
67-100	287 (25.0)
Employment Status: Employed	531 (47.1)
Unemployed	311 (27.6)
Retired	287 (25.4)
Biological Age	
Levine Phenotypic Age, mean ± SD (range)	
• at T0 (before chemotherapy)	59.1 ± 13.8 (19-95)
• at T1 (6 months after chemotherapy)	61.8 ± 14.1 (19-96)
• mean changes in biological age over time from T0 to T1	2.7 ± 1.2 (0.0-2.1)
Biological Age Acceleration	
(Differences from Levine Phenotypic Age to Chronological Age),	
mean ± SD	1.2 ± 0.5
• at T0 (before chemotherapy)	2.8 ± 0.7
• at T1 (6 months after chemotherapy)	1.6 ± 0.5
• mean changes in biological age acceleration over time	
from T0 to T1	
Chemotoxicity Incidences for 6 months of chemotherapy (n, %)	
Clinician-Reported Global Chemotoxicity	636 (56.3)
Clinician-Reported Gastrointestinal Chemotoxicity	460 (40.8)
Clinician-Reported Hematological Chemotoxicity	258 (22.8)

Note. SD = standard deviation.

3.2. Risk Factors of Levine Phenotypic Age

Table 2 describes the risk factors of biological aging as measured by Levine Phenotypic Age, at baseline, 6 months, and changes from baseline to 6 months. As expected, younger patients (age <50) showed significantly lower Levine Phenotypic Age at baseline (48.7 vs 69.9, $p<0.001$) and 6 months (51.7 vs 72.3, $p<0.001$). Notably, stage II patients experienced a greater Phenotypic Age increase over time as compared to stage III patients (2.3 vs. 1.8 years, $p=0.013$). Patients with higher comorbidity burdens showed greater Levine Phenotypic Age increases over time (3.0 vs. 2.4 years, $p = 0.013$). Socioeconomic factors significantly impacted aging trajectories: divorced/widowed/single, residents of disadvantaged neighborhoods (ADI 67-100), and unemployed/retired study participants, all demonstrated accelerated biological aging. These findings highlight how clinical and socioeconomic factors distinctly influence biological aging during cancer treatment.

Table 2. Factors related to the Biological Age (Levine Phenotypic Age) Over the Course of Chemotherapy.

		Biological Age (Levine Phenotypic Age mean ± SE)						
		At Baseline	<i>F, p^a</i>	6 months after chemotherap <i>y^c</i>	<i>F, p^a</i>	Changes paired overtim <i>e</i>	<i>t, p^b</i> Time effects	<i>F, p^a</i> group effects
Age group:								
Young Adult (18 ≤age <50, n=332)	48.7(0.4)	38.6,<.001	1	51.7 (0.4)	38.8,<.001	3.0 (0.06)	28.75,	7.98, .005
Older Adult (age ≥ 50, n =797)	69.9(0.9)			72.3 (0.3)		2.4 (0.05)	47.94, <.001	
Sex:								
Male	58.2(0.4)	3.35, .067		60.5(0.4)	3.35, .067	2.3 (0.02)	35.43,	0.08, .769
Female	60.0(0.3)			63.0(0.5)		3.0 (0.03)	43.71, <.001	
Race/Ethnicity:								
Non-Hispanic White	60.1 (0.4)	2.97, .051		63.3 (0.4)	3.23, .040	3.2 (0.03)	52.01,	1.12, .328
Non-Hispanic Black	59.6 (0.3)			62.1 (0.5)		2.5 (0.02)	18.73,	
Non-Hispanic Other	57.5 (0.4)			56.9 (0.4)		2.4 (0.01)	8.25,	
							<.001	
Cancer Site: Colon only	58.0 (0.5)	2.31, .101		60.8 (0.5)	3.32, .112	2.8 (0.02)	10.39,	1.18, .313
Rectal only	60.0 (0.4)			62.7 (0.3)		2.7 (0.03)	13.44,	
Colon and Rectal	59.3 (0.3)			61.6 (0.6)		2.3 (0.02)	16.51,	
							.101	
Cancer stages: II III	58.5(0.4) 59.7(0.5)	1.58, .162		61.5 (0.5) 62.1 (0.6)	1.79, .112	3.0 (0.02) 2.4 (0.03)	21.85, 49.33,	4.35, .013
5-FU (fluorouracil)- based chemotherapy								
FOLFOX (infusion)	57.6 (0.4)	1.11, .523		60.6 (0.5)	0.91, .941	3.0 (0.4)	5.6, .542	1.18, .313
FOLFIRI (infusion)	58.4 (0.9)			61.0 (0.8)		2.6 (0.8)	6.3, .481	
CAPEOX (oral)	60.8 (1.3)			63.2 (1.5)		2.4 (1.3)	2.1, .994	
Single-Agen 5- FU (infusion)	58.6 (1.7)			61.2 (1.8)		2.6 (1.5)	6.1, .501	

