Pharmacokinetics based individualised dosing of anticancer therapies for elderly patients

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Abstract

The overall aim of the research presented in this thesis was to explore and improve the outcomes of older adults with cancer with individualised dosing methods of anticancer therapy, and ways to increase the feasibility and uptake of such methods into routine clinical practice. Colorectal cancer and capecitabine chemotherapy were foci of the research.

Older adults with colorectal cancer (CRC), compared with younger adults with CRC, (colon n=1135, rectum n=714) in two retrospective cohort studies of a prospectively maintained database were found to have worse overall survival (OS) and cancer specific survival (CSS) and lower utilisation of adjuvant chemotherapy. Adjuvant chemotherapy independently predicted improved OS in older adults with stage III CRC. Ways to improve outcomes of older adults with CRC were highlighted as an area of unmet research need.

A systematic review identified 21 studies investigating the effects of ageing on the pharmacokinetic (PK) of anticancer therapies used in the treatment of older adults with CRC. The studies showed older age influences PK of irinotecan and, to some extent, that of capecitabine, 5-fluorouracil (5-FU) and panitumumab. An adjustment to the dose or frequency of these drugs prescribed to older adults with cancer may be needed.

A systematic review identified 23 studies that showed a high association between drug concentrations measured by microsampling and plasma or venous sampling for the majority of anticancer drugs (except mitotane). Overall, microsampling was a feasible and promising alternative to plasma or venous sampling for the therapeutic drug monitoring (TDM) of many anticancer drugs. Further research is needed to determine the accuracy of microsampling for the measurement of anticancer drugs.

A prospective observational study compared the PK of capecitabine and its metabolites in older adults and younger adults with cancer (n=26). This study found older adults had a greater exposure to 5-FU, the active metabolite of capecitabine. This was evident by a 17% increase in the area under the concentration-time curve (AUC) and a 14% increase in the maximal concentration (Cmax).The 5-FU Cmax was positively associated with *time up and go* (TUG), a measure of functional mobility, but not other geriatric assessment domains or severe toxicity. The findings of this study suggests that the increased toxicity in older adults on capecitabine might be due to higher exposure to 5-FU and warrants further study.

A pilot study (n=10) assessed the feasibility, acceptability and reliability of microsampling in the measurement of capecitabine concentrations for TDM in real world patients. Capecitabine concentrations measured by microsampling and plasma sampling were highly correlated, but consistently lower in microsampling. Microsampling was the preferred method by all participants with minimal pain. The study's findings suggest that microsampling may be a feasible alternative to plasma sampling for TDM of capecitabine in real-world patients.

In conclusion, older adults with cancer compared with younger adults experience worse outcomes like toxicity and receive less chemotherapy. Data on the effect of age on PK of chemotherapy in CRC is limited. Better dosing strategies may improve toxicity profile of anticancer therapy and increase the uptake of anticancer therapy by older adults with cancer. PK-guided dosing, using microsampling techniques, is a promising strategy that allows personalised dosing to reduce toxicity and improve efficacy. Further validation studies are required to determine the effectiveness of microsampling as a substitute for plasma sampling.

Statement of originality

This thesis is submitted to the University of Sydney in fulfilment of the regulations for the Degree of Doctor of Philosophy. The work presented in this thesis is to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

…… 06/09/2023

Mohsen Shafiei Date

Authorship attribution statement

I undertook this PhD as a Full-time PhD student between July 2017 and June 2020, and as a part-time PhD student between July 2020 and June 2023 at the Concord Medical School (Faculty of Medicine) University of Sydney under the supervision of Associate Prof Prunella Blinman and Prof Andrew McLachlan.

This thesis is presented as a hybrid thesis with a combination of traditional chapters (Chapters 1 and 8) and publications (Chapters 2, 3, 4, 5, 6 and 7). The study presented as Chapter 7 was submitted for publication and under peer review at the time of thesis submission for publication when I submitted my thesis.

I was responsible for the study design, data collection and analysis, interpretation of the findings, and drafting and revision of the manuscript of all studies in this thesis.

Dr Mohsen Shafiei 06/09/2023

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Associate Professor Prunella Blinman

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Publications arising from this thesis

Peer-reviewed journal publications

Chapter 2:

Shafiei M, Beale P, and Blinman P. **Utilisation of Adjuvant Chemotherapy and 5-Year Survival Analysis of Prospectively Recorded Cohort Data for Older Adults versus Younger Adults with Resected Primary Colon Cancer**. *J Gastrointest Cancer*. 2020; 51(3):988-997. https://doi.org/10.1007/s12029-019-00343-5.

Chapter 3:

Shafiei M, Beale P, and Blinman P. **Five Year Survival Outcomes of Prospectively Recorded Cohort Data for Older Adults versus Younger Adults with Resected Primary Rectal Cancer**. *J. Cancer Ther*.2021; 12, 437-452. https://doi.org/10.4236/jct.2021.127038

Chapter 4:

Shafiei M, Yoon R, McLachlan A, Boddy A, Beale P, Blinman P. **Pharmacokinetics of Anticancer Drugs Used in Treatment of Older Adults With Colorectal Cancer: A Systematic Review**. *Ther Drug Monit.* 2019; 41(5):553-560. https://doi.org/10.1097/ftd.0000000000000635.

Chapter 5:

Shafiei M, Mahmood A, Beale P, Galettis P, Martin J, McLachlan AJ, Blinman P. **Dried Blood Spot Sampling in the Monitoring of Anticancer Therapy for Solid Tumors: A Systematic Review.** *Ther Drug Monit.* 2023; 45(3):293-305. https://doi.org/10.1097/ftd.0000000000001082.

Chapter 6:

Shafiei M, Galettis P, Beale P, Reuter SE, Martin JH, McLachlan AJ, Blinman P. **Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: a pilot study.** Cancer Chemother. Pharmacol. 2023; 92(2):135-139. https://doi.org/10.1007/s00280-023-04552-5.

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Chapter 7:

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List of Abbreviations

5-DFCR	5-deoxy-5-fluorocytidine
5-DFUR	5-deoxy-5-fluorouridine
5-FU	5-fluorouracil
ALP	Alkaline phosphatase
ANOVA	Analysis of Variance
ASCO	American Society of Clinical Oncology
AUC	Area Under the Curve
BSA	Body Surface Area
BW	Body Weight
CI	Confidence Interval
CL/F	Apparent Clearance
CIRS-G	Cumulative Illness Rating Scale-Geriatric
Cmax	Maximum concentration
CRC	Colorectal Cancer
CrCL	Creatinine Clearance
CRP	C Reactive Protein
CRT	Chemoradiotherapy
CSS	Cancer Specific Survival
CTCAE	Common Terminology Criteria for Adverse Events

CV	Coefficient of Variation
DBS	Dried Blood Spot
DLT	Dose Limiting Toxicities
DN	Dose Normalised
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
FBAL	a-fluoro-b-alanine
FDA	Food and Drug Administration
GA	Geriatric Assessment
G8	Geriatric 8
h	Hour
Hb	Haemoglobin
HFS	Hand and Foot Syndrome
HNSCC	Head and Neck Squamous Cell Carcinoma
HQC	Higher Quality Control
HR	Hazard Ratio
INR	International Normalised Ratio
i.v.	Intravenous
ka	Absorption rate constant

kel	Elimination rate constant
LC–MS/MS	Liquid ChromatographyMass Spectrometry
LLOQ	Lower Limit Of Quantification
Ln	Natural logarithm
LoA	Limits of Agreement
mCRC	Metastatic Colorectal Cancer
MNA	Mini Nutritional Assessment
MOS	Medical Outcomes Study
MSI	Microsatellite Instability
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NLR	Neutrophil Lymphocyte Ratio
NYHA	New York Heart Association
OARS	Open questioning Affirming Reflecting Summarizing
OS	Overall Survival
PFS	Progression Free Survival
PD	Pharmacodynamic
PK	Pharmacokinetic
RT	Room Temperature

SCr	Serum Creatinine concentration
SEER	Surveillance Epidemiology and End Results
$t_{1/2}$	Half life
TDM	Therapeutic Drug Monitoring
THU	Tetrahydrouridine
Tmax	Time at which the maximum concentration occurs
TNM	Tumour, Nodes, Metastases
TUG	Time Up and Go
ULOQ	Upper Limit Of Quantification
UPLC-MS	Ultra Performance Liquid Chromatography-tandem Mass Spectrometry
V/F	Apparent volume of distribution
VAMS	Volumetric Absorptive Microsampling
VAS	Visual Analogue Scale
Vd	Volume of distribution
WB	Whole Blood

Chapter 1: Introduction

1.1 Background and rationale for the thesis

Cancer is predominantly a disease of older adults with an increasing incidence with advanced age. In Australia and worldwide, the absolute numbers of older adults with cancer is increasing due to the ageing of the population [1]. In patients with colorectal cancer, older age is associated with changes in clinical and pathologic characteristics of tumour and decreased overall and cancer specific survival [2, 3]. The definition of an older adult with cancer varies; many studies on patients with cancer have used an age limit of ≥70 years to define an older adult, however, other lower and higher age limits are also used (≥65 years,
≥75 years [4]. Older adults with cancer, compared with younger adults with cancer, generally derive similar benefit from chemotherapy but experience higher rates of chemotherapy- related toxicity [5]. This is due to multifactorial reasons including age-related physiological changes such as reduced renal clearance, increasing frailty and other geriatric syndromes, increased co-morbidities, and fewer social supports [6, 7].

Capecitabine, an oral fluoropyrimidine and a pro-drug of 5-fluorouracil, is a commonly used chemotherapy agent in the management of patients with gastrointestinal cancer and breast cancer. Capecitabine, as monotherapy, is a suitable agent for older adults and is commonly used for these patients [8-15]. Excess toxicity is frequently observed in older adults receiving capecitabine requiring dose modifications (delays, reductions, omissions), hospitalisations and other use of health care resources [16]. Capecitabine toxicity is often unpredictable and these factors make prescribing of capecitabine challenging in the older, frailer population.

Capecitabine is dosed with conventional body surface area (BSA) dosing. Whilst BSA dosing aims to reduce pharmacokinetic (PK) variability compared with fixed dosing, PK variability is still seen with BSA dosing of capecitabine. This means that some patients are unintentionally under-dosed with potentially compromised treatment, whilst others are unintentionally over-dosed resulting in severe chemotherapy-related toxicity. Hence alternative methods to BSA dosing of capecitabine are required. An alternative to BSA dosing is PK-guided dosing where measured PK parameters are used to adjust the dosing of chemotherapy in individual patients [17]. There is limited research evidence regarding PK- guided dosing of capecitabine in people with cancer, and the results of the few published studies are conflicting [18, 19]. Moreover, the association between PK of capecitabine and chemotherapy-induced toxicity, inflammatory markers and geriatric assessment (GA) tools is unknown.

PK-guided dosing of chemotherapy agents has been infeasible to date due to the need for additional multiple blood sampling over many consecutive hours from patients undergoing anticancer therapy, inadequate turn-around times of test results to enable dose adjustments within a chemotherapy cycle, the lack of established therapeutic concentration ranges and analytical challenges with pro-drugs like capecitabine [20, 21]. Recent technological and analytic advances, however, mean PK-guided dosing is now more feasible for use in the clinical setting [22]. Microsampling, using devices such as dried blood spot (DBS) cards or Mitra® devices, at point of care can potentially overcome some of the limitations of venous blood sampling and improve the feasibility of therapeutic drug monitoring (TDM) [23]. Microsampling techniques have been previously examined to measure capecitabine concentrations [24, 25] but correlation between capillary sampling concentrations and plasma

concentrations as well as patient's preferences between microsampling and venous blood sampling are, to our knowledge, unknown.

1.2 Ageing and its effect on drug metabolism

The PKs of chemotherapy in older adults with cancer vary widely due to the complexity and heterogeneity of ageing [26, 27]. Factors affecting the PKs include age-related physiological changes such as homeostasis impairment, organ dysfunction), the presence of geriatric syndromes like frailty, poly-pharmacy, falls and pharmacodynamics of the drug [28-30]. The many physiological changes associated with ageing that influence the PK of drugs including changes in distribution such as body composition, metabolism (ageing liver, hepatic blood flow) and elimination parameters (decline in renal function). Although altered absorption and bioavailability in older adults have not been documented to lead to significant or relevant clinical changes in PK of drugs, there are concerns regarding impaired gastric emptying, nutrition and adherence to treatment [28].

One of the most important factors associated with ageing affecting the PK of drugs is decline in renal function. Glomerular filtration rate (GFR) decreases by about 10 mL/minute/1.73 m2 with each decade of life, leading to an average 50% decline in GFR between the third and ninth decades of life [31, 32]. Dose reductions are therefore required for drugs with predominant renal excretion. Changes in liver function in older adults are also important in affecting drug clearance with consequent variability in response [33], for example, age-related decline in hepatic blood flow leading to a decrease in total clearance of high and low extraction ratio drugs [33]. The US FDA does not currently suggest specific dose adjustments based on older age per se due to insufficient data [34].

1.3 Capecitabine in the management of older adults with breast and

gastrointestinal cancer

Capecitabine is an oral, anti-metabolite in the fluoropyrimidine carbamate class that is a convenient alternative to intravenous 5FU. Capecitabine is commonly used in the management of patients with gastrointestinal cancers (eg colorectal, gastric, pancreatic, biliary) and breast cancer. As a prodrug, it is absorbed rapidly and unaltered through the small intestine and then metabolised primarily in the liver by carboxyl-esterase to 5'-deoxy-5-fluorocytidine (5'-DFCR). 5- DFCR is then converted to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine-deaminase, principally located in the liver and tumour tissue. Further metabolism of 5'-DFUR to the pharmacologically active agent 5-FU occurs mainly at the tumour site by thymidine phosphorylase, present in high levels in tumour tissues [35].

In colorectal cancer, capecitabine is used frequently in the older adults population due to its convenience and use as monotherapy as a less intensive chemotherapy regimen is preferred. It has equivalent efficacy with 5FU/ leucovorin (LV) in the adjuvant and palliative settings. In the adjuvant setting, for example, capecitabine was compared with bolus 5FU/LV in patients (n=1987) with stage III colon cancer (396 patients aged >70 years) with similar disease free survival (DFS) and overall survival (OS) across all age groups (HR for DFS: 0.88, 95% CI: 0.77-1.01, p-value for non-inferiority <0.0001; HR for OS: 0.86, 95% CI: 0.74–1.01, p-value for non-inferiority <0.001) [8]. Common toxicities of capecitabine include fatigue, hand foot syndrome and diarrhoea. Capecitabine had a fewer rate of toxicity, however, older patients required more dose reductions compare with the younger patients (51% in those aged ≥70 years and 39% in those aged <70 years). In the palliative setting, multiple phase III randomised control trials have shown equivalent OS and time to disease progression for capecitabine and 5FU/LV in the management of metastatic colorectal cancer [9, 36-38].

In breast cancer, capecitabine is used in both the metastatic setting, predominantly as first-line palliative chemotherapy in patients with hormone sensitive metastatic breast cancer [11, 12], and

now the adjuvant setting [15]. In metastatic breast cancer, a pooled data analysis from multiple phase II/III trials of capecitabine mono-therapy from 1996 to 2008 demonstrated acceptable response rates and higher objective response rates in the first-line compared with the later lines (ORR: 25.0 vs. 19.0 %, respectively, odds ratio 0.70; 95 % CI: 0.5-1.0) [13] with higher rates of OS and PFS in patients with HFS ($p < 0.0001$ PFS/OS) or diarrhoea ($p = 0.004$ OS; $p = 0.0045$ PFS) compare with patients without these toxicities.

1.4 Influence of ageing on pharmacokinetics of capecitabine and its metabolites

There are only a few studies concerning the PKs of capecitabine and its metabolites in the older adults with cancer, the results of which are inconsistent. Louie et al investigated the PKs of single agent capecitabine in the treatment of a small group of older adults with colorectal cancer (n=29). They showed a large variability in capecitabine clearance (CL/F) and volume of distribution (Vd/F) among older patients (>70 years), compared with younger patients (<60 years), but no difference in the PK parameters of 5'DFCR, 5'DFUR, or 5-FU between the two age groups [39]. Abdi et al compared the capecitabine PK data of 20 older patients with breast or colorectal cancer (aged >75 years) with 40 younger patients (aged <60 years) from two previous clinical trials [18, 40]. Capecitabine had a slower rate of absorption in older patients, but no difference in the clearance between older and younger patients. Higher PK parameters were correlated with a higher toxicity rate and a lower absorption rate constant (ka) of capecitabine in older versus younger patients [18]. Cassidy et al showed no impact of age, gender, BSA or creatinine clearance on PK parameters of capecitabine and its metabolites in adult patients (n=25) with solid tumours [41]. The sample size was, however, small and this study was not designed to compare the PKs of capecitabine between older and younger patients. The US FDA conducted a pooled data analysis of 505 patients with metastatic colorectal cancer, which demonstrated a 15% increase in AUC of FBAL, (a-fluoro-b-alanine) the major renally-excreted

metabolite, per 20% increase in age. There was no impact of age on the PK of 5-FU or other metabolites, and so the FDA does not recommend specific dose adjustments of capecitabine based on older age alone [34].

1.5 Aims and objectives

This thesis overall aims to improve the treatment outcomes of older adults with cancer receiving capecitabine by investigating aspects of individualised dosing to reduce inter- patient variability, significant chemotherapy-related toxicity, and under-dosing from this agent.

The specific objectives were to determine the:

1. long-term outcomes of older adults with resected primary colorectal cancer and the utilisation of adjuvant chemotherapy in this population

2. influence of ageing on the pharmacokinetics of anticancer therapy used in the treatment of patients with colorectal cancer

3. utilisation of microsampling in TDM of anticancer therapy used in the treatment of people with cancer

4. PK of capecitabine and its metabolites in adults with cancer and differences, if any, between older adults and younger adults with cancer, and potential associations of PK parameters with chemotherapy-related toxicity and GA tools

5. the feasibility and acceptability of microsampling for the TDM of capecitabine and the relationship between capillary samples and plasma samples in patients receiving capecitabine

1.6 Outline of Chapters

This thesis is presented in a hybrid format, combining traditional chapters (chapters 1 and 8) with journal publications (chapters 2, 3, 4, 5, 6 and 7).

Chapter 1 presents an introduction to the thesis, the aims and objectives of the thesis and an outline of each individual chapter.

Chapters 2 and 3 address objective 1 and present publications that determined the outcomes of older adults, compared with younger adults, with colorectal cancer and the utilisation of (adjuvant) chemotherapy. As such, these publications highlight the important role of chemotherapy in older adults with cancer and, in particular, capecitabine as a commonly used chemotherapy agent in colorectal cancer. These two chapters are published retrospective cohort studies conducted at a tertiary referral hospital (Concord Repatriation General Hospital) in Sydney, Australia. Only adult patients who had resection of a primary colon cancer or primary rectal cancer were included in the studies. In addition to survival outcomes and the role of chemotherapy, age-associated differences in patients, cancer, and treatment characteristics were determined.

Chapters 4 presents a published systematic review that addresses objective 2. This study summarises the existing evidence on influence of ageing on the pharmacokinetics of anticancer therapeutic agents used in the treatment of colorectal cancer such as fluoropyrimidines, oxaliplatin and irinotecan.