Body Mass Index (BMI)									
Obese (≥30)	58.8							5.4,	
	(0.9)							.499	
	59.4			61.8 (0.9)		3.0 (0.9)	6.1,		
	(0.8)	1.65, .209		62.0 (0.8)	1.11, .123	2.6 (0.8)	.312	2.01, .129	
	59.1			61.7 (0.6)		2.6 (0.5)	5.5,		
Normal (≥21, <25)	(0.6)			61.7 (1.3)		2.6 (1.2)	.561		
	59.1						6.5,		
Underweight (<21)	(1.3)						.209		
Modified Comorbidity Index:									
≥2	58.9(0.4)			61.7 (0.4)		2.9 (0.04)	<.001		
	58.1(0.5)	1.61, .203		60.6(0.5)	3.08, .080	2.5 (0.04)	39.15,	20.3, <.001	
< 2							<.001		
Radiation:									
Yes	58.5(0.4)			61.2 (0.5)		2.7 (0.5)	.121		
	59.7(0.6)	1.77, .233		62.5 (0.5)	1.66, .129	2.8 (0.4)	5.55,	1.87, .133	
No							.312		
Immunotherapy:									
Yes	58.1(0.4)			60.8 (0.4)		2.7 (0.02)	.121		
	60.1(0.5)	1.56, .122		62.8(0.6)	1.82, .132	2.7 (0.03)	11.15,	2.01, .122	
No							.212		
GI surgery:									
Yes	58.0(0.5)			60.7 (0.5)		2.7 (0.03)	<.001		
	60.2(0.6)	1.89, .132		62.9 (0.5)	1.55, .122	2.7 (0.04)	29.15,	2.22, .159	
No							<.001		
Current Smoking Status:									
Yes	58.9						51.45,		
	(0.4)	1.96, .195		61.3 (0.5)	2.71, .231	2.4 (0.04)	<.001	2.56, .222	
No	59.3			62.3 (0.4)		3.0 (0.01)	43.21,		
	(0.5)						<.001		
Current Heavy Alcohol Use:									
Yes	58.5			61.1 (0.4)		2.6(0.05)	<.001		
	(0.5)	1.75, .133		62.6(0.5)	1.88, .195	2.9(0.04)	41.21,	2.11, .298	
No	59.7(0.6)						<.001		
Routine Physical Activity:									
Yes	57.8(0.5)			60.2(0.4)		2.09, 2.4(0.05)	<.001		
	60.4(0.5)	1.79, .195		63.4(0.4)	.185	3.0(0.04)	39.41,	2.41, .187	
No							.004		
Primary Insurance Types:									
Private	58.9						12.41,		
	(0.4)			61.3(0.5)	2.11, .132	2.4 (0.4)	.496	2.41, .195	
Medicare/Medicaid	59.3	1.72, .194		62.5 (0.4)		3.2 (0.5)	19.31,		
	(0.5)						.312		
Marital status:									
Married/Partnered	58.5						44.07,		
	(0.5)	1.74, .175		60.0 (0.5)	2.21, .110	2.5 (0.05)	<.001	8.9, <.001	
Divorced/Widowed/Single	59.7			62.8(0.5)		2.8 (0.05)	36.91,		
	(0.5)						<.001		
ADI, Tertile:									
0-33	58.2(0.7)						22.8,		
34-66	58.7			60.7(0.7)		2.5 (0.08)	<.001	12.99,<.001	
67-100	(0.6)	1.98,.138		61.4(0.5)	3.19, .041	12.7 (0.06)	32.46,	1	
	60.4			63.4 (0.3)		3.0 (0.06)	<.001		
	(0.5)								

		40.21, <.001					
Employment Status:		35.12,					
Employed	57.6 (0.5)	7.21,<.001	60.4 (0.5)	7.60,<.001	2.6 (0.05)	<.001	4.88,<.001
Unemployed/Retired	60.6(0.7)	1	63.4 (0.7)	1	2.8 (0.07)	28.36, <.001	

Note. Characteristics of variables were described using means with standard error (SE). P-values in bold if they are <.05, as this is considered the statistical significance level, based on either using ^aANOVA for group differences or ^bpaired t-tests for time effects.