Chapters 5 presents a published systematic review that addresses objective 3. This study

summarises the existing evidence on the utilisation of microsampling like dried blood spot (DBS) sampling or volumetric absorptive microsampling (VAMS), in the measurement of concentrations of anticancer therapeutic agents in patients with solid tumour.

Chapter 6 presents a prospective observational study that addresses objective 4. The manuscript has been accepted for publication. This study explored the influence of ageing on pharmacokinetics of capecitabine and its metabolites in older adults with cancer, and determined associations between PK markers of capecitabine and chemotherapy-induced toxicity, inflammatory markers and GA tools.

Chapter 7 is a manuscript under review for publication that addresses objective 5 and presents a pilot study comparing capecitabine and its metabolites concentrations determined by microsampling (using Mitra® devices) versus venous blood and plasma sampling for therapeutic drug monitoring. This study also determined participants' preferences for the two sampling methods (finger prick sampling versus venous sampling).

Chapter 8 discusses the main findings of this thesis as a whole, including a summary of the principal findings in the context of current and emerging research, strengths and limitations, clinical and research implications, and concluding remarks.

1.7 References

1. Australian Institute of Health and Welfare & Australasian Association of Cancer Registries 2012.Cancer in Australia: an overview, 2012. Cancer series no. 74. Cat. no. CAN 70. AIHW.

2. Patel SS, Nelson R, Sanchez J, et al. Elderly patients with colon cancer have unique tumor characteristics and poor survival. *Cancer*.2013; 119:739-747.

3. Kotake K, Asano M, Ozawa H, Kobayashi H, Sugihara K. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. *Colorectal Dis*.2015; 17:205-215.

4. Wildiers H, Heeren P, Puts M, et al. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. *J Clin Oncol*.2014; 32:2595-2603.

5. Crombag MR, Joerger M, Thürlimann B, Schellens JH, Beijnen JH, Huitema AD. Pharmacokinetics of Selected Anticancer Drugs in Elderly Cancer Patients: Focus on Breast Cancer. *Cancers (Basel)*.2016; 8:6.

6. Korc-Grodzicki B, Boparai MK, Lichtman SM. Prescribing for older patients with cancer. *Clin Adv Hematol Oncol*.2014; 12:309-318.

7. Korc-Grodzicki B, Holmes HM, Shahrokni A. Geriatric assessment for oncologists. *Cancer Biol Med*.2015; 12:261-274.

8. Twelves C, Scheithauer W, McKendrick J, et al. Capecitabine versus 5-fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: final results from the X-ACT trial with analysis by age and preliminary evidence of a pharmacodynamic marker of efficacy. *Ann Oncol*.2012; 23:1190-1197.

9. Van Cutsem E, Hoff PM, Harper P, et al. Oral capecitabine vs intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomised, phase III trials. *Br J Cancer*.2004; 90:1190-1197.

11. Minea, L.N., Stănculeanu, D.L., Cringeanu, A., & Anghel, R.M. Capecitabine monotherapy for elderly patients with metastatic breast cancer. *J Clin Oncol*.2004. *22 14_suppl*, 797.

12. Oshaughnessy JA, Blum J, Moiseyenko V, et al. Randomized, open-label, phase II trial of oral capecitabine (Xeloda) vs. a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. *Ann Oncol*.2001; 12:1247-1254.

13. Blum JL, Barrios CH, Feldman N, et al. Pooled analysis of individual patient data from capecitabine monotherapy clinical trials in locally advanced or metastatic breast cancer. *Breast Cancer Res Treat*.2012; 136:777-788.

14. Shah M, Nunes MR, Stearns V. CDK4/6 Inhibitors: Game Changers in the Management of Hormone Receptor–Positive Advanced Breast Cancer?. *Oncology (Williston Park)*.2018; 32:216-222.

15. Masuda N, Lee SJ, Ohtani S, Im YH, et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. *N Engl J Med*.2017; 376:2147-2159.

16. Bajetta E, Procopio G, Celio L, et al. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. *J Clin Oncol*.2005; 23:2155-2161.

17. Griggs JJ, Mangu PB, Anderson H, et al. Appropriate chemotherapy dosing for obese adult patients with cancer: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol*.2012; 30:1553-1561.

18. Daher Abdi Z, Lavau-Denes S, Prémaud A, et al. Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. *Cancer Chemother Pharmacol*.2014; 73:1285-1293.

19. Gieschke R, Burger HU, Reigner B, Blesch KS, Steimer JL. Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. *Br J Clin Pharmacol*.2003; 55:252-263.

20. Alnaim L. Therapeutic drug monitoring of cancer chemotherapy. *J Oncol Pharm Pract*.2007; 13:207-221.

21. Paci A, Veal G, Bardin C, et al. Review of therapeutic drug monitoring of anticancer drugs part 1--cytotoxics. *Eur J Cancer*.2014; 50:2010-2019.

22. Gamelin E, Delva R, Jacob J, et al. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer [published correction appears in J Clin Oncol. 2013 Oct 1;31(28):3612]. *J Clin Oncol*.2008; 26:2099-2105.

23. Deprez S, Stove CP. Dried blood microsampling-assisted therapeutic drug monitoring of immunosuppressants: An overview. *J Chromatogr A*.2023; 1689:463724.

24. Singhal, P., P. A. Shah, J. V. Shah, et al. Determination of capecitabine-an anticancer drug in dried blood spot by LC-ESI-MS/MS. *Int J Pharma Pharmaceut Sci*.2015; 7: 238-45.

25. Radovanovic M, Schneider JJ, Shafiei M, et al. Measurement of 5- fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2022; 1188: 123075.

26. O'shaughnessy J, et al. Randomized, open-label, phase II trial of oral capecitabine (Xeloda®) vs. a reference arm of intravenous CMF (cyclophosphamide Wasil T, Lichtman SM. Clinical pharmacology issues relevant to the dosing and toxicity of chemotherapy drugs in the elderly. The oncologist. 2005;10(8):602-12.

27. Ferrucci L, et al. The frailty syndrome: a critical issue in geriatric oncology. Critical reviews in oncology/hematology. 2003;46(2):127-37.

28. Klotz U. Pharmacokinetics and drug metabolism in the elderly. Drug metabolism reviews. 2009;41(2):67-76.

29. Wynne H. Drug metabolism and ageing. The journal of the British Menopause Society. 2005;11(2):51-6.

30. Hilmer SN, et al. Clinical pharmacology in the geriatric patient. Fundamental & clinical pharmacology. 2007;21(3):217-30.

31. Gault MH, Cockcroft DW. Letter: Creatinine clearance and age. Lancet (London, England). 1975;2(7935):612-3.

32. Siersbaek-Nielsen K, et al. Rapid evaluation of creatinine clearance. Lancet (London, England). 1971;1(7709):1133-4.

33. Wilkinson GR. Drug metabolism and variability among patients in drug response. The New England journal of medicine. 2005;352(21):2211-21.

34. Food and Drug Administration Xeloda (Capecitabine), NDA 020896. 2015. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/020896s037lbl.pdf

35. Maroun JA. Capecitabine in the management of colorectal cancer. Expert review of anticancer therapy. 2001;1(3):327-33.

36. Twelves C. Capecitabine as first-line treatment in colorectal cancer. Pooled data from two large, phase III trials. European journal of cancer (Oxford, England : 1990). 2002;38 Suppl 2:15-20.

37. Van Cutsem E, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(21):4097-106.

38. Hoff PM, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(8):2282-92.

39. Louie SG, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). British journal of cancer. 2013;109(7):1744-9.

40. Urien S, et al. Pharmacokinetic modelling of 5-FU production from capecitabine--a population study in 40 adult patients with metastatic cancer. Journal of pharmacokinetics and pharmacodynamics. 2005;32(5-6):817-33.

41. Cassidy J, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. Cancer chemotherapy and pharmacology. 1999;44(6):453-60.

Chapter 2: Utilisation of adjuvant chemotherapy and 5-Year Survival analysis of prospectively recorded cohort data for older adults versus younger adults with resected primary colon cancer

2.1 Overview

This chapter is a published retrospective cohort study determining overall survival (OS) and cancer specific survival (CSS) and utilisation of chemotherapy among older adults versus younger adults with colon cancer. Older adults with colon cancer compared with younger adults had worse overall survival and received less adjuvant chemotherapy. This work highlighted poorer outcomes and ways to improve outcomes of older adults with colon cancer is an area of unmet research need. This manuscript is not quoted verbatim and formatted for publication as required by the Journal of Gastrointestinal Cancer.

2.1.1 Publication details

Shafiei M, Beale P, Blinman P. Utilisation of Adjuvant Chemotherapy and 5-Year Survival Analysis of Prospectively Recorded Cohort Data for Older Adults Versus Younger Adults with Resected Primary Colon Cancer. *J Gastrointest Cancer.* 2020 Sep; 51(3):988-997.

2.1.2 Contribution of authors to the work described in the Chapter

Mohsen Shafiei was responsible for the study concept, data analysis and interpretation of the findings, drafted and revised the manuscript.

Philip Beale contributed to the research proposal, interpretation of the findings and contributed to the revision of the manuscript.

Prunella Blinman was responsible for the study concept, data interpretation, drafting and review of the manuscript.

Abstract

Purpose

Colon cancer is predominantly a disease of older adults. Studies determining the influence of age on outcomes of colon cancer have conflicting results. We aim to determine the long-term outcomes and utilisation of adjuvant chemotherapy of older adults compared with younger adults who had had a resection of a primary colon cancer.

Methods

Consecutive patients who had resection of a primary colon cancer between January 1, 2000 and December 31, 2010 were identified from a prospective database and stratified into three age groups: ≤ 69 years, 70 to 79 years, and ≥ 80 years. Age related differences in patients, cancer, and treatment characteristics were determined by chi-square tests. Five-year overall survival and cancer-specific survival were determined by Kaplan-Meier method and by multivariable Cox regression analysis to adjust for potential confounding factors.

Results

Of 1135 included patients, 469 (41%) patients were aged ≤ 69 years, 382 (34%) were 70–79 years, and 284 (25%) were ≥80 years. Increasing age group predicted more comorbidity ($p < 0.001$), cardiac comorbidity ($p < 0.001$), right-sided cancers ($p < 0.001$), and less adjuvant chemotherapy (stage III only; $p < 0.001$). Increasing age group was associated with worse overall survival by stage ($p < 0.001$) but not cancer-specific survival by stage ($p = 0.83$).Adjuvant chemotherapy in patients with stage III colon cancer independently predicted improved overall survival ($p < 0.001$) and cancer-specific survival ($p = 0.01$).

Conclusions

Compared with younger adults, older adults with colon cancer had worse survival outcomes and received less adjuvant chemotherapy.

Keywords

Colon cancer. Adjuvant chemotherapy. Older adults. Overall survival. Cancer-specific survival

2.2 Introduction

Colon cancer is a common cancer predominantly affecting older adults. In 2018 worldwide, 1,096,601 individuals were diagnosed with colon cancer with the highest incidence in developed countries [1]. In Australia, the median age of diagnosis of colon cancer is 69 years, with more than 57% of the 10,000 effected individuals aged ≥70 years [2]. Given the ageing of the population, this means that an increasing number of older adults will be diagnosed with colon cancer and require treatment for this condition [3, 4].

Older adults with colon cancer present distinct challenges to cancer clinicians involved in their care. Ageing is a heterogeneous process with chronological age not always reflecting physiological age resulting in a wide range of suitability and fitness for cancer treatments. Non-cancer-related factors that affect decisions about cancer treatments in older adults include frailty, geriatric syndromes (e.g. poly-pharmacy, falls, malnutrition, cognitive impairment) and multiple comorbidities. In colon cancer, outcomes of older adults are also influenced by adverse tumour characteristics and differences in tolerance of treatment. Older adults with colon cancer are more likely to have right sided cancers [5, 6] and present at a more advanced cancer stage at the time of diagnosis [7], both of which are poor prognostics factors [7, 8]. Older adults also have a higher risk of peri-operative morbidity and mortality [9-11] and more frequent and severe chemotherapy toxicity [12] which may lead to underutilisation of the treatments.

Older adults with cancer are typically under-represented in clinical trials [13] meaning there is little available randomised data from trials including older adults to help guide their cancer care. Survival outcomes for older adults hence are often derived from subset analyses from clinical trials in which older adults only make up a small proportion of the trial population,

rather than from specific trials of older adults [14]. Older adults who participate in clinical trials are generally fitter and less frail than older adults with cancer seen in routine clinical practice, where most decisions about chemotherapy are made. Outcome studies can help fill this gap.

We aimed to determine the long-term outcomes of older adults who had resection for primary colon cancer compared with their younger counterparts and their utilisation of adjuvant chemotherapy at our local institution. We hypothesised that older adults, compared with younger adults, have worse long-term outcomes and lower rates of utilisation of adjuvant chemotherapy.

2.3 Methods

2.3.1 Study design

The study was a retrospective observational study of a prospectively maintained database of consecutive patients aged ≥18 years who had undergone curative or palliative resection of a primary colon cancer at Concord Repatriation General Hospital, Sydney, Australia. Patients registered between January 1, 2000 and December 31, 2010 were included in this study to allow at least 5-years of follow-up on all patients who had not died by December 31, 2016 for observed rather than estimated 5-year outcomes. The prospectively maintained database commenced in 1971 and includes details of patient characteristics, presentation, comorbidity, investigations, pathology, surgical management, complications, receipt of adjuvant therapy and follow-up data. Patients were excluded from the database if their tumour was not an invasive carcinoma or if they had inflammatory bowel disease or familial adenomatous polyposis coli. The database has ethics committee approval from the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206) and patients gave written consent for the use of their data and tumour specimens for research.

Patients were assigned to one of three age groups according to their age at the time of diagnosis with colon cancer: ≤69years, 70 to 79 years, and ≥ 80 years. This study included and explored the following variables: patient gender, previous history of colorectal cancer, number of comorbidities, cardiac comorbidity (New York Heart Association, NYHA), resection at urgent operation, histological type, staging TNM classification, tumour location, maximum surface dimension, number of nodes examined, distant metastasis, lymphatic vessel invasion, venous invasion, positive margin and adjuvant chemotherapy. Right-sided tumour was defined as tumour confined to caecum, ascending colon, hepatic flexure and

transverse colon and left-sided tumour was defined as tumour that involved splenic flexure, descending colon and sigmoid colon. Stage III was defined as colonic tumours with regional or apical nodal involvement and with no identifiable systemic metastatic disease (pTNM Stage III). The rationale for the focus on stage III colon cancer was this being the stage with a clear indication for adjuvant chemotherapy. The confounding factors of interest were chosen as evidence-based factors affecting the OS of patients with colorectal cancer (as described in the introduction).

2.3.2 Statistical analysis

Patient demographic, tumour and treatment characteristics between the three age-groups (≤69years, 70-79 years and ≥80 years) were compared by the chi-squared test. The failure event for overall survival (OS) was death from any cause and the failure event for cancer-specific survival (CSS) was death due to colon cancer, other cases being censored. The p-values were determined across the three age groups and values <0.05 were considered statistically significant.

5-year OS and CSS were assessed using Kaplan-Meier method and Cox regression. Patients were reviewed at six-monthly intervals for the first two years after resection and yearly thereafter until death or 31st December 2016. Analyses were conducted on the basis of intention to treat. Results are presented as 5-year OS and 5-year CSS curves by age group for the overall population and for the subset of patients with stage III colon cancer. In patients with stage III colon cancer, associations between dichotomised patient demographics, tumour and treatment characteristic and OS was determined using bivariate and multivariable Cox regression analysis. Statistical analysis was performed using SPSS version 24 (IBM Australia Limited, 2016). Two-sided tests were used with the level for significance set at 0.05.

2.4 Results

Of the 1135 included patients, the mean age was 70.5 years (range, 31-97 years) and just over half were male (604/1135, 53.2%). Patients predominantly had a resection for stage II (439/1135, 38.7%) or stage III (302, 26.6%) colon cancer. The primary site was more frequently right-sided (622/1135, 54.8%) than left-sided (513/1135, 45.2%).

Demographic, tumour and treatment characteristics are presented in Table 1. The total population by age group was 41% (469/1135) aged ≤69years, 34% (382/1135) aged 70-79 years, and 25% (284/1135) aged ≥80 years. Increasing age group was significantly associated with higher comorbidity ($p < 0.001$), cardiac comorbidity ($p < 0.001$), right-sided cancers ($p < 0.001$) and less adjuvant chemotherapy (stage III only; $p < 0.001$). In stage III colon cancer, 83% (114/138) of patients aged ≤69 years had adjuvant chemotherapy compared to 58% (55/94) for patients aged 70-80 years and 4% (3/70) for patients aged ≥80 years (Table 1). There was a trend towards a higher rate of larger tumours (>5cm) ($p=0.07$), resection at urgent operation ($p=0.08$) and lymphatic vessel invasion ($p=0.07$) among older patients compared with younger patients.

The OS and CSS by cancer stage and age group are presented in Table 2. Kaplan-Meier survival curves are presented in Figures 1 to 4. OS decreased significantly with increasing age group for all stages considered in total ($p<0.001$) (Table 2, Figure 1). For the smallest stage category of stage IV (150/1135, 13.2%), due to small numbers, OS was similar at 9% for patients aged 70-79 years (4/45), 8% for patients aged ≤69 years (5/66) and 5% for patients aged ≥80 years (2/39). OS for patients with stage III colon cancer, considered alone, also decreased for increasing age group (Figure 3). For CSS, there was no significant difference across age groups by stage considered in total ($p=0.83$) (Table 2, Figure 2). For

stage III, however, CSS for patients aged ≤69 years was significantly longer than CSS for patients ≥70 years (p=0.01) (Figure 4).

Predictors of OS in stage III colon cancer are presented in Table 3. Bivariate predictors of better OS were age ≤69 years (p<0.001), resection at non-urgent operation (p<0.001), no lymphatic vessel invasion (p=0.001), no positive margin (p=0.004), receipt of adjuvant chemotherapy (p<0.001) (Figure 5), maximum surface dimension of ≤ 5cm, number of comorbidities of ≤ 1(<0.001), left sided tumour (p=0.003) and no venous invasion (p=0.04). Adjuvant chemotherapy was also a predictor of better CSS in stage III colon cancer (HR 0.59, p=0.01) (Figure 6). Independent predictors of improved OS were age ≤69 years [HR (hazard ratio) 0.46, p=0.002], no resection at urgent operation (HR 0.30, p<0.001), no lymphatic vessel invasion (HR 0.70, p=0.03), no venous invasion (HR 0.65, p=0.04) and receipt of adjuvant chemotherapy (HR 0.52, p=0.001).

2.5 Discussion

The key findings of our study were that older adults, compared with younger adults, who had had a resection of a primary colon cancer of stage I to IV had higher comorbidity, more frequent right-sided cancers, and received less adjuvant chemotherapy. OS worsened with increasing age. CSS was similar across age groups other than in stage III where older adults had worse CSS.

Studies determining the effect of age on outcomes of colon cancer have conflicting results. Some studies show that older adults with colon cancer, compared with younger adults with colon cancer, have worse OS and CSS [4, 15]. Other studies show similar survival outcomes between older adults with colon cancer and younger adults with colon cancer especially in patients undergoing curative surgery for their colon cancer [16-18]. We had similar results with previous studies with regards to increasing age being associated with worse OS [4, 19, 20] more right-sidedness, [4, 8, 21, 22] comorbidity, [8, 17] and receipt of less adjuvant chemotherapy, [4, 8, 16, 17] but heterogeneous results with regards to CSS [18].

The largest two comparable outcome studies include Kotake et al who studied over 40000 patients with colorectal cancer from the Japanese cancer registry [8]. This study showed increasing age was associated with worse 5-year OS (50% in ≥80 years age group vs 73% in 50-64 years age group, $p<0.001$) and worse CSS (65% in ≥80 years age group vs 76% in 50-64 years age group, $p<0.001$) in patients with stage III disease. Similarly, Patel et al in a study of nearly 33000 patients with colon cancer also found increasing age was associated with lower 5-year OS (26% in ≥80 years age group vs 61% in 50-64 years age group, $p<0.001$) and lower 5-year CSS (50% in ≥80 years age group vs 69% in 50-64 years age group, $p<0.001$) in patients with stage III disease [4]. Two smaller studies by Devon et al

(n=623) and Widdison et al (n= 459) showed increasing age was a predictor of worse OS but not CSS, [17, 23] indicating that older patients are more likely to die from comorbid illnesses than cancer.

Increasing age was associated with worse OS but not CSS in our total study population indicating that older adults died of inter-current causes rather than of colon cancer. For stage III colon cancer, however, increasing age was associated with worse CSS, possibly due to the underutilisation of adjuvant chemotherapy in older adults. Worse OS may be due to the differences in tumour and patient characteristic and treatment disparity, but it seems unlikely that they account for all the differences in OS across the three age groups. Better understanding of the reasons behind these differences has important implications to prevent older adults with colon cancer being discriminated and denied standard of care treatment due to their chronological age alone or being treated unnecessarily. This is especially important in older adults with stage III colon cancer, where CSS of older adults was worse than in younger adults, where a careful selection approach should be adopted to consider surgery and adjuvant chemotherapy to improve their OS and CSS.

Right-sided tumour location is an established negative prognostic factor in patients with relapsed or stage IV colon cancer, but its impact on outcomes in patients with stage I-III colon cancer is unclear [24, 25]. In our study, right-sided tumour location did not independently predict OS in patients with stage III colon cancer. Kennecke et al investigated the prognostic impact of tumour sidedness in patients with colon cancer (n= 5378) and showed that right-sided tumour location was a favourable prognostic factor in patients with stage II colon cancer and a negative prognostic factor in stage IV colon cancer, but not a prognostic factor in stage III colon cancer [24]. The latter finding may be ameliorated, in part,

by the presence of high microsatellite instability (MSI) in 20% of right-sided tumours given the favourable prognostic effect of MSI-high cancers [26]. In a meta-analysis of 66 studies with more than 1.4 million patients with colon cancer, Petrelli et al found that tumour left-sidedness was associated with a significantly decreased risk of death (hazard ratio, 0.82, 95% CI, 0.79-0.84; $p<0.001$) independent of stage [25]. Therefore, the actual impact of tumour sidedness on the outcomes of older patients with colon cancer, particularly those with early stage disease, remains unclear.

Comorbidity is very relevant to the management of older adults with colon cancer as it weighs strongly in decisions about cancer treatments. Whilst higher comorbidity has previously been associated with worse OS in patients with colon cancer, [27-29] we did not find this in our study. This is possibly due to the selection bias of patients needing to be fit enough to have had a resection of colon cancer to be included in the database, and hence the smaller proportion of patients with >1 comorbid conditions (76/226, 33%). The impact of more comorbidity on CSS, as opposed to OS, is unclear because often OS has been the survival endpoint in the available studies not CSS, but it is likely less significant [27, 30-32].

Older adults in our study, compared with younger patients, received less adjuvant chemotherapy. Older adults (aged >80 years) had also a lower adjuvant chemotherapy utilisation rate (4%) than older patients in large database studies (8% and15 %) [4, 8]. Reasons why patients did not receive adjuvant chemotherapy would have been useful to review, but these were not captured in the database and is a limitation of the study. The receipt of adjuvant chemotherapy predicted better OS in stage III colon cancer. The demonstrated OS benefit of adjuvant chemotherapy in this study is from non-randomised data, and hence minimal emphasis is placed on this result. The under-utilisation of adjuvant

chemotherapy may be due to the lack of robust data supporting the use of adjuvant chemotherapy for colon cancer in older adults (aged ≥70 years) [33]. Other reasons include clinician nihilism and unwillingness to refer or treat older adults with adjuvant chemotherapy, inadequate skills in assessing older adults' suitability for chemotherapy, and concerns about excess toxicity even with standard doses [34]. Fit older patients with colon cancer benefit equally from adjuvant chemotherapy without a significant increase in toxicity [35, 36]. Ways to increase utilisation of adjuvant chemotherapy in older adults include conducting trials specifically in older adults, the use of geriatric assessments and risk predicting tools to assist oncologists in assessing older adults' suitability for chemotherapy [37-39] and studies determining the optimal adjuvant dosing of chemotherapy agents in older adults [38, 40].

The main strength of our study lies in it being performed on a large prospectively maintained surgical database over one decade with minimal missing data. Limitations include the database only involving a single institution meaning that the surgical and oncological management, patient selection, surgical techniques, post-operative care, and selection for adjuvant chemotherapy may differ from other institutions or health care settings. Generalisability of the study may also be limited by the sample bias of only including patients who had had a resection of a primary colon cancer and hence excludes patients who were not suitable or fit for surgery or chose not to have surgery. Details of chemotherapy regimen, completion or toxicities were also not readily available and required retrieval of individual patient records for which the study was not resourced.

2.6 Conclusion

Older adults who had a resection of a stage I-IV colon cancer had higher comorbidity, more frequent right-sided cancers, and received less adjuvant chemotherapy. Older adults had

worse OS across all stages and worse CSS in stage III disease. These results highlight the need to optimise the treatment of older adults with colon cancer and ways to increase the utilisation of adjuvant chemotherapy.

Figure 1. Kaplan Meier OS curve by age group for all stages

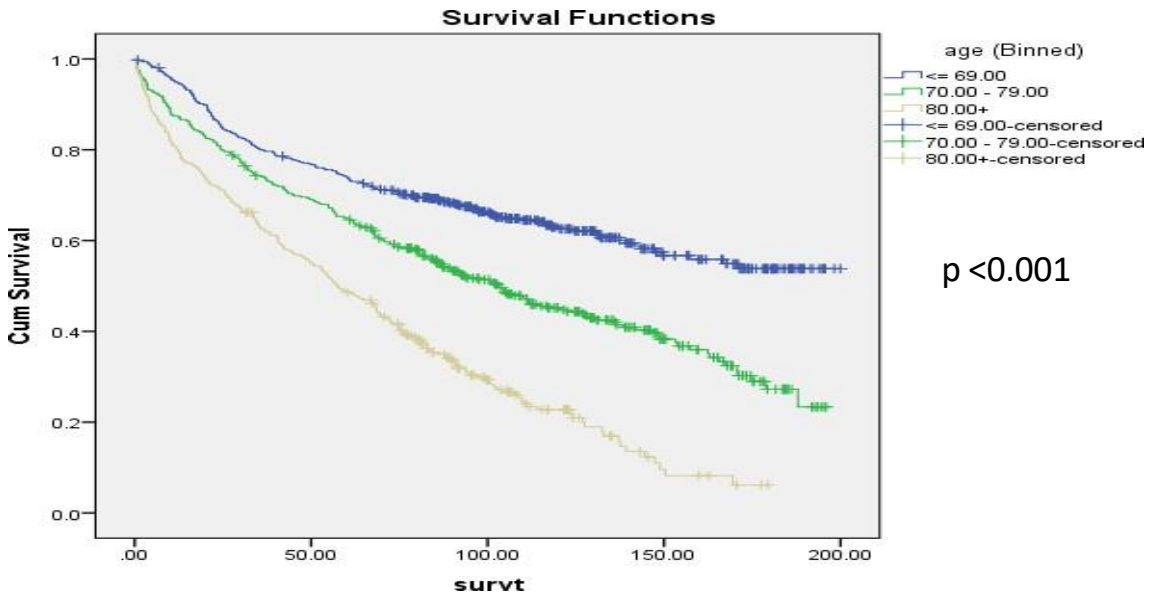
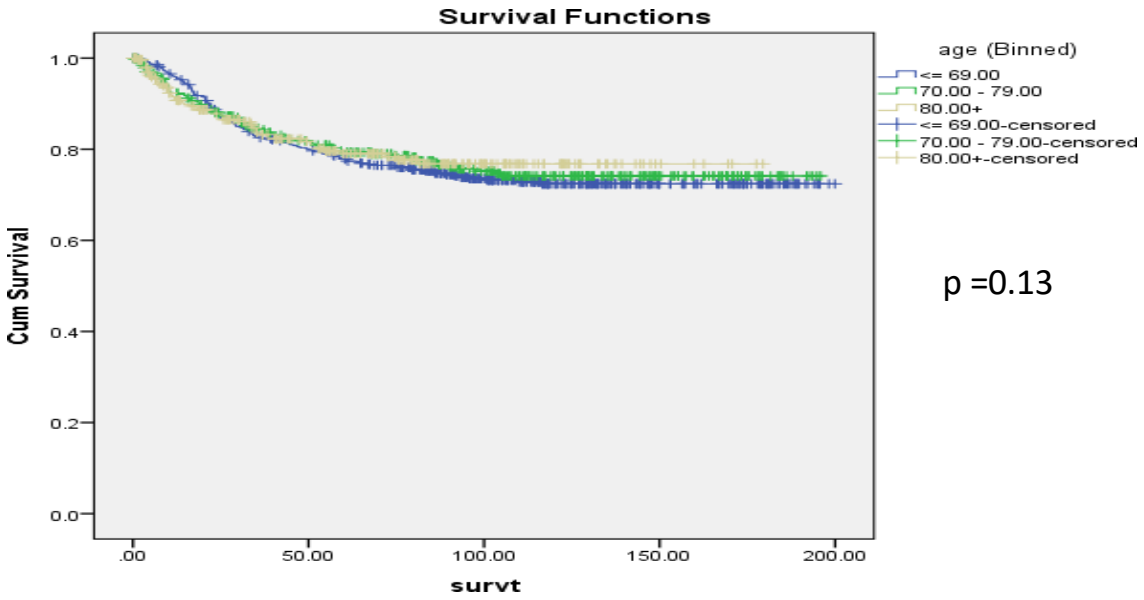
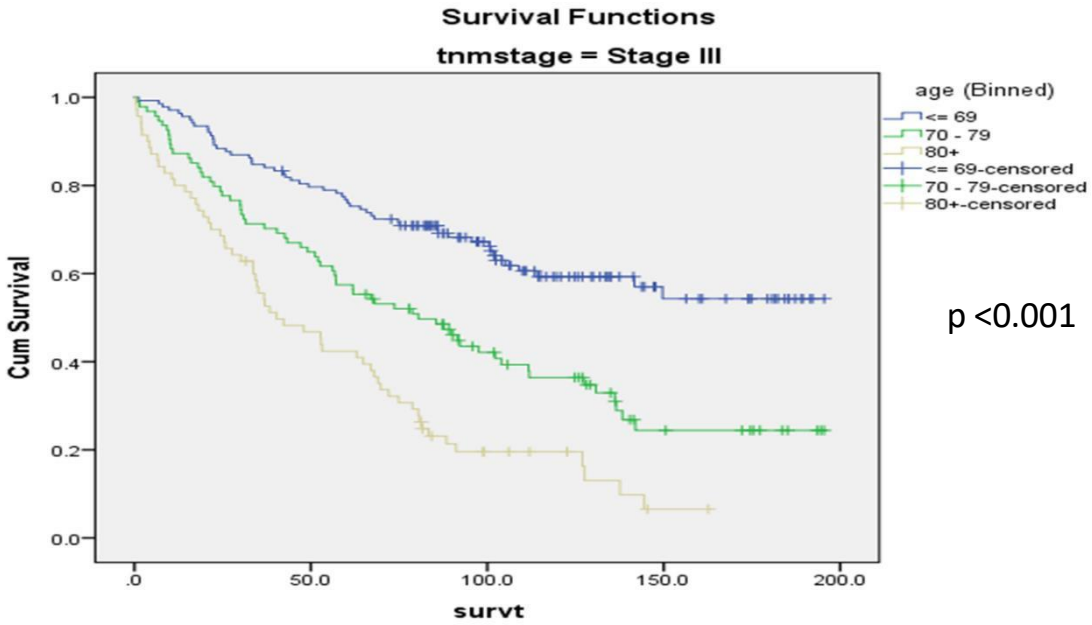


Figure 2. Kaplan Meier CSS curve by age group for all stages



p =0.13

Figure 3. Kaplan Meier OS curve by age group for stage III only



p <0.001

Figure 4. Kaplan Meier CSS curve by age group in stage III only

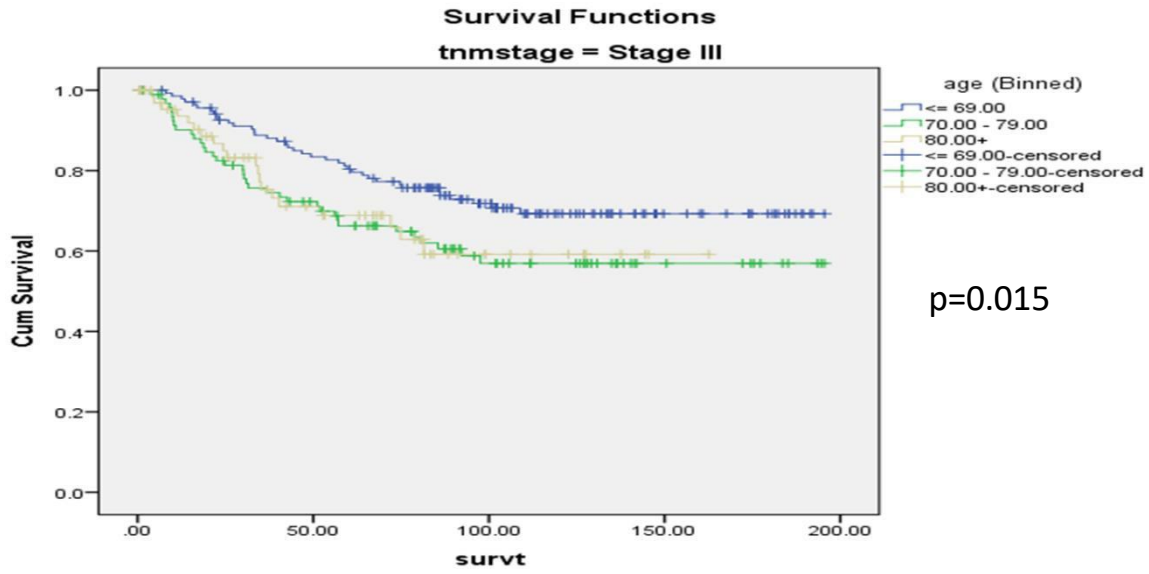


Figure 5. Kaplan Meier OS curve by adjuvant chemotherapy in stage III

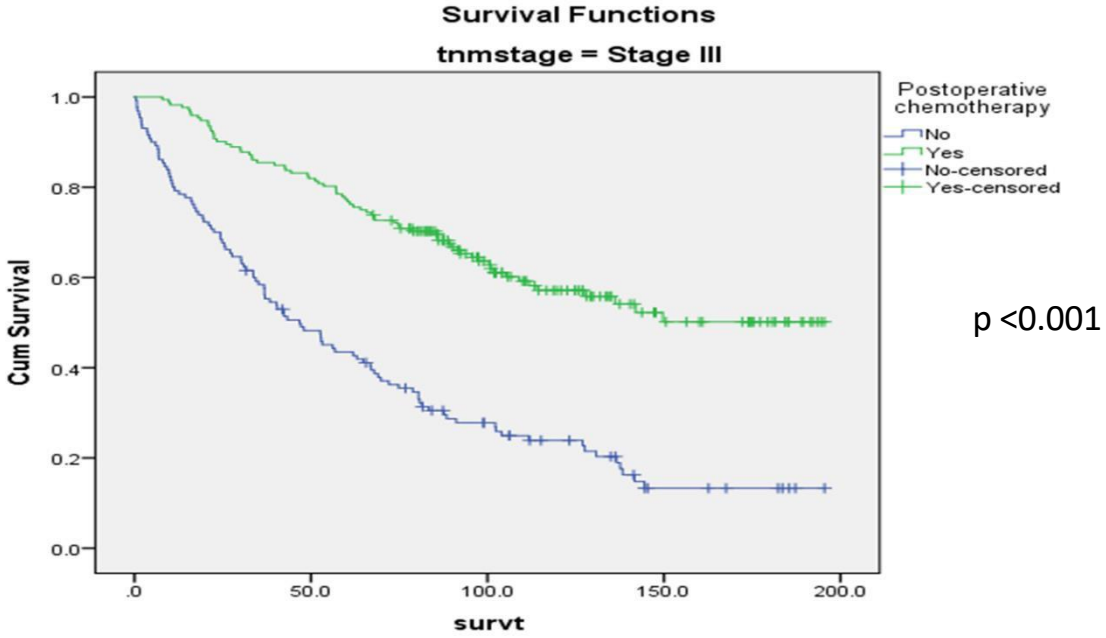


Table 1. Tumour and treatment characteristics stratified by age

Characteristics	Age group years				
	Total N=1135 Mean 70.5	≤69 N=469 Mean 58.7	70-79 N= 382 Mean 74.7	≥80 N= 284 Mean 84.4	P for 3 age groups
Gender					
Male	604 (3.2%)	257 (54.8%)	209 (54.7%)	138 (48.6%)	0.20
Female	531 (46.8%)	212 (45.2%)	173 (45.3%)	146 (51.4%)	
Previous CRC resected					
No	1073 (94.5%)	447 (95.3%)	357 (93.5%)	269 (94.7%)	0.49
Yes	62 (5.5%)	22 (4.7%)	25 (6.5%)	15 (5.3%)	
No. of comorbidities					
≤1	784 (69.1%)	385 (82.1%)	238 (62.3%)	161 (56.7%)	<0.001
>1	351 (30.9%)	84 (17.9%)	144 (37.7%)	123 (43.3%)	
Heart problem*					
No	737 (71.1%)	398 (87.3%)	234 (67.2%)	105 (45.1%)	<0.001
Yes	300 (28.9%)	58 (12.7%)	114 (32.8%)	128 (54.9%)	
Resection at urgent operation					
No	1050 (92.5%)	442 (94.2%)	353 (92.4%)	255 (89.8%)	0.08
Yes	85 (7.5%)	27 (5.8%)	29 (7.6%)	29 (10.2%)	

Site of primary tumour					
Right	622 (54.8%)	209 (44.6%)	217 (56.8%)	196 (69.0%)	<0.001
Left	513 (45.2%)	260 (55.4%)	165 (43.2%)	88 (31.0%)	
Histological type of primary					
Adenocarcinoma	1007 (88.7%)	418 (89.1%)	346 (90.6%)	243 (85.6%)	0.12
Mucinous Adenocarcinoma/ Signet ring	128 (11.3%)	51 (10.9%)	36 (9.4%)	41 (14.4%)	
Maximum surface dimension – cm					
≤ 5	755 (66.5%)	326 (69.5%)	255 (66.8%)	174 (61.3%)	0.07
>5	380 (33.5%)	143 (30.5%)	127 (33.2%)	110 (38.7%)	
Number of nodes examined					
≤ 11	307 (27%)	113 (24.1%)	121 (31.7%)	73 (25.7%)	0.04
12+	828 (73%)	356 (75.9%)	261 (68.3%)	211 (74.3%)	
Distant metastasis					
No	985 (86.8%)	403 (85.9%)	337 (88.2%)	245 (86.3%)	0.60
Yes	150 (13.2%)	66 (14.1%)	45 (11.8%)	39 (13.7%)	
Lymphatic vessel permeation					
No	904 (79.6%)	366 (78.0%)	319 (83.5%)	219 (77.1%)	0.07
Yes	231 (20.4%)	103 (22.0%)	63 (16.5%)	65 (22.9%)	
Venous invasion					

None	999 (88.0%)	408 (87.0%)	343 (89.8%)	248 (87.3%)	0.79
Yes	136 (12.0%)	61 (13.0%)	39 (10.2%)	36 (12.7%)	
Tumour in line of resection					
No	1108 (97.6%)	458 (97.7%)	372 (97.4%)	278 (97.9%	0.91
Yes	27 (2.4%)	11 (2.3%)	10 (2.6%)	6 (2.1%)	
Adjuvant chemotherapy Stage III					
No	130 (43%)	24 (17.4%)	39 (41.5%)	67 (95.7%)	<0.001
Yes	172 (57%)	114 (82.6%)	55 (58.5%)	3 (4.3%)	
TNM stage					
Stage I	244 (13.2%)	95 (20.3%)	98 (25.7%)	51 (18%)	0.09
Stage II	439 (38.7%)	170 (36.2%)	145 (38%)	124 (43.7%)	
Stage III	302 (26.6%)	13 8(29.4%)	94 (24.6%)	70 (24.6%)	
Stage IV	150 (21.5%)	66 (14.1%)	45 (11.8%)	39 (13.7%)	
*98 missing cases were missing data for New York Heart Association evaluation.					