3.3. Risk Factors of Age Acceleration

Table 3 provides the risk factors for age acceleration (the age gap between biological age measured by Levine Phenotypic Age and chronological age during chemotherapy. The younger adult group showed greater age acceleration compared to the older adult group (with differences of 2.3 vs. 0.9 years at baseline and 4.1 vs. 2.3 years after treatment, Ps <0.001). Both age groups showed similar increases in age acceleration over the treatment period, with younger patients gaining 1.8 years and older patients gaining 1.4 years. Clinical characteristics showed important associations with age acceleration. Patients with stage III cancer presented with lower baseline age acceleration than stage II patients (1.3 vs 1.9 years, p<0.001), and experienced more pronounced increases in aging during treatment (3.8 vs 2.6 years, p<0.001). Patients with more comorbidities experienced a more substantial increase in age acceleration during chemotherapy (1.8 vs 1.4 years, p<0.001). Socioeconomic factors revealed disparities in biological aging patterns. Unmarried patients maintained higher age acceleration values than their married counterparts at both baseline (2.2 vs 1.0 years, p=0.006) and post-treatment (3.8 vs 2.6 years, p<0.001). Neighborhood disadvantage showed strong associations with age acceleration, as residents of the most disadvantaged areas (ADI: 67-100) had higher age acceleration values of 2.1 years at baseline and 4.1 years post-treatment, compared to residents of the least disadvantaged areas (p=0.008 and p<0.001, respectively). Employment status significantly influenced age acceleration over time, with unemployed or retired patients experiencing greater increases than employed patients (1.7 vs 1.5 years, p<0.001).

Table 3. Factors related to Age Acceleration (i.e., Differences from Biological Age to Chronological Age) Over the course of Chemotherapy.

Differences from Biological Age to Chronological Age (mean ± SE)							
		At Baseli ne	F, p ^a	6 months after chemother apy ^c	F, p ^a	Chang es over time paired t, p ^b Time effects	F, p ^a group effects
Age group:							
Young Adult (18 ≤age <50, n=332)	2.3(0.3)	30.371, <.001		4.1(0.3)	19.558, <.001	1.8(0.1) <.001	7.985, .005
Older Adult (age ≥ 50, n =797)	0.9(0.2)			2.3(0.2)		1.4(0.1) <.001	
Sex:							
Male	1.4 (0.2)	0.064, .800		3.0 (0.2)	0.098, .754	1.6 (0.1) 1.6(0.1)	40.31, .001 39.51, .003
Female	1.8 (0.2)			3.4 (0.2)			.086, .769
Race/Ethnicity:							
Non-Hispanic White	1.7 (0.2)	0.436, .647		3.5 (0.2)	0.785, .456	1.8 (0.1) 1.4 (0.1)	49.65, .001 51.55, .004
Non-Hispanic Black				2.7 (0.4)			1.115, .328
Non-Hispanic Other				3.4 (0.8)			

	1.8 (0.7)				1.6 (0.2)	48.43, .003	
Cancer Site: Colon only	1.5 (0.2)				1.3 (0.3)	24.51, .121	
Rectal only	1.8 (0.2)	0.351, .641	2.8(0.2) 3.5 (0.3)	0.841, .533	1.7 (0.3)	51.55, .006	0.549, .299
Colon and Rectal	1.5 (0.1)		3.3 (0.5)		1.8 (0.3)	21.39, .149	
Cancer stages: II	1.9 (0.2)	4.958, <.001	3.8(0.4) 2.6 (0.3)	6.582, <.001	1.9 (0.1)	49.55, .005	4.281, <.001
III	1.3 (0.3)				1.3 (0.8)	29.41, .134	
5-FU (fluorouracil)-based chemotherapy	1.3 (0.5)				1.3 (0.5)	13.12, .156	
FOLFOX (infusion)	1.5 (0.7)	1.312, .985	2.6 (0.5) 3.1 (0.8)	0.412, .512	1.6 (0.7)	14.61, .952	0.102, .132
FOLFIRI (infusion)	1.9 (0.9)		3.9 (0.9) 3.3 (1.1)		2.0 (0.9)	13.05, .121	
CAPEOX (oral)	1.7 (1.1)				1.9 (1.1)	19.11, .232	
Single-Agen 5-FU (infusion)							
Body Mass Index (BMI)	1.7 (0.6)				1.4(0.6)	5.6, .988	
Obese (≥30)	1.6 (0.7)	0.192, .542	3.1 (0.6) 3.4 (0.6)	0.293, .133	1.8 (0.6)	7.1, .999	1.102, .432
Overweight(≥25, <30)	1.5(0.4)		3.0 (0.5) 3.3 (1.1)		1.5 (0.4)	11.2, .516	
Normal (≥21, <25)						13.5, .309	
Underweight (<21)	1.6 (1.1)				1.7(1.1)		
Modified Comorbidity Index: ≥2	1.3 (0.2)	0.025, .875	3.1(0.2) 3.3(0.2)	1.679, .195	1.8 (0.1)	39.41, .005	20.33, <.001
< 2	1.9 (0.2)				1.4(0.1)	45.44, .001	