Table 2. 5-year OS and CSS by age group and pathological stage

Stage	Age group	Cases (n)	5 Year OS rate (%)	P value	5 Year CSS rate	P value
Stage I	< 70	95	92%	<0.001	99%	0.13
	70-79	98	79%		99%	
	≥ 80	51	74%		97%	
	All	244				
Stage II	< 70	170	87%		91%	
	70-79	145	78%		93%	
	≥ 80	124	55%		93%	
	All	439				
Stage III	< 70	138	77%		80%	
	70-79	94	57%		66%	
	≥ 80	70	42%		69%	
	All	302				
Stage IV	< 70	66	8%		9%	
	70-79	45	9%		12%	
	≥ 80	39	5%		11%	
	All	150				

Table 3. Bivariate and multivariable OS analysis for stage III colon cancer

Variable	Number	Bivariate hazard ratio (95% CI)	p	Multivariable hazard ratio (95% CI)	p
Female	135				
Male	167	1.01(0.75-1.37)	0.93		
Age < 70 years	138				
Age ≥ 70 years	164	0.26 (0.18-0.38)	<0.001	0.46 (0.29-0.75)	0.002
No Previous CRC	289				
Previous CRC	13	1.04 (0.49-2.21)	0.92		
No Resection at urgent operation	283				
Resection at urgent operation	19	0.21 (0.13-0.34)	<0.001	0.30 (0.17-0.51)	<0.001
No Lymphatic vessel invasion	195				
Lymphatic vessel invasion	107	0.61 (0.45-0.82)	0.001	0.70 (0.51-0.96)	0.03
Adenocarcinoma	270				
Other histology	32	0.73 (0.46-1.14)	0.16		

No Tumour in line of resection Tumour in line of resection	294 8	0.33 (0.15-0.70)	0.004	0.62 (0.26-1.48)	0.29
Adjuvant chemotherapy No Adjuvant chemotherapy	172 130	0.33 (0.24-0.45)	<0.001	0.52 (0.35-0.77)	0.001
Maximum surface dimension ≤ 5cm Maximum surface dimension > 5cm	206 96	0.70 (0.51-0.95)	0.02	0.87 (0.63-1.20)	0.39
Number of nodes examined < 12 Number of nodes examined ≥ 12	79 223	0.87 (0.62-1.22)	0.41		
Number of Comorbidities ≤ 1 Number of Comorbidities > 1	226 76	0.54 (0.39-0.74)	<0.001	0.87 (0.62-1.23)	0.44
Right Sided Left sided	161 141	1.57 (1.16-2.13)	0.003	1.32 (0.96-1.81)	0.09
No Venous invasion Venous invasion	253 49	0.67 (0.45-0.10)	0.04	0.65 (0.43-0.99)	0.04

CRC, Colorectal cancer

2.7 References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J. Clin.*2018; 68:394-424.

2. Australian Institute of Health and Welfare. Colon Cancer ACIM. https://www.aihw.gov.au/reports/cancer/cancer-data-in-australia/acim-books. 2018 Accessed 16 May 2019.

3. Audisio RA, Veronesi P, Ferrario L, Cipolla C, Andreoni B, Aapro M. Elective surgery for gastrointestinal tumours in the elderly. *Ann Oncol.*1997; 8:317-326.

4. Patel SS, Nelson R, Sanchez J, Lee W, Uyeno L, Garcia-Aguilar J et al. Elderly patients with colon cancer have unique tumor characteristics and poor survival. *Cancer.*2013; 119:739-747.

5. Kamran SC, Clark JW, Zheng H, Borger DR, Blaszkowsky LS, Allen JN et al. Primary tumor sidedness is an independent prognostic marker for survival in metastatic colorectal cancer: Results from a large retrospective cohort with mutational analysis. *Cancer Med.*2018; 7:2934-2942.

6. Loupakis F, Yang D, Yau L, Feng S, Cremolini C, Zhang W et al. Primary tumor location as a prognostic factor in metastatic colorectal cancer. *J Natl Cancer Inst.*2015; 107.

7. Marusch F, Koch A, Schmidt U, Zippel R, Gastmeier J, Ludwig K et al. Impact of age on the short-term postoperative outcome of patients undergoing surgery for colorectal carcinoma. *Int J Colorectal Dis*.2002; 17:177-184.

8. Kotake K, Asano M, Ozawa H, Kobayashi H, Sugihara K. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. *Colorectal Dis.*2015; 17:205-215.

9. Baquero GA, Rich MW. Perioperative care in older adults. *J Geriatr Cardiol*.2015; 12:465-469.

10. McNicol L, Story DA, Leslie K, Myles PS, Fink M, Shelton AC et al. Postoperative complications and mortality in older patients having non-cardiac surgery at three Melbourne teaching hospitals. *Med J Aust*.2007; 186:447-452.

11. Sieber FE, Barnett SR. Preventing postoperative complications in the elderly. *Anesthesiol Clin*.2011; 29:83-97.

12. Crombag MR, Joerger M, Thurlimann B, et al. Pharmacokinetics of Selected Anticancer Drugs in Elderly Cancer Patients: Focus on Breast Cancer. *Cancers* (Basel).2016; 8.

13. Hurria A, Dale W, Mooney M, et al. Designing therapeutic clinical trials for older and frail adults with cancer: U13 conference recommendations. *J Clin Oncol*.2014; 32:2587-2594.

14. Moth EB, Kiely BE, Martin A, et al. Older adults' preferred and perceived roles in decision-making about palliative chemotherapy, decision priorities and information preferences. *J geriatr oncol*.2019; 30190:1879-4068.

15. Kune GA, Kune S, Field B, et al. Survival in patients with large-bowel cancer. A population-based investigation from the Melbourne Colorectal Cancer Study. *Dis Colon Rectum*.1990; 33:938-946.

16. McMillan DC, McArdle CS. The impact of young age on cancer-specific and non-cancer-related survival after surgery for colorectal cancer: 10-year follow-up. *Br. J. Cancer.* 2009; 101:557.

17. Devon KM, Vergara-Fernandez O, Victor JC, et al. Colorectal cancer surgery in elderly patients: presentation, treatment, and outcomes. *Dis Colon Rectum*.2009; 52:1272-1277.

18. Chandrasinghe PC, Ediriweera DS, Nazar T, et al. Overall Survival of Elderly Patients Having Surgery for Colorectal Cancer Is Comparable to Younger Patients: Results from a South Asian Population. *Gastroenterol Res Pract.*2017; 9670512-9670512.

19. Dekker JW, van den Broek CB, Bastiaannet E, et al. Importance of the first postoperative year in the prognosis of elderly colorectal cancer patients. *Ann Surg Oncol*.2011; 18:1533-1539.

20. Jessup JM, McGinnis LS, Steele GD, et al. The National Cancer Data Base. Report on colon cancer. *Cancer*.1996; 78:918-926.

21. Holt PR, Kozuch P, Mewar S. Colon cancer and the elderly: from screening to treatment in management of GI disease in the elderly. *Best Pract Res Clin Gastroenterol*.2009; 23:889-907.

22. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med*.2005; 352:2211-2221.

23. Widdison AL, Barnett SW, Betambeau N. The impact of age on outcome after surgery for colorectal adenocarcinoma. *Ann R Coll Surg Engl.*2011; 93:445-450.

24. Kennecke HF, Yin Y, Davies JM, et al. Prognostic effect of sidedness in early stage versus advanced colon cancer. *Health Sci Rep.* 2018; 1:e54.

25. Petrelli F, Tomasello G, Borgonovo K, et al. Prognostic survival associated with left-sided vs right-sided colon cancer: A systematic review and meta-analysis. *JAMA Oncol.* 2017; 3:211-219.

26. Iacopetta B, Grieu F, Amanuel B. Microsatellite instability in colorectal cancer. *Asia Pac J Clin Oncol*.2010; 6:260-269.

27. Sarfati D, Hill S, Blakely T, et al. The effect of comorbidity on the use of adjuvant chemotherapy and survival from colon cancer: a retrospective cohort study. *BMC Cancer*.2009; 9:116.

28. Sarfati D, Tan L, Blakely T, et al. Comorbidity among patients with colon cancer in New Zealand. *N Z Med J*.2011; 124:76-88.

29. Tooth L, Hockey R, Byles J, et al. Weighted multimorbidity indexes predicted mortality, health service use, and health-related quality of life in older women. *J Clin Epidemiol*.2008; 61:151-159.

30. He X, Clarke SJ, McLachlan AJ. Clinical pharmacology of chemotherapy agents in older people with cancer. *Curr Gerontol Geriatr Res.*2011; 2011:628670.

31. Rieker RJ, Hammer E, Eisele R, et al. The impact of comorbidity on the overall survival and the cause of death in patients after colorectal cancer resection. *Langenbecks Arch Surg*.2002; 387:72-76.

32. Søgaard M, Thomsen RW, Bossen KS, et al. The impact of comorbidity on cancer survival: a review. *Clin Epidemiol*.2013; 5 (Suppl 1):3-29.

33. Nitsche U, Stöss C, Friess H. Effect of Adjuvant Chemotherapy on Elderly Colorectal Cancer Patients: Lack of Evidence. *Gastrointest Tumors*.2017; 4:11-19.

34. Burdette-Radoux S, Muss HB. Adjuvant chemotherapy in the elderly: whom to treat, what regimen? *Oncologist*.2006; 11:234-242.

35. Iwashyna TJ, Lamont EB. Effectiveness of adjuvant fluorouracil in clinical practice: a population-based cohort study of elderly patients with stage III colon cancer. *J Clin Oncol*.2002; 20:3992-3998.

35. Sargent DJ, Goldberg RM, Jacobson SD, Macdonald JS, Labianca R, Haller DG, et al. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. N Engl J Med. 2001;345(15):1091–7.

36. Hurria A, Siccion EP. Assessing the 'fit' older patient for chemotherapy. *Oncology* (Williston Park).2014; 28:594, 596, 598-599

37. Lichtman SM, Villani G. Chemotherapy in the elderly: pharmacologic considerations. *Cancer Control*.2000; 7:548-556

38. Millan M, Merino S, Caro A, et al. Treatment of colorectal cancer in the elderly. *World J Gastrointest Oncol*.2015; 7:204-220.

39. Field KM, Kosmider S, Jefford M, et al. Chemotherapy Dosing Strategies in the Obese, Elderly, and Thin Patient: Results of a Nationwide Survey. *J Oncol Pract.* 2008; 4:108-113.

Chapter 3: Five year survival outcomes of prospectively recorded cohort data for older adults versus younger adults with resected primary rectal cancer

3.1 Overview

This chapter is a published retrospective cohort study determining overall survival and cancer specific survival of older adults versus younger adults with resected rectal cancer. Older adults with stage III rectal cancer, compared with younger adults, had worse overall survival (OS) and cancer specific survival (CSS) and received less adjuvant chemotherapy. This work highlighted that ways to improve outcomes of older adults with rectal cancer is an area of unmet research need.

This manuscript is formatted according to the style of this thesis.

3.1.1 Publication details

Shafiei M, Beale P, and Blinman P. (2021) Five Year Survival Outcomes of Prospectively Recorded Cohort Data for Older Adults versus Younger Adults with Resected Primary Rectal Cancer. *J. Cancer Ther.* 12, 437-452. doi: 10.4236/jct.2021.127038.

3.1.2 Contribution of authors to the work described in the Chapter

Mohsen Shafiei was responsible for the study concept, data analysis and interpretation of the findings, drafted and revised the manuscript.

Philip Beale contributed to the research proposal, interpretation of the findings and contributed to the revision of the manuscript.

Prunella Blinman was responsible for the study concept, data interpretation, drafting and review of the manuscript.

Abstract

Background

Rectal cancer predominantly occurs in older adults. We aimed to compare the long-term outcomes of older adults (≥70 years) versus younger adults (<70 years) who had had a primary resection for stage I-IV rectal cancer.

Methods

Consecutive patients who had resection of a primary rectal cancer between January 1, 2000 and December 31, 2010 were identified from a prospective database at the Concord Repatriation General Hospital and stratified into two age groups: <70 years and ≥70 years. Age-related differences in patients, cancer, and treatment characteristics were determined by Chi-square tests. 5-year overall survival (OS) and cancer-specific survival (CSS) were determined by Kaplan-Meier method and by multivariable Cox regression analysis.

Results

Of 714 included patients, the mean age was 65.8 years (range, 21-92 years). 407 (57%) patients were aged <70 years and 307 (43%) were aged ≥70 years. Older age (>70 years) predicted more comorbidity (p<0.001) and earlier stage (p=0.01). Of the patients with stage III rectal cancer, older adults (>70 years), compared with younger adults (<70 years), received less neoadjuvant chemotherapy [7/86 (8.1%) vs 25/147 (17.0%), p=0.058], less neoadjuvant radiotherapy [8/86 (9.3%) vs 42/147 (28.6%), p=0.001] and less adjuvant chemotherapy [30/86 (34.9%) vs 117/147 (79.6%), p<0.001]. Older age was associated with worse OS and CSS in

stage III ($p<0.001$ and $p=0.02$ respectively). Adjuvant chemotherapy independently predicted improved OS ($p<0.001$) and CSS ($p=0.008$) regardless of age.

Conclusion

Older adults who had had a resection of stage I-IV primary rectal cancer received less neoadjuvant and adjuvant therapy and had worse OS and CSS than their younger counterparts.

Keywords

Rectal cancer. Chemotherapy. Radiotherapy. Overall survival. Cancer specific survival

3.2 Introduction

Rectal cancer predominantly occurs in older adults with an increasing incidence with increasing age [1]. Worldwide, there were an estimated 704,000 new cases of rectal cancer in 2018 [2] with the highest risk in developed countries. In Australia, there were an estimated 5238 new cases of rectal cancer in 2019 with over half of these patients (58%) aged over 65 years [3]. With increasing life expectancy and the general ageing of the population [4], the number of older adults diagnosed with rectal cancer is expected to increase, making optimisation of the management of rectal cancer in older adults an important priority for clinicians involved in their care.

The treatment of locally advanced rectal cancer (stage II, ≥T3-N0 or stage III, any T ≥N1) has evolved over the last two decades. Surgery is the mainstay of curative treatment with the addition of neoadjuvant and/or adjuvant therapy for resectable locally advanced disease. For fit patients, one standard approach is trimodality treatment with neoadjuvant radiotherapy +/- chemotherapy followed by a total mesorectal excision (TME) and adjuvant chemotherapy. This approach is based on several randomized clinical trials that showed neoadjuvant radiotherapy +/- chemotherapy improved local control ranged from 7% (4.4-11%, p=0.004) to 16% (11-27%, p<0.001) without consistent improvement in overall survival (OS) [5, 6]. The addition of adjuvant chemotherapy improved disease-free survival (DFS) (HR 0.59, 95% CI 0.40-0.85) and distant recurrence (HR 0.61, 95% CI 0.40-0.94) particularly in patients with a tumour 10-15 cm from the anal verge [7]. The NCCN and ESMO guidelines recommend adjuvant chemotherapy as standard treatment for all patients with locally advanced rectal cancer after neoadjuvant radiotherapy or chemoradiotherapy (CRT) and surgery [8, 9].

Older adults with rectal cancer, compared with younger adults with rectal cancer, may be challenging to treat with triple modality therapy due to the intensity and toxicity of the treatment. Older adults have more comorbidities and geriatric syndromes such as falls, polypharmacy, cognitive impairment and malnutrition that reduce their fitness for standard cancer therapy [10, 11]. Older adults are also more likely to discontinue therapy earlier than younger adults due to the higher rates of treatment toxicity [12]. Older adults are less likely to be referred for neoadjuvant and adjuvant therapy for rectal cancer [13] and, when referred, they may not be offered similar treatment as their younger counterparts [13-15]. Another key factor affecting the management of older adults with rectal cancer include their underrepresentation in pertinent clinical trials. The abovementioned trials of neoadjuvant CRT and adjuvant chemotherapy in rectal cancer included mostly younger (median age of 60-61) and fitter adults (ECOG performance status of 0 or 1) rather than the frail, older adults typical of routine clinical practice [16]. This means little specific randomised evidence in older adults with rectal cancer to help clinicians guide their care.

Observational studies have a role in determining the impact of age on outcomes of rectal cancer when older adults are underrepresented in randomised clinical trials. The results of observational studies determining overall survival (OS) and cancer-specific survival (CSS) for rectal cancer generally show worse OS with increasing age, but inconsistent results for CSS [17-19].

We conducted an observational study to determine the long-term outcomes of older adults who had had a resection of a primary rectal cancer and their utilisation of neoadjuvant CRT and adjuvant chemotherapy, compared with their younger counterparts in our local institution. We hypothesised that older adults, compared with younger adults, had worse long-term outcomes and lower rates of utilisation of neoadjuvant and adjuvant therapy.

3.3 Methods

3.3.1 Study design

Consecutive patients over the age of 18 who had undergone curative or palliative surgery for a diagnosis of rectal cancer at the Concord Repatriation General Hospital, Sydney, Australia between 2000 and 2011 were included. Data were extracted from a prospectively collected colorectal cancer (CRC) database maintained since 1971 and received approval of the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206). This database included patient characteristics, comorbidity, presentation, investigations, pathology, neoadjuvant therapy, surgical management, complications, receipt of adjuvant therapy and follow-up data. This project included and explored the following variables: patient gender, previous history of colorectal cancer, number of comorbidities, cardiac comorbidity, resection at urgent operation, histological type, maximum surface dimension, staging, lymphatic vessel invasion, venous invasion, positive margin, neoadjuvant therapy and adjuvant chemotherapy. Patients were stratified to two age groups, <70 years and ≥70 years, at the time of diagnosis.

3.3.2 Statistical analysis

Patient demographics, tumour and treatment characteristics between the two age-groups (<70 years and ≥70 years) were compared by the use of the log-rank test. Demographic, tumour and treatment characteristics were compared with use of the chi-squared test for association for categorical factors. Kaplan-Meier method was used to construct overall and rectal cancer specific survival curves in patients with stage III rectal cancer.

For 5-year CSS and 5-year OS analysis in patients with stage III rectal cancer, the two age groups (<70 years and ≥70 years) were further stratified by gender, resection at urgent

operation, lymphatic vessel invasion, positive margin, venous invasion, number of comorbidities and receipt of neoadjuvant CRT and adjuvant chemotherapy. To determine the association between these factors and patient OS and CSS, multivariate cox regression analysis was performed. SPSS (version 24) was used for all statistical analyses. All p values were 2-sided and values <0.05 were considered statistically significant.

3.3.3 Results

714 patients were included in the study. The mean age was 65.9 years (range, 21-92 years). 407 (57%) patients were aged <70 years and 307 (43%) were ≥70 years. There were more males than females in both the younger (271/407, 67%) and older (182/307, 60%) age groups. Demographic information, presentation and treatment characteristics are presented in Table 4.

Older age group (≥70 years) predicted more comorbidity (p<0.001), cardiac comorbidity (p<0.001), lymphatic vessel invasion (p=0.03), early stage tumour (p=0.01), less neoadjuvant radiotherapy (p 0.001), less neoadjuvant chemotherapy (p<0.001) and less adjuvant chemotherapy (stage III only; p<0.001).

In patients with stage III rectal cancer, older adults (≥70 years), compared with younger adults (<70 years), received less neoadjuvant chemotherapy [7/86 (8.1%) vs 25/147 (17.0%), p=0.058], less neoadjuvant radiotherapy [8/86 (9.3%) vs 42/147 (28.6%), p=0.001] and less adjuvant chemotherapy [8/86 (9.3%) vs 42/147 (28.6%), p=0.001].

The 5-year OS and 5-year CSS between the two age groups stratified by cancer stage are shown in Table 5. Kaplan-Meier survival curves are presented in Figures 6 to 10. Five-year OS was significantly lower in the older age group irrespective of cancer stage (p<0.001) (Table 5, Figure 6). In patients with stage III rectal cancer, increasing age group was associated with worse 5-year OS [44.2% (≥70 years) vs 71.9% (<70 years), p<0.001], and worse 5-year CSS [62.3% (≥70 years) vs 76.2% (<70 years), p=0.02] (Figure 8 and 9).

In patients with stage III rectal cancer, bivariate predictors of improved OS were age <70 years (p<0.001), no lymphatic vessel invasion (p<0.001), no positive margin (p<0.001), receiving adjuvant chemotherapy and less comorbidity (p=0.002) (Table 6). Neoadjuvant radiotherapy

did not improve OS (p=0.41) but significantly improved CSS (p=0.038) (Figure 10). On multivariable analysis, improved OS was independently predicted by age <70 years (hazard ratio, 0.44, p<0.001), no lymphatic vessel invasion (hazard ratio, 0.47, p<0.001), no positive margin (hazard ratio, 0.23 p<0.001) and receiving adjuvant chemotherapy (hazard ratio, 0.50, p=0.001). Improved CSS was predicted by adjuvant chemotherapy in stage III rectal cancer (p=0.008) (Figure 11).

3.4 Discussion

The key findings of our study were that older adults (≥70 years), compared with younger adults (<70 years), who had had a resection of a primary rectal cancer of stage I to IV had higher comorbidity and cardiac comorbidity, more lymphatic vessel invasion and more early stage cancers. Older adults, compared with younger adults, received less neoadjuvant radiotherapy, less neoadjuvant chemotherapy and less adjuvant chemotherapy. 5-year OS declined significantly with increasing age group. 5-year CSS was significantly worse in older adults with stage III rectal cancer.

The survival outcomes in our study are similar to other published studies. Chang et al conducted an observational study using the Surveillance, Epidemiology, and End Results (SEER) database to examine more than 21,000 patients with locally advanced rectal cancer and found a 31% increase in the relative risk for cancer-specific mortality with each 5-year increase in age ≥70 years (RR = 1.31; 95% CI, 1.25–1.36; P < 0.0001) [18]. Kotake et al studied included 16147 patients with rectal cancer in a large study from the Japanese cancer registry and found older age predicted worse 5-year OS (50% in ≥80 years vs 73% in 50-64 years, p<0.001) and worse 5-year CSS (65% in ≥80 years vs 76% in 50-64 years, p<0.001) [17]. Jung et al studied 15,104 patients with rectal cancer from the Swedish Rectal Cancer Registry 1995–2004 of whom more than 11000 had had curative surgery (stages I-IV). Older adults (≥75 years), compared with younger adults (<75 years), had worse 5-year OS (0.52, 95% CI, 0.50–0.54 vs 0.62, 95% CI, 0.61–0.63) [19]. Devon et al studied 373 adults undergoing curative surgery for their rectal cancer at the Mount Sinai Hospital, Canada between 1997 and 2006. Older adults (aged >75 years), compared with younger adults (aged 50 – 75 years), had worse 5-year OS (68.7% vs 57.3%, p=0.036) but no difference in 5-year CSS (74.0% vs. 74.7%, p=0.277) [20].

Similarly Widdison et al studied 218 patients with rectal cancer and showed older age was not a predictor of worse 5-year CSS (72% for younger and older groups) [21].

It was unsurprising that older adults had worse OS in our study, like in the observational studies discussed above, given competing risks for death in older adults. More concerning was that CSS, or the chance of surviving cancer in the absence of other causes of death, was worse for older adults in stage III rectal cancer. Possible reasons for this result highlighted by our study are increased comorbidities and low utilisation rates of neoadjuvant and adjuvant therapy. Other possible reasons include increased toxicity from radiotherapy and chemotherapy and increased post-surgical complications.

The utilisation of neoadjuvant radiotherapy (7.8%) and neoadjuvant chemotherapy (5.9%) in older adults in our study was low, however, similar to other studies [17, 19]. The role of neoadjuvant radiotherapy and CRT in rectal cancer, however, is now well established. Multiple randomised trials and population based studies have shown that neoadjuvant radiotherapy and CRT improve local control in patients aged >70 years [6, 22-25]. The large Swedish Rectal Cancer Study Group trial (n=1168) showed neoadjuvant radiotherapy (25 Gy in 5 fractions), compared with surgery alone, reduced local recurrence by 16% (from 27% to 11%, $p<0.001$) and improved both five-year OS by 10% (48% to 58%, $p=0.004$) and CSS by 9% (65% to 74%, $p=0.002$) (ref Swedish rectal trial). One possible explanation for the low utilisation rates in our study was the dates of data extraction being 2000-2011 (to allow for 5 years of follow-up for survival outcomes) when neoadjuvant radiotherapy +/- chemotherapy for older adults was likely a less accepted standard of care. Utilisation rates of neoadjuvant radiotherapy for rectal cancer for older adults have likely increased over time as clinicians have become familiar with the treatment and are generally more confident treating older adults with cancer. The older

observational studies such as Kotake et al (1995 to 2004) showed rates of 0.3% in patients aged ≥80 years and 34% in patients aged ≥75 years by Jung et al (1995 to 2004) [7, 26]. Later studies such as Zhao et al that analysed rectal cancer data from the SEER database between 2004 and 2016, showed a utilisation rate of neoadjuvant radiotherapy of 53% for patients aged >60 years, lower than the 67% rate of patients aged ≤60 years [27]. Other reasons for the low utilisation rates include patient preference for no neoadjuvant and/or adjuvant therapy, and patient and clinician concerns about excess toxicity such as faecal incontinence and sexual dysfunction, which are more pronounced in older patients [28-30].

In our study, older adults with rectal cancer received less adjuvant chemotherapy (9.3%) than younger adults (28.6%) with rectal cancer like in previous studies [31]. Irrespective of age, there is no clear OS benefit of adjuvant chemotherapy for rectal cancer, and the treatment is largely a translation from the DFS and OS benefit of adjuvant chemotherapy in colon cancer [7, 31-35]. A meta-analysis of four pivotal randomised control trials examining the benefit of adjuvant chemotherapy for patient with locally advanced rectal cancer demonstrated that adjuvant 5-fluorouracil/capecitabine improves DFS (HR 0.59, 95% CI: 0.40-0.85, p=0.005) and rate of distant recurrence (HR 0.61, 95% CI: 0.40-0.94, p=0.025) in those patients with a tumour 10 to 15 cm above the anal verge but no improvement in OS (HR 0.97, 95% CI: 0.81-1.17, p=0.775) [7] Common clinical practice, supported by guidelines, is four months of adjuvant chemotherapy for patients who had long course CRT and six months of adjuvant chemotherapy for patients who have not had neoadjuvant therapy [8].

Possible reasons for the low utilisation rates in our study include the paucity of robust evidence supporting the benefit of such therapy in patients of all ages and in older adults (>70 years), referrer bias against the treatment resulting in reduced referrals for adjuvant chemotherapy, and

concerns about the increased toxicity of chemotherapy in older adults [36]. Fit older adults with rectal cancer, however, benefit equally from adjuvant chemotherapy without a significant increase in toxicity [37].

Increasing treatment utilisation in older adults with rectal cancer involves optimal assessment of their fitness for treatment to minimise their exclusion from treatment based on their chronological age. This is particularly important in older adults with stage III rectal cancer where the worse CSS in our study highlights the need to improve outcomes and where trimodality treatment, requiring careful patient selection, is a standard of care. Optimal assessment of older adults can be achieved by the use of formal geriatric assessments and risk predicting tools, as recommended by ASCO guidelines [38, 39]. Integrated geriatric assessment in the care of older adults with cancer have recently been shown to improve quality of life, reduce hospital admissions and reduce early discontinuation of anti-cancer therapy [40-42]. The key ways to improve treatment utilisation in older adults with rectal cancer include conducting trials and studies specific to older adults, for example, the optimal dosing of adjuvant chemotherapy.

The main strength of our study is the prospective, large surgical database with minimal missing data. Limitations of our study include the database involving a single institution meaning that the surgical and oncological management, patient selection, surgical techniques, pre-operative and post-operative care, and selection for neoadjuvant radiotherapy or chemo-radiotherapy and adjuvant chemotherapy may differ from other institutions or health care settings. Details of radiotherapy (dose, fractionation, completion) and chemotherapy (regimen, dose, toxicities, completion) were not readily available and required manual searching through medical records for which the study was not adequately resourced. Generalisability of the study is limited due

to the inclusion of patients who had had a resection of a primary rectal cancer and hence excludes patients who were not suitable or fit for surgery or who chose not to have surgery.

3.5 Conclusion

In conclusion, older adults who had a resection of a stage I-IV rectal cancer had higher comorbidity, cardiac comorbidity, more lymphatic vessel invasion, early stage tumour, and received less neoadjuvant radiotherapy, less neoadjuvant chemotherapy and less adjuvant chemotherapy. Older adults had worse OS and worse CSS in stage III disease. These results highlight the need to optimise the treatment of older adults with rectal cancer and ways to increase the utilisation of adjuvant chemotherapy.