Table 3. Continued.	Differences from Biological Age to Chronological Age (mean ± SE)						
	At Baseline	F, p ^a	6 months after chemotherapy ^c	F, p ^a	Changes over time	paired t, p ^b Time effects	F, p ^a group effects
Radiation: Yes	1.1 (0.3)	0.412, .521	2.9 (0.4)	0.334, .563	1.8 (0.2)	40.55, .005	0.007, .934
No	2.1 (0.1)		3.5 (0.2)		1.4 (0.1)	51.55, .007	
Immunotherapy: Yes	1.8 (0.3)	5.078, .024	3.6 (0.3)	0.385, .726	1.8 (0.1)	43.59, .001	1.035, .309
No	1.4 (0.2)		2.8(0.2)		1.4(0.1)	45.61, .010	
GI surgery: Yes	1.5 (0.2)	0.684, .408	3.3(0.2)	0.699, .403	1.8 (0.1)	40.99, .005	0.056, .812
No	1.7(0.4)		3.1(0.4)		1.4 (0.1)	25.61, .112	
Current Smoking Status: Yes	1.4(0.1)	0.415, .122	2.8(0.1)	0.423, .412	1.4(0.1)	59.55, .009	0.322, .599
No	1.8(0.2)		3.6(0.1)		1.8(0.1)	61.15, .003	
Current Heavy Alcohol Use: Yes	1.3(0.1)	0.333, .233	2.5(0.1)	0.012, .999	1.2(0.1)	55.69, .010	0.043, .891
No	1.5(0.3)		2.9(0.2)		1.4(0.1)	63.52, .009	
Routine Physical Activity: Yes	1.2(0.1)	0.513, .431	2.8 (0.2)	0.333, .444	1.6 (0.1)	54.59, <.001	0.019, .981
No	2.0(0.2)		3.6 (0.1)		1.6 (0.1)	55.61, .010	
Primary Insurance Types: Private	1.5(0.2)	0.222, .132	3.3(0.2)	0.122, .159	1.8 (0.1)	13.73, .232	0.233, .481
Medicare/Medicaid	1.7(0.1)		3.1(0.2)		1.4(0.1)	19.65, .167	
Marital status: Married/Partnered	1.0(0.2)	5.174, .006	2.6 (0.2)	8.351, <.001	1.6 (0.1)	65.05, .001	8.984, <.001
Divorced/Widowed/Single	2.2 (0.2)		3.8(0.2)		1.6 (0.1)	49.10, .010	
ADI, Tertile: 0-33	1.1 (0.3)	4.819, .008	2.5(0.1)	8.324, <.001	1.4 (0.1)	49.89, .009	12.993, <.001
34-66	1.6 (0.2)		3.2(0.2)		1.6 (0.1)	59.11, .012	
67-100	2.1 (0.2)		4.1(0.2)		2.0 (0.1)	48.51, .019	
Employment Status: Employed	1.1 (0.2)	1.842, .118	2.8(0.2)	2.319, .055	1.5 (0.1)	53.59, .013	4.889, <.001
Unemployed/Retired	1.7(0.2)		3.5(0.3)		1.7 (0.1)	47.51, .005	

Note. Characteristics of variables were described using means with standard error (SE). P-values in bold if they are <.05, as this is considered the statistical significance level, based on either using ^aANOVA for between-group differences or ^bpaired t-tests for within-group change from baseline to 6-months post-chemotherapy. ^cAge acceleration at 6 months = Biological age at 6 months – (baseline chronological age + 0.5 years).

3.4. Associations of Biological Age with Chemotoxicity

Table 4 shows the results of associations of biological age (baseline and changes over time) with chemotoxicity outcomes. Patients who experienced global chemotoxicity showed markedly higher biological age (Levine Phenotypic Age) compared to those without toxicity, with values of 60.5 versus 57.7 years at baseline (p<0.001) and 63.8 versus 59.8 years after treatment (p<0.001). These patients also demonstrated greater increases in Levine Phenotypic Age over time (3.3 vs. 2.1 years, p<0.001). Patients reported GI and hematological toxicities had higher baseline Levine Phenotypic Age, and greater increases in Levine Phenotypic Age over time, as compared to the patients without these adverse events.

Similar patterns emerged for age acceleration, where global chemotoxicity cases had substantially higher age acceleration at baseline (2.1 vs. 0.3 years, p<0.001), post-treatment (4.3 vs. 1.3 years, p<0.001), and over time (2.2 vs. 1.1 years, p<0.001). Similar results were found in GI and hematological toxicities. Despite this, age acceleration rates (i.e., age acceleration changes over time) were similar among groups with GI (1.9 years accelerated) and hematological toxicities (1.9 years accelerated). The age acceleration differences were even more pronounced, with hematological toxicity cases showing higher baseline (2.3 vs. 0.1 years, p<0.001) and post-treatment (4.2 vs. 1.4 years, p<0.001) values than those without hematological toxicity.

Table 4. Associations of Biological Age with Chemotoxicity.

Chemotoxicity	Biological Age (Levine Phenotypic Age), mean ± SE					
	At Baseline	F, p ^a	6 months after	F, p ^a	Changes over time	paired t, p ^b Time effects F, p ^a group effects

			chemothera py					
Global.	Yes	60.5 (0.4)	81.71, <.001	63.8 (0.5)	129.01,<.0	3.3 (0.04)	78.1, .005 69.5, .001	542.71, <.001
	No	57.7 (0.5)		59.8 (0.5)	.01	2.1 (0.04)		
GI.	Yes	60.6 (0.8)	7.53, .006	62.8 (0.1)	8.53, .004	2.2 (0.2)	33.5, .009	107.52, .077
	No	57.6 (0.4)		60.8 (0.1)		3.2 (0.4)	49.5, .010	
Hematological.	Yes	60.1 (0.6)	6.19, .013	63.5 (0.7)	8.52, .004	3.4 (0.7)	39.6, .015	17.92, <.001
	No	58.1 (0.4)		60.1 (0.4)		2.0 (0.4)	40.5, .032	
Age Acceleration (Differences from Levine Phenotypic Age to Chronological Age)								
Chemotoxicity		At Baseline	F, p ^a	6 months after chemotherapy ^c	F, p ^a	Changes over time	paired t, p ^b Time effects	F, p ^a group effects
Global.	Yes	2.1 (0.2)	190.07, <.001	4.3 (0.2)	373.40,<.0	2.2(0.1)	55.1, .009	542.74,<.00
	No	0.3 (0.2)		1.3 (0.2)	.01	1.1(0.1)	59.3, .013	1
GI.	Yes	1.5 (0.2)	3.13, .077	3.4 (0.4)	5.78, .016	1.9 (0.1)	53.1, .007	8.53, .004
	No	0.9 (0.4)		2.2 (0.2)			1.3 (0.4)	
Hematological.	Yes	2.3 (0.3)	27.81, <.001	4.2 (0.3)	36.50,	1.9 (0.1)	66.3, .010	17.87,<.001
	No	0.1 (0.2)		1.4 (0.2)	<.001	1.3 (0.1)	65.5, .005	

Note. Characteristics of variables were described using means with standard error (SE). P-values in bold if they are <.05, as this is considered the statistical significance level, based on either using ^aANOVA for group differences or ^bpaired t-tests for time effects. ^cAge acceleration = Biological age at 6 months – (chronological age + 0.5 years).

3.5. Impact of Biological Age on Chemotoxicity

Given the significant replationship between biological age and chemotoxicity outcomes shown in Table 4, we performed further logistic regression analyses to examine the impact of biological age on chemotoxicity (i.e., strengths of the associations) in Table 5. The analysis revealed significant associations between biological aging measures and chemotoxicity risk in both unadjusted and adjusted models. For global chemotoxicity, baseline Levine Phenotypic Age showed strong predictive value with an adjusted odds ratio (aOR) of 1.27 (95% CI: 1.22-1.32, p<0.001), indicating a 27% increased risk per year of higher biological age. Changes in Phenotypic Age over time demonstrated an even greater association with risk (aOR=2.74, 95% CI: 2.45-3.04, p<0.001). Similar patterns emerged for age acceleration measures, with baseline values (aOR=1.27, p<0.001) and changes over time (aOR=2.74, p<0.001) both showing significant links to global toxicity risk.

For GI and hematological toxicities, results were similar showing significant associations between biological measures and chemotoxicity. Overall, compared to Levine Phenotypic Age and its age acceleration at baseline, changes in these biological aging measures were more strongly associated with chemotoxicity outcomes. Most of the biological aging measures at baseline and changes over time (Levine Phenotypic Age and age acceleration) in unadjusted models reached significance in the adjusted models.

Table 5. Impact of Biological Age (Levine Phenotypic Age) on Chemotoxicity.