Figure 6. OS curve by age group for all stages, P<0.001

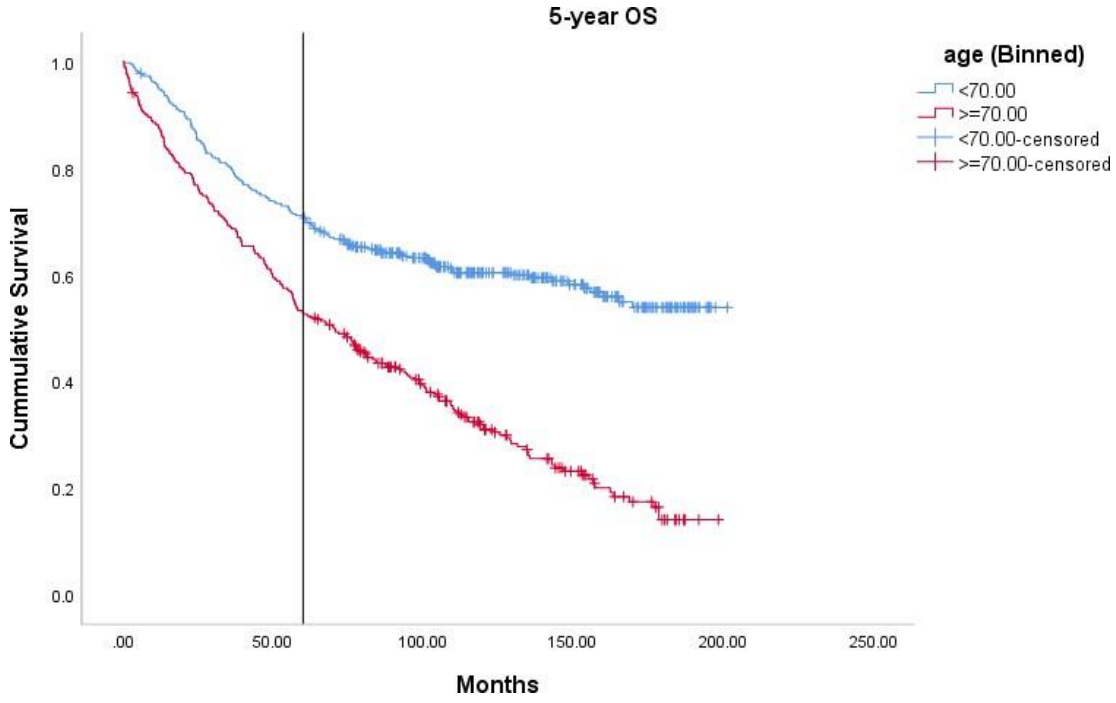


Figure 7. CSS curve by age group for all stages, P=0.65

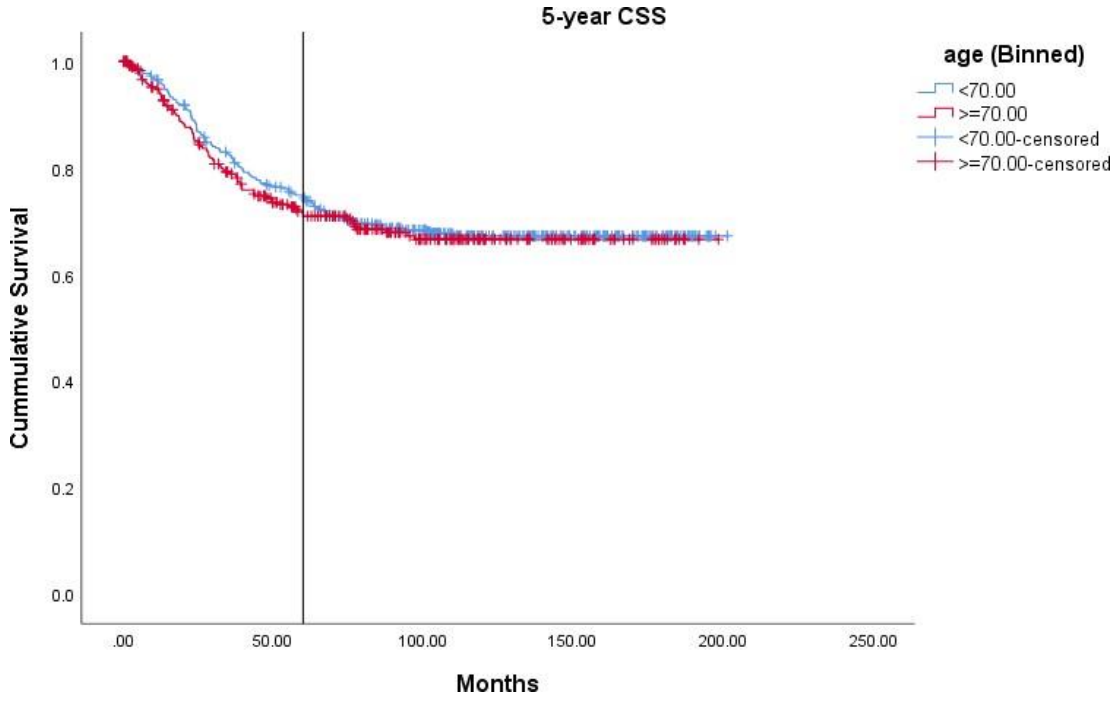


Figure 8. OS curve by age group for stage III, P< 0.001

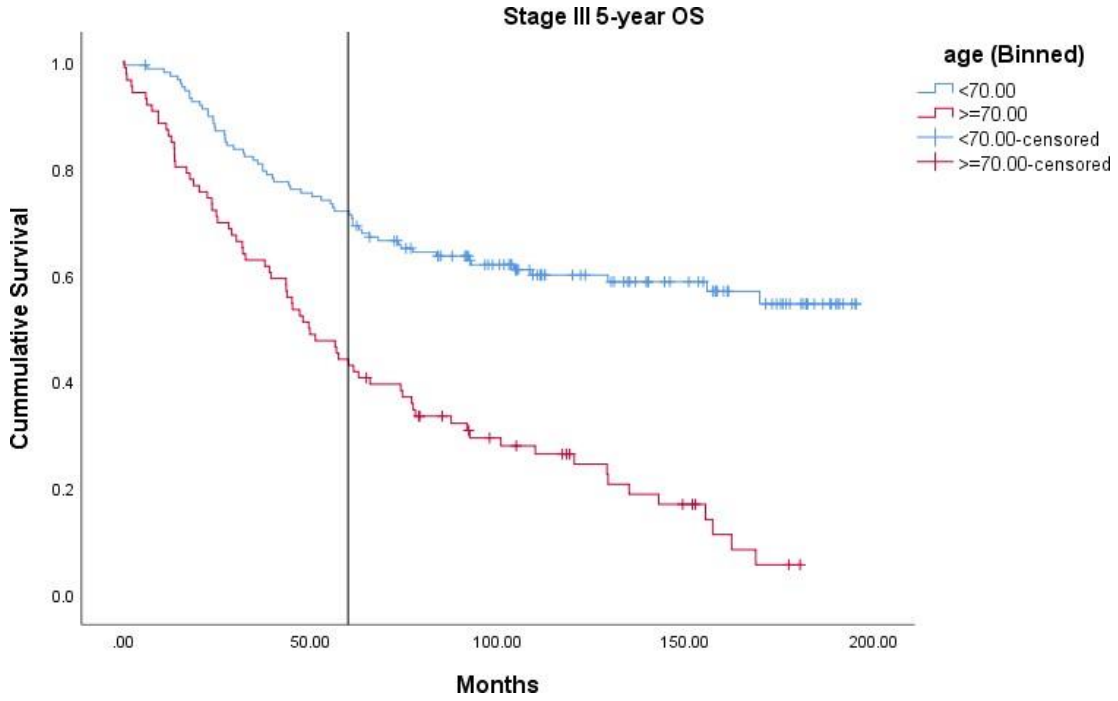


Figure 9. CSS curve by age group for stage III, P=0.02

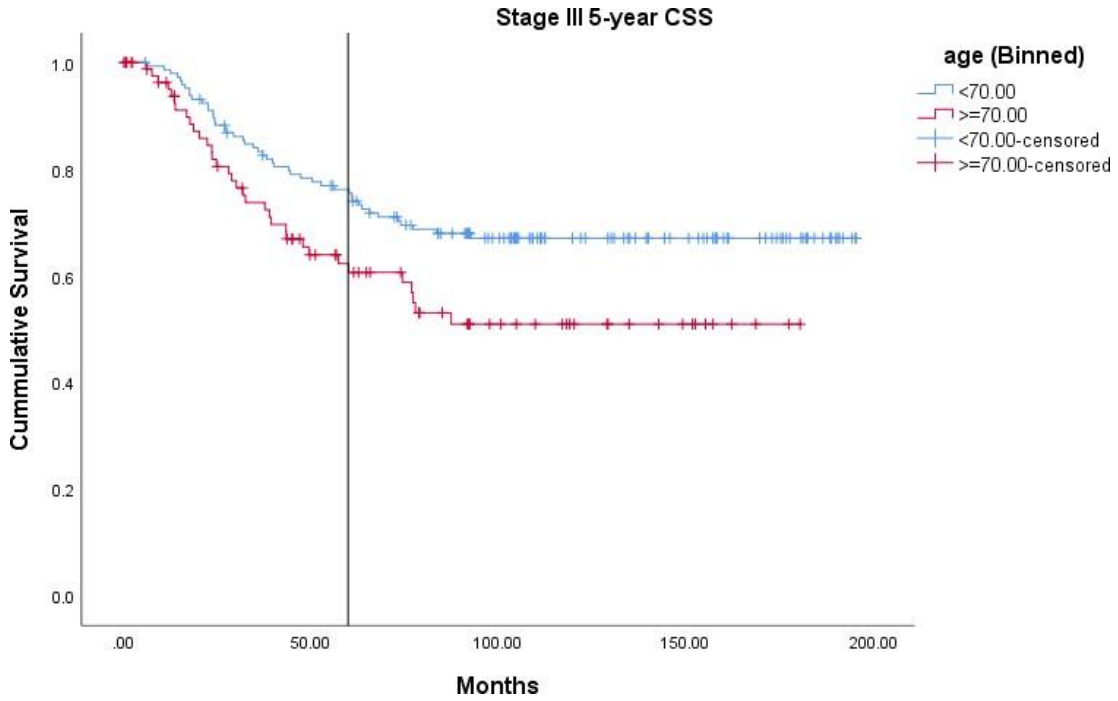


Figure 10. CSS curve by neoadjuvant radiotherapy in stage III rectal cancer, P=0.038

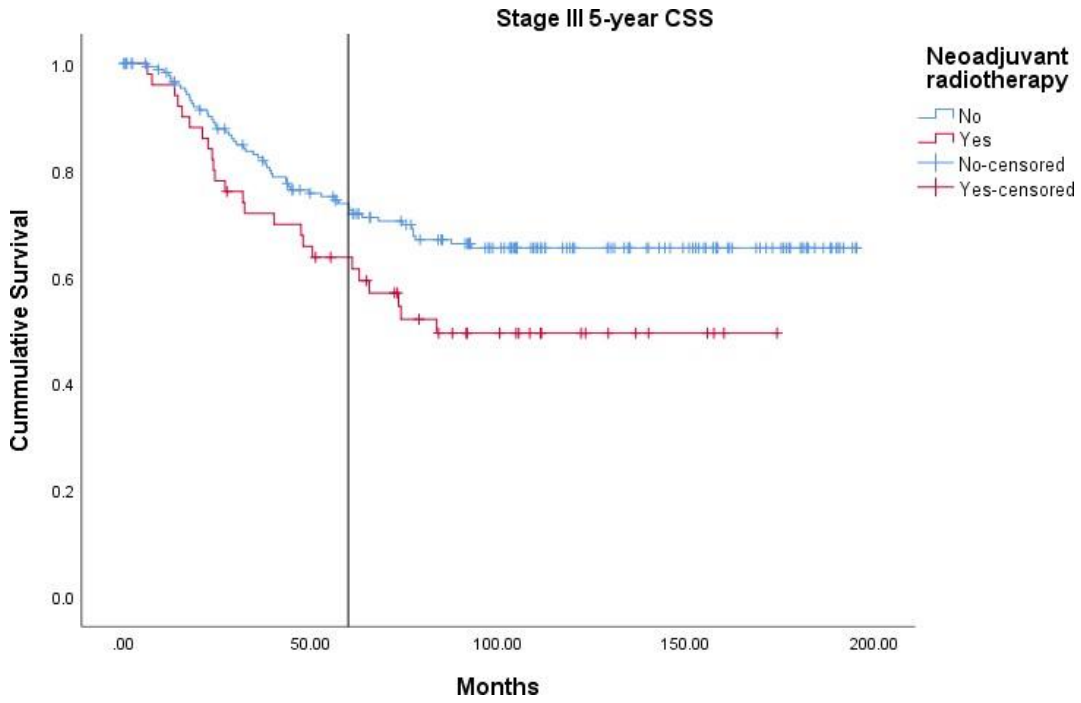


Figure 11. CSS curve by adjuvant chemotherapy in stage III rectal cancer, P=0.008

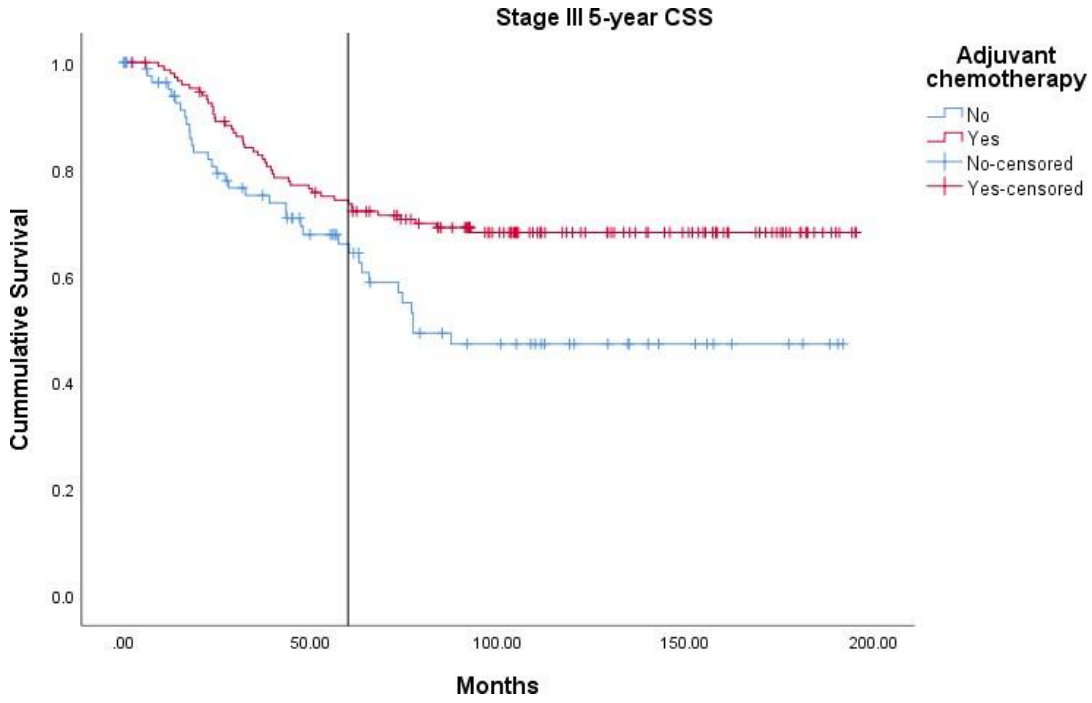


Table 4. Tumour and treatment characteristics stratified by age

Characteristics	Age group years			
	Total N=714 Mean	<70 N=407 Mean	≥70 N= 307 Mean	P difference between <70 and ≥70
Previous CRC resected				
No	702 (98.3%)	399 (98.0%)	303 (98.7%)	P=0.49
Yes	12 (1.7%)	8 (2%)	4 (1.3%)	
No. of comorbidities				
≤1	545 (76.3%)	341 (83.8%)	204 (66.4%)	P<0.001
>1	169(23.7%)	66 (16.2%)	103(33.6%)	
Cardiac comorbidity*				
No	526 (77.8%)	355 (89%)	171 (61.7%)	P< 0.001
Yes	150(22.2%)	44 (11%)	106(38.3%)	
Resection at urgent operation				
No	707 (99%)	403 (99%)	304 (99%)	P=0.99
Yes	7 (1%)	4 (1%)	3 (1%)	
Histological type of primary				
Adenocarcinoma	661 (92.6%)	371 (91.2%)	290 (94.5%)	P=0.09
Mucinous Adenocarcinoma/ Signet ring	53 (7.4%)	36 (8.8%)	17 (5.5%)	
Distant metastasis				
No	621 (87.0%)	347 (85.3%)	274 (89.3%)	P=0.12
Yes	93 (13.0%)	60 (14.7%)	33 (10.7%)	

Lymphatic vessel permeation				
No	569 (79.7%)	313 (76.9%)	256 (83.4%)	P=0.03
Yes	145(20.3%)	94 (23.1%)	51 (16.6%)	
Venous invasion				
None	582 (81.5%)	326 (80.1%)	256 (83.4%)	P=0.26
Yes	132(18.5%)	81 (19.9%)	51 (16.6%)	
Positive margin				
No	667 (93.4%)	380 (93.4%)	287 (93.4%)	P=0.95
Yes	47 (6.6%)	20 (6.5%)	27 (6.6%)	
Preoperative radiotherapy				
No	594 (83.2%)	311 (76.4%)	283 (92.2%)	P<0.001
Yes	120 (16.8%)	96 (23.6%)	24 (7.8%)	
Preoperative chemotherapy				
No	633 (88.7%)	344 (84.5%)	289 (94.1%)	P<0.001
Yes	81 (11.3%)	63 (15.5%)	18 (5.9%)	
Postoperative radiotherapy				
No	691 (96.8%)	395 (97.1%)	296 (96.4%)	P=0.64
Yes	23 (3.2%)	12 (2.9%)	11 (3.6%)	
Postoperative chemotherapy				
No	487 (68.2%)	225 (55.3%)	262 (85.3%)	P<0.001
Yes	227 (31.8%)	182 (44.7%)	45 (14.7%)	
TNM stage				
Stage I	187 (26.2%)	95 (23.3%)	92 (30.0%)	P=0.01
Stage II	201 (28.2%)	105 (25.8%)	96 (31.3%)	

Stage III	233 (32.6%)	147 (36.1%)	86 (28.0%)	
Stage IV	93 (13.0%)	60 (14.7%)	33 (10.7%)	

*There were 38 missing cases for New York Heart Association evaluation.

Table 5. 5-year overall and cancer specific survival after surgery by age group and pathological stage

Stage	Age group	No of cases	5 Year OS rate	P value	5 Year CSS rate	P value
Stage I	< 70	95	94.7%	<0.001	97.8%	0.001
	≥ 70	92	72.8%		91.1%	
	All	187				
Stage II	< 70	105	81.9%		87.3%	
	≥ 70	96	60.0%		82.6%	
	All	201				
Stage III	< 70	147	71.9%		76.2%	
	≥ 70	86	44.2%		62.3%	
	All	233				
Stage IV	< 70	60	11.7%		11.9%	
	≥ 70	33	0%		0%	
	All	93				

Table 6. Bivariate and multivariable survival analysis for only stage III rectal cancer

Variable	Number	Bivariate hazard Ratio (95% CI)	p	Multivariable hazard Ratio (95% CI)	p
Female Male	86 147	1.13 (0.79-1.63)	0.47		
Age < 70 years Age ≥ 70 years	147 86	0.34 (0.24-0.48)	<0.001	0.44 (0.30-0.65)	<0.001
No Previous CRC Previous CRC	228 5	0.61 (0.19-1.93)	0.40		
No Resection at urgent operation Resection at urgent operation	230 3	0.44 (0.11-1.77)	0.25		
No Venous invasion Venous invasion	181 52	0.70 (0.48-1.04)	0.08		
No lymphatic vessel invasion Lymphatic vessel invasion	156 77	0.49 (0.34-0.69)	<0.001	0.47 (0.32-0.68)	<0.001
No positive margin Positive margin	212 21	0.16 (0.10-0.26)	<0.001	0.23 (0.14-0.39)	<0.001

Adenocarcinoma Mucinous adenoCa/ Signet ring	208 25	0.68 (0.41-1.13)	0.14		
Neoadjuvant radiotherapy No neoadjuvant radiotherapy	50 183	1.19 (0.78-1.80)	0.41		
Neoadjuvant chemotherapy No neoadjuvant chemotherapy	32 201	1.07 (0.64-1.78)	0.79		
Adjuvant radiotherapy No adjuvant radiotherapy	14 219	1.40 (0.73-2.67)	0.31		
Adjuvant chemotherapy No adjuvant chemotherapy	147 86	0.34 (0.24-0.50)	<0.001	0.50 (0.34-0.74)	0.001
Number of nodes examined <12 Number of nodes examined ≥12	60 173	1.30 (0.89-1.90)	0.17		
Number of comorbidities ≤1 Number of comorbidities > 1	179 54	0.55 (0.38-0.81)	0.002	0.76 (0.51-1.12)	0.16

CRC, Colorectal cancer

3.6 References

1. Matanoski G, Tao X, Almon L, et al. Demographics and tumor characteristics of colorectal cancers in the United States, 1998-2001. *Cancer.* 2006; 107: 1112-1120.

2. Society. A C. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21492. Accessed September 16, 2020

3. Australia. C. 2019. Cancer incidence. https://ncci.canceraustralia.gov.au/diagnosis/cancer-incidence/cancer-incidence. Accessed September 16, 2020.

4. Crimmins E M. Lifespan and Healthspan: Past, Present, and Promise. *Gerontol.* 2015; 55: 901-911.

5. Quirke P, Steele R, Monson J, et al. Effect of the plane of surgery achieved on local recurrence in patients with operable rectal cancer: a prospective study using data from the MRC CR07 and NCIC-CTG CO16 randomised clinical trial. *Lancet.* 2009; 373: 821-828.

6. Cedermark B, Dahlberg M, Glimelius B, et al. Improved survival with preoperative radiotherapy in resectable rectal cancer. *N Engl J Med*.1997; 336: 980-987.

7. Breugom A J, Swets M, Bosset J F, et al. Adjuvant chemotherapy after preoperative (chemo)radiotherapy and surgery for patients with rectal cancer: a systematic review and meta-analysis of individual patient data. *Lancet Oncol.* 2015; 16: 200-207.

8. Network. N C C. 2020. NCCN clinical practice guidelines in oncology. Rectal cancer. https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf. Accessed September 16, 2020.

9. Oncology E S f M. 2020. Rectal Cancer: ESMO Clinical Practice Guidelines. https://www.esmo.org/guidelines/gastrointestinal-cancers/rectal-cancer. Accessed September 12, 2020.

10. Hurria A, Browner I S, Cohen H J, et al. Senior adult oncology. *J Natl Compr Canc Netw*.2012; 10: 162-209.

11. Farrington N, Richardson A, Bridges J. Interventions for older people having cancer treatment: A scoping review. *J Geriatr Oncol.* 2020; 11: 769-783.

12. Kahn K L, Adams J L, Weeks J C, et al. Adjuvant chemotherapy use and adverse events among older patients with stage III colon cancer. *JAMA.* 2010; 303: 1037-1045.

13. Luo R, Giordano S H, Freeman J L, Zhang D, Goodwin J S. Referral to medical oncology: a crucial step in the treatment of older patients with stage III colon cancer. *Oncologist.* 2006; 11: 1025-1033.

14. Samet J, Hunt W C, Key C, Humble C G, Goodwin J S. Choice of Cancer Therapy Varies With Age of Patient. *JAMA.* 1986; 255: 3385-3390.

15. Goodwin J S, Hunt W C, Samet J M. Determinants of cancer therapy in elderly patients. *Cancer.* 1993; 72: 594-601.

16. Moth E B, Kiely B E, Martin A, et al. Older adults' preferred and perceived roles in decision-making about palliative chemotherapy, decision priorities and information preferences. *J Geriatr Oncol*. 2020; 11: 626-632.

17. Kotake K, Asano M, Ozawa H, et al. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. *Colorectal Dis* 2015; 17: 205-215.

18. Chang G J, Skibber J M, Feig B W, et al. Are we undertreating rectal cancer in the elderly? An epidemiologic study. *Ann Surg.* 2007; 246: 215-221.

19. Jung B, Pahlman L, Johansson R, et al. Rectal cancer treatment and outcome in the elderly: an audit based on the Swedish Rectal Cancer Registry 1995-2004. *BMC Cancer* 2009; 9: 68.

20. Devon K M, Vergara-Fernandez O, Victor J C, et al. Colorectal cancer surgery in elderly patients: presentation, treatment, and outcomes. *Dis Colon Rectum.* 2009; 52: 1272-1277.

21. Widdison A L, Barnett S W, Betambeau N. The impact of age on outcome after surgery for colorectal adenocarcinoma. *Ann R Coll Surg Engl*.2011; 93: 445-450.

22. Gérard J P, Conroy T, Bonnetain F, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol.* 2006; 24: 4620-4625.

23. Bosset J F, Calais G, Mineur L, et al. Enhanced tumorocidal effect of chemotherapy with preoperative radiotherapy for rectal cancer: preliminary results--EORTC 22921. *J Clin Oncol*.2005; 23: 5620-5627.

24. Boulis-Wassif S, Gerard A, Loygue J, et al. Final results of a randomized trial on the treatment of rectal cancer with preoperative radiotherapy alone or in combination with 5-fluorouracil, followed by radical surgery. Trial of the European Organization on Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. *Cancer.* 1984; 53: 1811-1818.

25. McCarthy K, Pearson K, Fulton R, et al. Pre-operative chemoradiation for non-metastatic locally advanced rectal cancer. *Cochrane Database Syst Rev.*2012; 12: Cd008368.

26. Holm T, Rutqvist L E, Johansson H, et al. Postoperative mortality in rectal cancer treated with or without preoperative radiotherapy: causes and risk factors. *Br J Surg.* 1996; 83: 964-968.

27. Zhao F, Wang J, Yu H, et al. Neoadjuvant radiotherapy improves overall survival for T3/4N+M0 rectal cancer patients: a population-based study of 20300 patients. *Radiat Oncol.* 2020; 15: 49.