Timepoints	Unadjusted Models ^a	Adjusted Models ^{a,b}
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Baseline (T0); Change over time (T1-T0): from baseline to 6 months post-chemotherapy	OR (95% CI)	Wald, p	aOR (95% CI)	Wald, p
Global Chemotoxicity				
Levine Phenotypic Age at T0	1.03 (1.02, 1.04)	73.29, <.001	1.27(1.22, 1.32)	72.30, <.001
Changes in Levine Phenotypic Age over time	2.70 (2.42, 2.97)	110.58, <.001	2.74 (2.45, 3.04)	336.96, <.001
Age Acceleration (Differences from Biological Age to Chronological Age) at T0	1.30 (1.21, 1.31)	131.52, <.001	1.27(1.22, 1.32)	137.28, <.001
Changes in Age Acceleration over time	2.70 (2.41, 2.97)	346.79, <.001	2.74(2.45, 3.05)	336.19, <.001
GI Chemotoxicity				
Levine Phenotypic Age at T0	1.03 (1.01, 1.04)	7.47, .006	1.03(1.01, 1.05)	5.33, .021
Changes in Levine Phenotypic Age over time	1.12 (1.04, 1.22)	8.42, .004	1.10(1.01, 1.20)	5.66, .042
Age Acceleration (Differences from Biological Age to Chronological Age) at T0	1.02 (0.99, 1.04)	3.14, .076	1.03(0.94, 1.05)	3.46, .059
Changes in Age Acceleration over time	1.12(1.04, 1.22)	8.42, .004	1.10 (1.02, 1.20)	5.66, .042
Hematological Chemotoxicity				
Levine Phenotypic Age at T0	1.01(1.01, 1.03)	6.73, .010	1.06(1.03, 1.08)	33.03, <.001
Changes in Levine Phenotypic Age over time	1.17(1.09, 1.26)	18.18, <.001	1.15(1.06, 1.24)	29.01, .005
Age Acceleration (Differences from Biological Age to Chronological Age) at T0	1.06(1.04, 1.08)	25.91, <.001	1.06(1.03, 1.08)	23.02, <.001
Changes in Age Acceleration over time	1.17(1.09, 1.26)	18.22, <.001	1.15(1.05, 1.22)	13.12, <.001

Note. P-values are bolded if they are <.05,.^aWhen testing baseline biological age variables, we did not adjust for changes in biological age variables. When testing changes in biological age variables, we adjusted baseline biological age variables as confounders for all regression models.^b For adjusted models, we controlled for age groups, employment status, cancer stages, comorbidities, marital status, and ADI.

4. Discussion

This study provides compelling evidence that chemotherapy in CRC patients is associated with measurable increases in biological aging, as captured by Levine’s Phenotypic Age using EHR data. Our findings demonstrate that biological aging progresses significantly over six months of treatment, with Phenotypic Age increasing by an average of 2.7 years, while age acceleration increased from 1.2 to 2.8 years. Our study also demonstrates that biological aging during chemotherapy is influenced by a combination of clinical factors, including comorbidity burden, along with socioeconomic determinants such as marital status, neighborhood characteristics, and employment status. The findings identify specific patient subgroups who may be particularly vulnerable to accelerated biological aging during cancer treatment, suggesting potential targets for more personalized treatment approaches and supportive care interventions. Below, we interpret our findings in the context of prior research, discuss clinical implications, and explore potential biological mechanisms.

Biological aging during chemotherapy. Our study provides evidence that **5-FU-based chemotherapy accelerates biological aging in CRC patients**, aligning with findings from breast cancer research and preclinical models [9,37]. Although cancer therapies—including chemotherapy, radiotherapy, and immunotherapy—are designed to induce apoptosis or senescence in malignant cells, they also inadvertently promote senescence in healthy tissues, contributing to systemic

biological aging [9,37]. Chemotherapy-induced biological aging likely arises through multiple interconnected pathways [9,13,27,29,38,39]. First, chemotherapy agents cause **DNA damage and cellular senescence**, as demonstrated by elevated expression of senescence markers such as *p16^{INK4A}* in breast cancer patients following treatment [40]. Second, chemotherapy is associated with **epigenetic aging and telomere attrition**. For instance, large-scale studies reveal that breast cancer survivors treated with chemotherapy exhibit **1-2 years of accelerated epigenetic aging** (measured by the Horvath and GrimAge clocks) compared to non-chemotherapy controls [38]. Additionally, chemotherapy patients show **shortened leukocyte telomere length in breast cancer** [41] and increased inflammatory markers (e.g., IL-6, CRP) in CRC [42], which correlate with reduced survival and worse cancer health outcomes. Lastly, agents such as 5-FU and oxaliplatin generate **reactive oxygen species (ROS)**, overwhelming endogenous antioxidant defenses and accelerating cellular aging [43]. Our findings highlight the potential utility of **Levine Phenotypic Age**, a biomarker derived from EHR-based inflammatory and clinical measures, in predicting chemotoxicity. Several mechanisms may explain its predictive power. Chemotherapy induces a **senescence-associated secretory phenotype (SASP)**, leading to the release of pro-inflammatory cytokines (e.g., IL-6, CRP) that exacerbate tissue damage and systemic aging [44]. Patients with elevated baseline Levine Phenotypic Age may have diminished stress response capacity, rendering them more vulnerable to treatment toxicity [32]. The integration of biological aging biomarkers, such as Levine Phenotypic Age, into clinical practice could improve risk stratification and guide therapeutic decisions. Further research is needed to elucidate the precise mechanisms linking chemotherapy-induced aging and adverse treatment outcomes.

Risk factors for biological aging during chemotherapy. Our findings align with prior work showing that cancer treatment accelerates aging biomarkers [45], but we extend this by identifying key risk factors for biological aging. *The elevated biological aging in stage III versus stage II patients in our study may reflect combined effects of more aggressive therapies (e.g., chemotherapy-induced senescence) and greater tumor burden (e.g., pro-inflammatory signaling), both known to accelerate aging-related processes [4].* In our study, older adults exhibited higher baseline biological age, while younger adults showed greater age acceleration. Unlike chronological age, biological age may be influenced by factors such as lifestyle and stressors [46]. The younger group may be aging more quickly than anticipated due to more aggressive treatment plans, and chronic exposure to various risk factors of aging, such as higher work-and-life stress levels, unhealthy lifestyles, and low resilience [47]. This suggests that younger individuals are also vulnerable to chemotherapy-induced premature aging, possibly due to stronger treatment regimens or less physiological reserve. Greater comorbidities, advanced cancer stages, higher ADI (i.e., higher area-level SDOH disparities), non-employment status, and marital status (divorced, widowed, or single) were also associated with biological age and age acceleration. Potential mechanisms can be considered to explain our findings. Older adults' elevated baseline biological age likely reflects the lifelong accumulation of comorbidities and chronic inflammation [48]. In contrast, younger adults' accelerated aging may stem from acute stressors, unhealthy lifestyles, and more aggressive cancer treatments [49]. Social factors associated with biological aging in our study are supported by other studies: poor socioeconomic conditions (e.g., poor area-level SDOH, unemployment status, and family support) support heightened chronic stress, dysregulating epigenetic repair [50]. Poor income status, loneliness, unhealthy lifestyle, and poor environmental conditions are also associated with biological aging [50]. These findings suggest that chemotherapy may amplify biological aging, which various mechanisms can influence. This underscores the need for age-tailored interventions, such as comorbidity management, socioeconomic support, to mitigate chemotherapy-associated aging risks. Further investigations are needed to determine how biological aging differs in various chronological age groups.

Biological age is a risk factor for chemotoxicity. Our study is also the first to examine associations between biological aging and 5-FU-based chemotoxicity outcomes in CRC using Levine Phenotypic Age. Patients with higher baseline Phenotypic Age and greater increases over time had elevated risk of global, GI, and hematological toxicities. Several studies support our findings

showing associations between biological aging and chemotoxicity in other cancer types. In patients with breast cancer, T cell $p16^{INK4a}$ expression was positively associated with peripheral neuropathy after 1.5 years of treatment with paclitaxel and docetaxel [51], and fatigue after one month of taxane-based chemotherapy [52]. Multiple studies have shown a link between baseline telomere length and chemotherapy-induced toxicities in breast cancer and lymphoma patients [53-55].

Additionally, recent research identified a connection between $p16^{INK4a}$ expression and epigenetic aging markers such as the Hannum and PhenoAge DNA methylation clocks, along with various mRNA signatures of T cell senescence. This study suggests that aging-related biomarkers, particularly $p16^{INK4a}$ and epigenetic clocks, may help predict physical and cognitive frailty in older adults with blood cancers [56]. *Despite the current literature on biological aging and chemotoxicity mentioned above, indicators of epigenetic age (e.g., DNA methylation clocks) and T cell expression profiles remain limited for routine clinical use due to insufficient clinical validation, high costs, and the technical complexity of measuring these markers [9].* Therefore, our study enhances the existing literature by demonstrating the potential utility of the blood-based Levine Phenotypic Age algorithm to predict various types of chemotoxicity (GI and hematological) commonly observed in patients with CRC. These results demonstrate that easy-to-use biological aging measures (i.e., Levine PhenoAge) can serve as robust predictors of various chemotoxicities in CRC. This suggests that biological aging assessments could help identify high-risk patients before treatment and monitor toxicity risk during chemotherapy. The maintained significance in adjusted models in our study underscores the independent predictive value of these aging measures beyond sociodemographic and clinical factors [15].