28. Dahlberg M, Glimelius B, Graf W, et al. Preoperative irradiation affects functional results after surgery for rectal cancer: results from a randomized study. *Dis Colon Rectum.*1998; 41: 543-549; discussion 549-551.

29. Lange M M, den Dulk M, Bossema E R, et al. Risk factors for faecal incontinence after rectal cancer treatment. *Br J Surg.*2007; 94: 1278-1284.

30. Birgisson H, Pahlman L, Gunnarsson U, et al. Late adverse effects of radiation therapy for rectal cancer - a systematic overview. *Acta Oncol.*2007; 46: 504-516.

31. Liu S L, O'Brien P, Zhao Y, et al. Adjuvant treatment in older patients with rectal cancer: a population-based review. *Curr Oncol.* 2018; 25: e499-e506.

32. Bosset J F, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med.*2006; 355: 1114-1123.

33. Sainato A, Cernusco Luna Nunzia V, Valentini V, et al. No benefit of adjuvant Fluorouracil Leucovorin chemotherapy after neoadjuvant chemoradiotherapy in locally

advanced cancer of the rectum (LARC): Long term results of a randomized trial (I-CNR-RT). *Radiother Oncol.*2014; 113: 223-229.

34. Breugom A J, van Gijn W, Muller E W, et al. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo) radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. *Ann Oncol.*2015; 26: 696-701.

35. Glynne-Jones R, Counsell N, Quirke P, et al. Chronicle: results of a randomised phase III trial in locally advanced rectal cancer after neoadjuvant chemoradiation randomising postoperative adjuvant capecitabine plus oxaliplatin (XELOX) versus control. *Ann Oncol*.2014; 25: 1356-1362.

36. Nitsche U, Stöss C,Friess H. Effect of Adjuvant Chemotherapy on Elderly Colorectal Cancer Patients: Lack of Evidence. *Gastrointest Tumors.*2017;4: 11-19.

37. Millan M, Merino S, Caro A, et al. Treatment of colorectal cancer in the elderly. *World J Gastrointest Oncol*.2015; 7: 204-220.

38. Hurria A,Siccion E P. Assessing the 'fit' older patient for chemotherapy. *Oncology.* (Williston Park) 2014; 28: 594, 596, 598-599.

39. Lichtman S M,Villani G. Chemotherapy in the elderly: pharmacologic considerations. *Cancer Control*.2000; 7: 548-556.

40. Mohile S G, Mohamed M R, Culakova E, et al. A geriatric assessment (GA) intervention to reduce treatment toxicity in older patients with advanced cancer: A University of Rochester Cancer Center NCI community oncology research program cluster randomized clinical trial (CRCT). *J Clin Oncol.*2020; 38: 12009-12009.

41. Li D, Sun C-L, Kim H, et al. Geriatric assessment-driven intervention (GAIN) on chemotherapy toxicity in older adults with cancer: A randomized controlled trial. *J Clin Oncol*.2020; 38: 12010-12010.

42. Soo W-K, King M, Pope A, et al. Integrated geriatric assessment and treatment (INTEGERATE) in older people with cancer planned for systemic anticancer therapy. *J Clin Oncol*.2020; 38: 12011-12011.

Chapter 4: Pharmacokinetics of anticancer drugs used in treatment of older adults with colorectal cancer: a systematic review

4.1 Overview

The purpose of this chapter is to explore the impact of ageing on the pharmacokinetics of anticancer therapy as a potential cause for the observed higher rate of toxicity in older adults. This chapter is a published systematic review of studies that examined the effect of ageing on the pharmacokinetics of anticancer therapy used in the treatment of colorectal cancer. Whilst age was shown to influence pharmacokinetics of irinotecan, capecitabine, 5-flourouracil and panitumumab, the effects were small and not easily translated into recommended dose modifications of these drugs for older adults.

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4.1.1 Publication details

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4.1.2 Contribution of authors

Mohsen Shafiei developed the research proposal and research methods, performed the systematic review and data analysis, interpreted the findings, and drafted and revised the manuscript.

Robert Yoon performed the systematic review, and contributed to data analysis.

Andrew McLachlan contributed to the research proposal contributed to the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Philip Beale contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Alan Boddy contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Prunella Blinman contributed to the research proposal and the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Abstract

Purpose

Older adults with cancer experience more toxicity from anti-cancer therapy, possibly due to age-related changes in the pharmacokinetic (PK) profile of anti-cancer therapies. We aimed to evaluate studies investigating the effect of ageing on the PK of anti-cancer therapies used in the treatment of colorectal cancer.

Methods

A systematic literature search of the electronic databases EMBASE and PUBMED was performed to find eligible studies that assessed the effect of age on the PK of anti-cancer therapies used in the treatment of colorectal cancer.

Results

The 21 eligible studies included 17 prospective studies and 4 pooled analyses of prospective studies. Of these, PK of 5-fluorouracil was determined in 7 studies, oxaliplatin in 2 studies, capecitabine in 3 studies, irinotecan in 4 studies, bevacizumab in 1 study, cetuximab in 3 studies, and panitumumab in 1 study. Studies included a median of 44 patients and had varying definitions of older adults: aged ≥65 years (3 studies), >70 years (3 studies) or >75 years (1 study). Increasing age significantly affected PK parameters of irinotecan only with a 7.2% reduction in CL ($p < 0.001$) for every 10 years older than 60, AUC ($p=0.007$) and Cmax ($p= 0.009$).

Conclusion

Older age influences PK of irinotecan, but there is limited evidence for age-related changes in PK of other anti-cancer therapies used in the management of older adults with colorectal cancer. Factors other than PK may be responsible for the greater toxicity of these agents experienced by older adults.

4.2 Introduction

Colorectal cancer (CRC) is a common cancer of older adults, and a common cause of cancer death. In 2012, there were an estimated 1.4 million cases of CRC and 693,900 deaths from colorectal cancer worldwide [1]. More than 60% of patients diagnosed with colorectal cancer aged ≥70 years and absolute numbers of older adults increasing due to the ageing of the population. The management of older adults with CRC is thus an increasing issue for clinicians providing their care.

Older adults with colorectal cancer, compared with younger adults with colorectal cancer, experience more toxicity from anti-cancer therapy [2]. Data from randomised trials and large pooled analyses in the adjuvant setting show older adults experiencing more chemotherapy related toxicity with 5-fluorouracil [3], capecitabine [4], FOLFOX [5-FU/leucovorin (LV) and oxaliplatin] [5, 6], and XELOX (capecitabine and oxaliplatin) [5]. In the metastatic setting, older adults experience more chemotherapy toxicity with capecitabine [4, 7], and irinotecan [8-10], and regimens such as FOLFOX, [8, 11] XELOX [12] and FOLFIRI (5-FU/LV and irinotecan) [8-10, 12]. Of the targeted therapies in the metastatic setting, older adults experience more toxicity with bevacizumab [13].

Ageing is a heterogeneous process with often little relationship with chronological age, and variable decline in physiological reserve and functional status. Prescribing anti-cancer therapies to older adults can be challenging with wide variation in response, more treatment toxicity as described above and worse survival regardless of the cancer stage [14-17]. Older adults are also underrepresented in clinical trials [18, 19], meaning dosing and efficacy data are predominantly derived from clinical trials of younger, fitter patients, physiologically distinct from the majority of older adults seen in routine clinical practice.

Ageing is associated with changes in the clinical pharmacology of anti-cancer therapies, namely pharmacokinetics (PK) and pharmacodynamics (PD) [20]. PK is the study of 'what the body does to a drug', that is, the uptake of a drug by the body and its time course of absorption, distribution, metabolism, and excretion. PD, however, is the study of what a drug does to the body meaning the relationship between the concentration of a drug at the site of action in the body and its biochemical and physiological effects [21, 20]. Age-related changes in the PK of anti-cancer therapies occur due to physiological changes affecting the absorption, distribution, metabolism and excretion of drugs [21, 22]. Renal clearance, for example, typically declines with increasing age impairing the excretion of renally-excreted drugs with resulting increased drug exposure and toxicity [23]. Changes in PK associated with ageing are important for medical oncologists to understand as they are potentially ameliorated, at least in part, by dose modifications or use of a less toxic alternative [20].

To better understand the role of aged-associated changes in PK and its impact on toxicity of anti-cancer therapies used in older adults with CRC, we conducted a systematic literature review aiming to investigate and evaluate trials studying the effect of ageing on the PK of anti-cancer therapies commonly used in the treatment of CRC.

4.3 Methods

Two independent reviewers (MS, RY) conducted a systematic literature search of the electronic databases EMBASE and PUBMED. Studies were included if they assessed the effect of age on the PK of the following chemotherapy or biologic anti-cancer therapies used in the treatment of CRC: The key words, “elderly”, “aging”, “ageing”, ”geriatrics”, “old”, AND “metabolism”, “pharmaco*”, “AUC” (area under the curve), “Cmax” (maximum concentration) were used and then the results were combined with each of the anticancer agents used in the treatment of patients with CRC: ”Irinotecan”, “5-Fluorouracil”, “capecitabine”, “panitumumab”, “oxaliplatin”, “bevacizumab” and “cetuximab”. All solid cancer types were included (see Table 7), not just CRC. Results were limited to studies in humans, and publication dates through to December 2017.

The independent reviewers extracted and tabulated data for pre-planned data fields for each study. Results were then reviewed together for consensus on each data field for each study. Disagreements were resolved with discussion and repeat review of the relevant study as needed.

4.4 Results

Twenty-one publications met the eligibility criteria and were included in the review. All results are presented in Table 7. Seventeen publications were prospective studies and four studies were pooled PK data analyses of prospective studies. The PK of irinotecan were assessed in 3 studies, [24-26] 5- fluorouracil in 7 studies [27-33], capecitabine in 2 studies [34,35], oxaliplatin in 2 studies [36,37], bevacizumab in 1 study [38] and cetuximab in 2 studies [39,40]. Four studies examined the PK of panitumumab, irinotecan, capecitabine and cetuximab from pooled analysis of prospective studies [41-44]. Studies included a median of 44 patients (range 19 to 1200) with the age definition of an older adult varying across studies (≥65 years, 70 years or >75 years). Six studies determined the PK of the anti-cancer therapies in CRC, while 15 studies concerned other cancer types. PK parameters significantly affected by age were CL (drug clearance), AUC, Cmax and Vmax (maximum rate of process) across 8 studies [25, 42, 26, 24, 29, 30, 35 and 41].

Irinotecan

The doses of irinotecan ranged from 20 mg/m2 to 340 mg/m2. Iirinotecan is commonly dosed as 180 mg/m2 every 2 weeks [45] or 350 mg/m2 every three weeks to treat patients with CRC in clinical practice [46]. Three of the four studies found a significant association between the PK of irinotecan and increasing age. Klein et al conducted a dose escalation study of irinotecan (n=78) in solid tumours and found a 7.2% reduction in irinotecan clearance (CL) for every 10 years older than 60 years ($p<0.001$). [42] Miya et al investigated factors influencing PK of irinotecan and showed increased AUC ($r=0.44$) and Cmax ($r=0.42$) of irinotecan with increasing age (age range 29-75 years, $p=0.007$, $p=0.009$ respectively)

[25]. Poujol et al showed a significant 8% decline in the CL of irinotecan with increasing age (median age 62 years, r =0.42, p=0.009) [26].

5-fluorouracil

The doses of 5-fluorouricil ranged from 320 mg/m2 to 2400 mg/m2. 5-fluorouricil is commonly dosed from 400 mg/m2 (bolus) to 2400 mg/m2 (46-hour continuous infusion) every 2 weeks in clinical practice [47]. Denham et al (n=44) found an increasing AUC of 5-fluorouricil with increasing age (p=0.02) [30]. Etienne et al (n=104) assessed the effect of patient factors on the PK of 5-fluorouricil and found a statistically significant decrease in the CL of 5-fluorouricil with increasing age (p<0.001) [29]. The other five studies showed no association between the PK of 5-fluorouricil and increasing age.

Capecitabine

All studies used the same dose of capecitabine (1000 mg/m2). Capecitabine is commonly dosed from 1000 mg/m2 to 1250 mg/m2 twice daily for 14 days every 3 weeks in clinical practice [48]. Louie et al investigated the PK of capecitabine in older adults with colorectal cancer (n=29) and found that older patients (aged >70 years), compared with younger patients (aged <60 years), had a statistically significant 71% decline in CL (p=0.03) and a 150% increase in the AUC (p=0.04) of capecitabine, but no difference in the PK parameters of the metabolites (5'DFCR, 5'DFUR, 5-FU) of capecitabine [35]. Abdi et al compared the PK data of capecitabine in 20 older patients with breast or colorectal cancer (aged >75 years) with 40 younger patients (aged <60 years) from two previous clinical trials [43]. Elimination parameters of capecitabine and its metabolites were not affected by age. Significantly higher median exposures of capecitabine and its metabolites occurred in older patients who

experienced hand foot syndrome, compared with older patients who did not experience hand foot syndrome. Cassidy et al, in a small study (n=25) of adults with solid tumours showed age, gender, BSA or creatinine clearance did not affect PK parameters of capecitabine and its metabolites [34].

Oxaliplatin

The doses of oxaliplatin ranged from 50 mg/m2 to 130 mg/m2. Oxaliplatin is commonly dosed as 85 mg/m2 every two weeks and 130 mg/m2 every 3 weeks in clinical practice [47]. Bastian et al investigated the effect of age on the PK of oxaliplatin in 56 patients (41y-79y) with solid tumours (majority CRC) from phase I and phase I/II studies. CL of oxaliplatin was not affected by age, but decreased CL was correlated with lower body weight and higher serum creatinine [36]. Delord et al conducted an observational phase I study in 40 patients aged 29 years to 82 years with CRC, exploring the impact of multiple covariates including age, gender, anaemia, BSA and renal function on the PK of oxaliplatin. PK of oxaliplatin was not affected by age, but increased CL was significantly correlated with increased SCr, higher BSA and haemoglobinaemia [37].

Panitumumab

The doses of panitumumab ranged from 0.01 mg/kg to 9 mg/kg. Panimtumumab is dosed as 6 mg/kg every 2 weeks in clinical practice [49]. Ma et al investigated the PK of panitumumab in a pooled data analysis of 14 prospective clinical trials including 1200 patients with solid tumours [41]. The population PK of panitumumab was explained by both linear (dose-proportional manner) and non-linear (saturable binding to EGFR) elimination pathways. Age was negatively correlated with Vmax of panitumumab (non-linear clearance) with an increase

in age from 50 years to 70 years yielding a 15.3% decrease in Vmax. However, the contribution of age to the variance of area under the curve at steady state (AUC_{SS}) was small at only 0.7% compared with those of the weight-based dose regimen around 69.2%.

Bevacizumab

In the only published relevant study, the dose of bevacizumab was 5 mg/kg. Bevacizumab is dosed at 5mg/kg every two weeks to 7.5mg/kg every three weeks in clinical practice. [50]. Panoilia et al conducted a small study (n=19) primarily designed to characterise bevacizumab's population PK [38]. In this study age had no significant effect on bevacizumab PK.

Cetuximab

All three studies of cetuximab used the same dose of 250 mg/m2. Cetuximab is dosed as 500 mg/m2 every two weeks in clinical practice [51]. In each case, the PK of cetuximab was not influenced by age [40, 39, 44]. Azzopardi et al examined patient factors that influenced PK of cetuximab in a PK-guided dose intensification study of cetuximab in 96 patients with metastatic CRC aged 38 to 80 years. Only BSA and initial serum albumin concentration were significantly correlated with CL of cetuximab, but not other covariates including age [40]. Tan et al (n=40) and Dirks et al (n=156) investigated the effect of patient factors on the PK of cetuximab and showed that patients' BSA and weight affected PK parameters, but not age [39, 44].

4.5 Discussion

Older age was associated with PK parameters in all studies concerning irinotecan [24, 42, 26, 25], the one study concerning panitumumab [41], and some, but not all, of the studies concerning 5-fluorouracil [30, 29] and capecitabine [35]. No association between increasing age and PK parameters were found in the included studies concerning oxaliplatin, bevacizumab, or cetuximab [36-38, 40, 39, 44]. There were overall few studies that determined the effect of age as a primary outcome on the PK of anti-cancer therapies use in the management of CRC.

We conducted this review to help determine whether changes in PK are responsible for the increased toxicity such as fatigue, diarrhoea, myelosuppression, dehydration and consequent hospitalisations, [8] experienced by older adults with colorectal cancer with these drugs. The most consistent findings for an effect of older age on PK were in the studies concerning irinotecan. Where age-related PK changes were found, however, the reported effect sizes were small, all less than 10% and so unlikely to be of clinical significance. There are no guidelines for the interpretation and clinical significance of PK parameters, but Joerger et al have suggested a minimum change of at least 20% in major PK parameters, mainly drug elimination, to be considered as clinically significant [52].

The most consistent finding of changes in PK of anti-cancer therapies with older age is a decline in CL. The Louie et al study included in this review showed a 71% decline in CL of capecitabine in older adults. Studies in other cancer types have also shown a decline in CL in older adults such as a 31% decline in the CL of carboplatin in lung cancer [53] and a 30% decline in the CL of doxorubicin in breast cancer in two studies which both defined older adults as aged > 70 years [54]. Such knowledge of PK of anti-cancer therapies in older adults

provides an opportunity to overcome the heterogeneity of the ageing process and to refine prescribing, by better understanding treatment-related toxicity and optimise dosing for maximum efficacy.

Factors other than age-related changes in PK of anti-cancer therapies are likely responsible for the excess treatment toxicity seen in older adults with CRC. Age-related changes in PD can explain, for example, the greater haematological toxicity from chemotherapy due to reduced haematopoiesis with increasing age. Geriatric syndromes are another likely cause. The presence of multiple comorbidities in older adults can lead to frailty, vulnerability, and limited physiological reserve to tolerate serious treatment toxicities [55]. Polypharmacy and cognitive impairment can cause confusion and impede compliance with usual medications and oral chemotherapy, such as capecitabine, usually taken independently at home. Limited social support and social isolation can cause late presentations to medical care, leading to more severe and prolonged toxicity.

Optimal selection of older adults for anti-cancer therapy and tailored prescribing are imperative for quality care of older adults with cancer. Patient selection can be aided by the use of Complex Geriatric Assessment (CGA) or an abbreviated version of such, and/or the use of risk prediction tools that estimate the risk of severe chemotherapy toxicity [56, 57]. CGAs are recommended for all older adults with cancer [58] and have been shown to identify impairments and frailty, predict survival and treatment toxicity, and help develop appropriate supportive care interventions. There are no studies determining the relationship, if any, between the results of geriatric assessments and PK.

Prescribing of anti-cancer therapies involves careful consideration and application of relevant cancer treatment guidelines. International cancer treatment guidelines do not recommend dose modifications for older age per se for the anti-cancer therapies in our review [48, 59-60].

The Australian EVIQ guidelines recommend a lower starting dose of capecitabine when used as monotherapy in the metastatic setting in 'elderly patients and other patients considered at risk of toxicity' (from 1250 mg/m2 to 1000 mg/m2 bid) [61]. Dose modifications across guidelines are recommended in people with renal and hepatic impairment, commonly seen in older adults, for 5-fluorouracil, capecitabine, irinotecan, and oxaliplatin. Importantly, older age should never be seen as a reason to not actively treat an older people with CRC, especially where there is genuine consideration for a positive outcome [62]. What is clear from these PK data is the need to consider dose individualisation and careful monitoring to guide dosing in older people [20].