Clinical Implications

Our findings suggest that biological aging assessments could improve risk stratification before chemotherapy. For example, clinicians can consider identifying patients at high risk of chemotoxicity by utilizing biological aging and related factors. Furthermore, researchers can consider developing risk screening tools by integrating biological aging markers and relevant clinical and social factors to guide treatment decisions. Second, patients with high Levine Phenotypic Age and age acceleration may benefit from dose modifications, comorbidity management, enhanced supportive care, or anti-aging interventions such as antioxidants (e.g., coenzyme Q10) or lifestyle interventions to mitigate toxicity [57]. Third, Levine Phenotypic Age can be used to monitor long-term survivorship care in patients with biological aging. For example, the high-risk groups of aging may need earlier surveillance and rehabilitation for aging-related chronic diseases, such as cognitive impairment and cardiovascular diseases.

Limitations and Future Directions

This is a secondary data analysis, so several factors relevant to our study were not available, such as education levels, annual household income, which can better reflect individual socioeconomic status. Furthermore, our study was not able to address some confounders such as stress levels and diet quality, in our analyses. Since clinicians do not use the CTCAE tool or patient-reported (PRO) CTCAE on a routine basis to report chemotoxicity at the many tertiary cancer centers, this could limit the accurate capture of chemotoxicity data. Therefore, routine use of the clinician-reported CTCAE tool as well as PRO data should be considered in future studies. Future validation of the Levine Phenotypic Age in a separate cohort of CRC patients is needed to confirm our findings. Using only two timepoints of follow-up data has limitations in drawing clear causal relationships between biological aging and chemotoxicity outcomes. Addressing long-term follow-up is required to determine if accelerated aging persists post-treatment and impacts long-term survivorship outcomes (e.g., frailty, quality of life, function, fatigue and secondary cancers). Furthermore, before considering anti-aging interventions for CRC patients, future research should prioritize evaluating existing aging metrics, including molecular biological markers and clinical frailty, to determine the optimal tool or develop novel aging-based screening and chemotoxicity management strategies for

use in CRC. Lastly, current knowledge about aging in cancer survivors is largely based on studies of older adults with non-CRC, underscoring the need to expand investigations to both young and older adults with CRC.

5. Conclusions

This study demonstrates that chemotherapy accelerates biological aging in CRC patients. Levine's Phenotypic Age, calculated using routine blood test results from EHR, served as a strong predictor of treatment toxicity (global, GI, and hematological). Both young and older adults, those with greater comorbidities, more advanced cancer stages, and socioeconomic disadvantaged (including area-level disparities) individuals are at higher risk for chemotoxicity. Future research should validate the current findings in different cohorts of patients with CRC to facilitate the use of blood-based biological aging markers in routine clinical use, develop aging-based chemotoxicity screening tools, and explore whether targeting aging mechanisms (e.g., anti-inflammatories) can improve treatment tolerance and survivorship outcomes.

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Institutional Review Board Statement: This study employed de-identified electronic health records from the Ohio State University Comprehensive Cancer Center. The institutional review board determined this retrospective analysis of existing anonymized data qualified as non-human subjects research under 45 CFR 46 regulations, granting both an exemption and waiver of informed consent. All data handling complied with HIPAA Privacy Rule requirements. Access to these records was controlled through the Cancer Center's data governance policies and required execution of a formal data-use agreement.

Informed Consent Statement: Not applicable due to secondary data analyses using de-identified EHR data.

Data Availability Statement: The datasets analyzed in this study are not publicly available due to privacy restrictions but can be accessed by qualified researchers upon reasonable request to the corresponding author. As a secondary analysis of existing de-identified EHR data, our study did not generate new datasets. However, the dataset contains potentially identifiable information (including zip codes and ages above 80) that could risk patient re-identification under HIPAA regulations. Access requires approval through Ohio State University's formal data request process, including execution of a Data Use Agreement and institutional review board approval.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADI	Area Deprivation Index
aOR	Adjusted Odds Ratio
BMI	Body Mass Index
CBC	Complete Blood Count

CAPEOX	Capecitabine, Oxaliplatin
CI	Confidence Interval
CRC	Colorectal Cancer
CTCAE	Common Terminology Criteria for Adverse Events
EHR	Electronic Health Record
5	FU
5	Fluorouracil
FOLFIRI	Folinic Acid, Fluorouracil, Irinotecan
FOLFOX	Folinic Acid, Fluorouracil, Oxaliplatin
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
ICD9/10	International Classification of Diseases, 9th/10th Revisions
IRB	Institutional Review Board
OR	Odds Ratio
ROS	Reactive Oxygen Species
SASP	Senescence Associated Secretory Phenotype
SD	Standard Deviation
SDOH	Social Determinants of Health
SE	Standard Error

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