An important limitation of the available PK studies is their tendency to be conducted in clinical trials enrolling predominantly younger, fitter patients. Even where older adults are eligible and included in clinical trials, they typically comprise only a small proportion of the total trial population, and the included older adults are also a very fit subset of the entire population of older adults with cancer [63]. These limit the generalisability of the PK results and consequent dosing recommendations to the typical older adults in clinical practice. This limitation applies to several of the studies included in our review.

Other limitations of our review include the methodological heterogeneity across studies, the small number of studies for each drug included in the review, the small number of patients in the included studies, and the variable definitions of ageing and older adults. Some studies, for example, used 65 years as a dichotomous cut-off for older versus younger patients,

whereas other studies used 75 years. These limitations make it difficult to draw firm conclusions and reduce the applicability of the results to typical older adults having anti-cancer therapy for CRC in clinical practice. The small sample sizes of the included studies and age typically explored as a potential predictor in subgroup analyses rather than as a primary outcome reduce the power to detect age as a significant covariate.

4.6 Conclusion

In conclusion, older age was significantly associated with PK parameters of anti-cancer therapies used in older adults with CRC, but the effects were small and not easily translated into recommended dose modifications of these drugs for older adults. PK and PD studies including older adults typical of routine clinical practice to optimise dosing of anti-cancer therapy are warranted.

Table 7. Pharmacokinetics of anti-cancer drugs in older adults

Anti-cancer drug	Participants Median age (range)	Cancer type	Study purpose	Findings and comments	Author Year
Irinotecan	n=28 <65y: n=16, ≥65y: n=12 63y (29y-82y)	CRC, Unknown primary , uterus, renal cell	Phase I dose-escalation PK study of oral irinotecan in patients with solid tumours to characterize the MTD, DLTs, PK profile, and antitumor effects.	High inter-individual PK variability. Advanced age was associated with reduced drug tolerance; Patients aged ≥65y had DLT at lower dose (66 mg/m^2/d) than patients aged <65y (80 mg/m^2/d).	Drengler et a 1999 [24]
	n=36 60y (29y-75y)	Lung, head and neck, colon and uterus	Observational study examining influence of gender, age, BSA and SCr on PK of irinotecan and its metabolites.	Irinotecan AUC significantly increased with increasing age (p=0.007), male gender (p=0.008) and poor SCr. Irinotecan Cmax significantly increased with increasing age (p= 0.009), male gender (p=0.007) and BSA (p= 0.023).	Miya et al 2001 [25]
	n=78 61y (31y-80y)	Solid tumours and lymphoma	PK analysis of 2 dose-escalation studies to develop population PK model.	Increasing age and poorer performance status significantly correlated with decreased irinotecan CL (p<0.01).Irinotecan CL decreased 2.1 L/h (7.2%) for every 10y older than 60y.	Klein et al 2002 [42]

	n=35 62y (mean)	Digestive system	Prospective observational PK study investigating the effect of patient factors on irinotecan CL.	Irinotecan CL significantly declined with increasing age and explained 8% of the inter-individual variability in CL.	Poujol et al 2005 [26]
5FU	n=26 (≥70y: n=4, <70y: n=22) 53y (43y-75y)	CRC, breast and oesophagus	Observational study investigating the effect of gender, age and BSA on the PK of 5FU.	Advanced age correlated with reduced 5FU CL but not statistically significant. ↑ 5FU CL was associated with ↑ BSA, male gender and ↑ dose ($p<0.001$).	Port et al 1991 [27]
	n=360 (>70 y: n=58 51–70 y: n=245 <50 y: n=57) 62y (25y–91y)	HNSCC	Prospective observational study examining the effect of sex & age on 5FU CL.	5FU CL was not influenced by age ($p=0.45$), but was 10% lower in females ($p= 0.0005$).	Milano et al 1992 [28]
	n=104 59y (31y-84y)	Head and neck and oesophagus	Prospective study investigating the effect of patient factors including age on 5FU CL.	Increasing age correlated with reduced 5-FU CL ($p<0.001$).	Etienne et al 1998 [29]
	n=44 72y (42y-91y)	Oesophageal	Observational study investigating causes of increased rate of myelosuppression in older patients on chemo-radiotherapy including PK of 5FU (as 5FU/cisplatin).	Advanced age correlated with higher 5FU AUC ($p= 0.02$).	Denham et a 1999 [30]

	n=181 65y (34y-87y)	CRC	Observational study examining patient factors including age on 5FU AUC and association with toxicity in adjuvant setting.	5FU AUC or CL not influenced by age. ↑ Drug dose ($p< 0.0001$), ↑ body weight ($p< 0.0001$) and female gender ($p< 0.0001$) were correlated with ↑ 5FU AUC.	Gusella et al 2006 [31]
	n=103 <65y: n=55 59y (33y-64y) ≥65y: n=48 70y (65y-80y)	mCRC	Prospective study of PK-guided dosing of 5FU in patients with mCRC assessing the impact of age on PK of 5FU.	5FU CL, Vd, $t_{1/2}$ and AUC not influenced by age ($p = 0.1$). Patients aged ≥65y tolerated dose intensification similar to the patients aged <50y ($p = 0.9$).	Duffour et al 2010 [32]
	n=31 ≥65 y: n=14 <65 y: n=17 63y (31y–81y)	Gastro-intestinal	Prospective single arm study investigating the effect of gender, age, BSA, SCr, liver dysfunction and DPYD genotype on PK (AUC, CL & Vd) of 5FU.	5FU CL was ↑ in male gender ($p<0.01$) and not effected by age.	Mueller et al 2012 [33]
Capecitabine	n=25 62y (41y-80y)	CRC and breast	Randomised crossover bioequivalence study of two capecitabine tablet formulations, examining the effect of age, gender, BSA and creatinine CL on PK of capecitabine PK.	PK of capecitabine and its metabolites not influenced by age ($p> 0.15$), BSA ($p = 0.03$), or creatinine CL ($p = 0.29$), but were only ↑ in female gender ($p = 0.001$).	Cassidy et al 1999 [34]
	n=29 A: ≥70y: n=24 76 y (mean) B: <60y: n=5 55y (mean)	Unresectable CRC	Prospective study investigating the influence of age on PK of capecitabine and its metabolites.	Advanced age was associated with 71% decrease in capecitabine CL ($p= 0.03$) and 150% increase in capecitabine AUC ($p= 0.04$).	Louie et al 2013 [35]

	n=60 <75y: n=40 54y (30y-73y) ≥75y: n=20 80y (75y-92y)	Breast and CRC	Prospective observational study examining effect of age on PK of capecitabine and its metabolites and investigating the exposure–effect relationship in older age group (>75y).	PK of capecitabine not influenced by age (p= 0.59). Higher exposure of capecitabine and its metabolites was observed in patients developed hand and foot syndrome in cycle 2 of treatment (p= 0.01)	Abdi et al 2014 [43]
Oxaliplatin	n=56 59y (41y-79y)	Solid tumours (majority CRC)	Prospective phase I and phase I/II studies to develop population PK model and to investigate the influence of covariates (including age) on PK of oxaliplatin.	Oxaliplatin CL not influenced by age, but was positively correlated with body weight (p<0.001), negatively correlated with SCr (p<0.001), and was greater in male patients (p< 0.01).	Bastian et al 2003 [36]
	n=40 59y (29y-82y)	CRC	Prospective observational phase I study to explore association between patient factors and PK parameters of oxaliplatin.	PK parameters of oxaliplatin not influenced by age, but ↑ CL was significantly correlated with↑ SCr, ↑ BSA and ↓ Hb.	Delord et al 2003 [37]
Panitumumab	n=1200 Male 62y (mean) Female 59y (mean)	CRC, lung and kidney	Pooled data analysis to determine population PK modelling of panitumumab from 14 prospective trials and to explore the impact of baseline covariates on PK parameters of panitumumb.	Advanced age was correlated with reduced panitumumab Vmax (p< 0.001) but effect size was small (0.7% of variance in AUC versus weight-based dose regimen effect of 69.2%).	Ma et al 2009 [41]

Bevacizumab	n=19 60y (37y-73y)	CRC	Prospective study to develop population PK model for bevacizumab.	PK of bevacizumab not influenced by age (p>0.01).	Panoilia et a 2015 [38]
Cetuximab	n=40 60y (22y-85y)	CRC	Prospective study to evaluate the PK of cetuximab given as to different dose. Effects of patient factors on cetuximab CL were assessed.	Cetuximab CL not influenced by age, but increased with BSA (p = 0.002), weight (p = 0.002) and dose (p < 0.0001).	Tan et al 2006 [39]
	n=156 56y (23y-77y)	HNSCC	Pooled data analysis of PK of cetuximab from early phase I/II & II studies to evaluate the PK of cetuximab and to identify the effects of covariates on its PK.	Cetuximab PK parameters not influenced by age. Cetuximab CL predicted by Ideal Body Weight (p<0.001) and white blood cell count (p< 0.001).	Dirks et al 2008 [44]
	n=96 63y (38y–80y)	mCRC	Prospective phase II study, investigating influence of inter-individual variability in cetuximab PK on progression free survival of patient with CRC.	Cetuximab PK parameters not influenced by age. ↑ BSA correlated with ↑cetuximab Vd (p=0.01) and ↑ pre-treatment serum albumin correlated with↓ cetuximab CL (p= 0.006).	Azzopardi et al 2011 [40]

Abbreviations: AUC, area under the curve; BSA, body surface area; BW, body weight; CL, clearance; Cmax, maximum concentratio; CRC, colorectal cancer; DLT, dose-limiting toxicities; Hb, haemoglobin; HNSCC, head and neck squamous cell carcinoma; mCRC, metastatic colorectal cancer; MTD, maximum-tolerated dose; PK, pharmacokinetic; SCr, serum creatinine; t1/2, half-life; Vd, volume of distribution.

Figure 12. PRISMA Flow Diagram

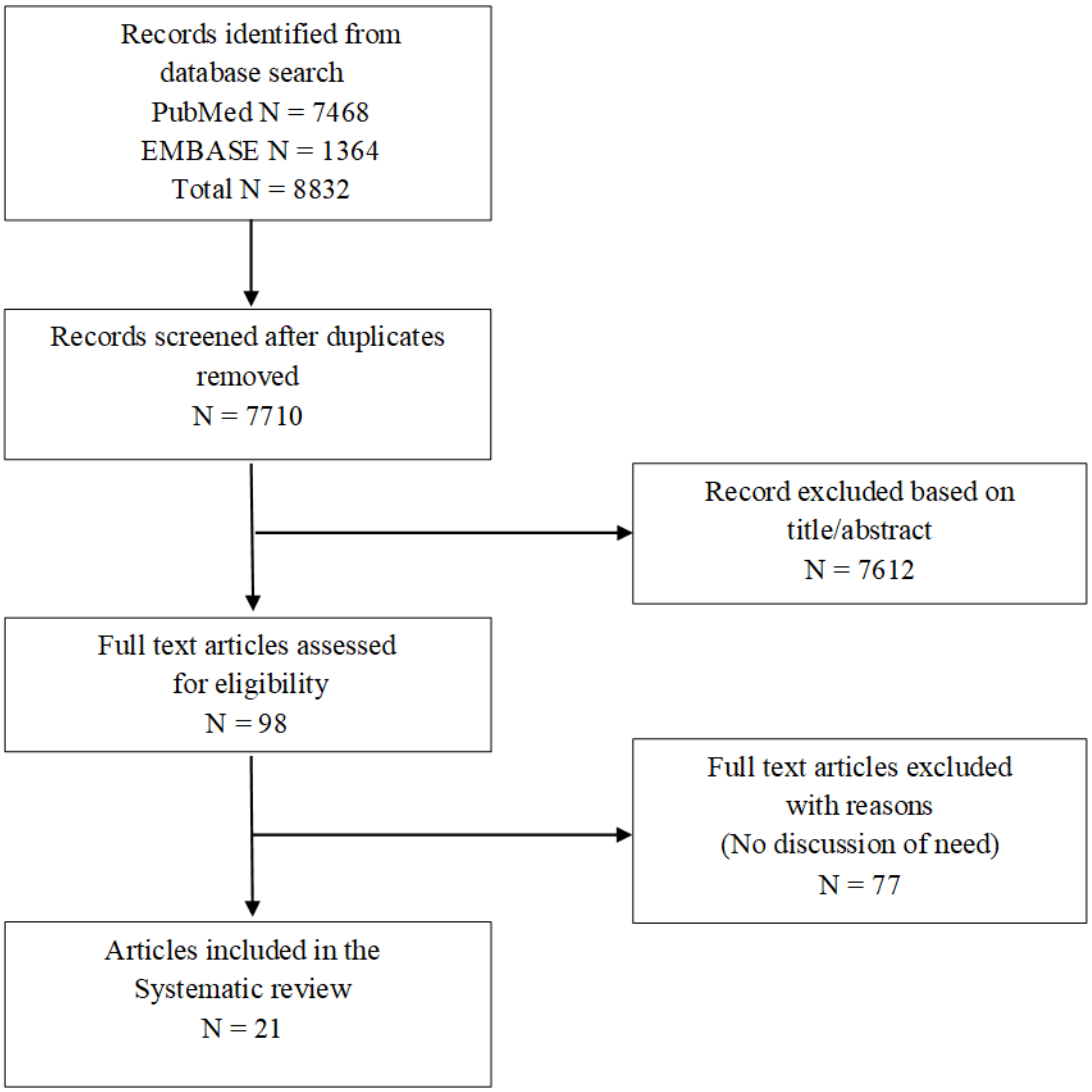


Figure 1. PRISMA Flow Diagram.

4.7 References

1. Arnold M, MS Sierra, M Laversanne, et al. Global patterns and trends in colorectal cancer incidence and mortality. *Gut.*2017; 66:683-691.

2. Millan M, S Merino, A Caro, et al. Treatment of colorectal cancer in the elderly. *World J Gastrointest Oncol.*2015; 7: 204-220.

3. Stein BN, NJ Petrelli, HO Douglass, et al. Age and sex are independent predictors of 5-fluorouracil toxicity. Analysis of a large scale phase III trial. *Cancer.*1995*;* 75:11-17

4. Hall M, D Krell, R Glynne-Jones. Single-agent capecitabine use in patients age 70 or older with colorectal cancer. *J Clin Oncol*.2015; 33 (3_suppl):764-764.

5. Cen P, C Liu, XL Du. Comparison of toxicity profiles of fluorouracil versus oxaliplatin regimens in a large population-based cohort of elderly patients with colorectal cancer. *Ann Oncol*.2012; 23: 1503-1511.

6. Kim J-Y, YJ Kim, K-W Lee, et al. Practical Outcome of Adjuvant FOLFOX4 Chemotherapy in Elderly Patients with Stage III Colon Cancer: Single-center Study in Korea. *J Clin Oncol.*2013; 43:132-138.

7. Cassidy J, C Twelves, E Van Cutsem, et al. First-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. *Ann Oncol*.2002; 13:566-575.

8. McKibbin T, CR Frei, RE Greene, et al. Disparities in the use of chemotherapy and monoclonal antibody therapy for elderly advanced colorectal cancer patients in the community oncology setting. *Oncologist.*2008; 13: 876-885.

9. Folprecht G, P Rougier, L Saltz, et al. Irinotecan in first line therapy of elderly and non-elderly patients with metastatic colorectal cancer: Meta-analysis of four trials investigating 5-FU and irinotecan. *J Clin Oncol*.2006; 24 (18_suppl):3578-3578.

10. Tam VC, S Rask, T Koru–Sengul, et al. Generalizability of toxicity data from oncology clinical trials to clinical practice: toxicity of irinotecan-based regimens in patients with metastatic colorectal cancer. *Curr Oncol*.2009; 16: 13-20.

11. Figer A, N Perez-Staub, E Carola, et al. FOLFOX in patients aged between 76 and 80 years with metastatic colorectal cancer: an exploratory cohort of the OPTIMOX1 study. *Cancer*.2007; 110: 2666-2671.

12. Lichtman SM, G Villani. Chemotherapy in the elderly: pharmacologic considerations. *Cancer Control*.2000; 7: 548-556.

13. Cunningham D, I Lang, E Marcuello, et al. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. *Lancet Oncol*.2013; 14: 1077-1085.

14. Korc-Grodzicki B, MK Boparai, SM Lichtman. Prescribing for older patients with cancer. *Clin Adv Hematol Oncol*.2014; 12: 309-318.

15. Korc-Grodzicki B, HM Holmes, A Shahrokni. Geriatric assessment for oncologists. *Cancer Biol Med* 2015; 12: 261-274.

16. Kotake K, M Asano, H Ozawa, et al. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. *Colorectal Dis.*2015; 17: 205-215.

17. Patel SS, R Nelson, J Sanchez, et al. Elderly patients with colon cancer have unique tumor characteristics and poor survival. *Cancer.*2013; 119: 739-747.

18. Moth EB, J Vardy, P Blinman. Decision-making in geriatric oncology: systemic treatment considerations for older adults with colon cancer. *Expert Rev Gastroenterol Hepatol.*2016; 10: 1321-1340.

19. Zulman DM, JB Sussman, X Chen, et al. Examining the evidence: a systematic review of the inclusion and analysis of older adults in randomized controlled trials. *J Gen Intern Med.*2011; 26: 783-790.

20. He X, SJ Clarke, AJ McLachlan. Clinical pharmacology of chemotherapy agents in older people with cancer. *Curr Gerontol Geriatr Res.*2011; 2011: 628670.

21. Hilmer SN, AJ McLachlan, DG Le Couteur. Clinical pharmacology in the geriatric patient. *Fundam Clin Pharmacol.*2007; 21: 217-230.

22. McLachlan AJ, SN Hilmer, DG Le Couteur. Variability in response to medicines in older people: phenotypic and genotypic factors. *Clin Pharmacol Ther.*2009; 85: 431-433.

23. Wasil T, SM Lichtman. Clinical pharmacology issues relevant to the dosing and toxicity of chemotherapy drugs in the elderly. *Oncologist*.2005; 10: 602-612.

24. Drengler RL, JG Kuhn, LJ Schaaf, et al. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. *J Clin Oncol*.1999; 17: 685-696.

25. Miya T, T Goya, H Fujii, et al. Factors affecting the pharmacokinetics of CPT-11: the body mass index, age and sex are independent predictors of pharmacokinetic parameters of CPT-11. *Invest New Drugs*.2001; 19: 61-67.

26. Poujol S, F Bressolle, J Duffour, et al. Pharmacokinetics and pharmacodynamics of irinotecan and its metabolites from plasma and saliva data in patients with metastatic digestive cancer receiving Folfiri regimen. *Cancer Chemother Pharmacol*.2006; 58: 292-305.

27. Port RE, B Daniel, RW Ding, et al. Relative importance of dose, body surface area, sex, and age for 5-fluorouracil clearance. *Oncology*.1991; 48: 277-281.

28. Milano G, MC Etienne, E Cassuto-Viguier, et al. Influence of sex and age on fluorouracil clearance. *J Clin Oncol*.1992; 10: 1171-1175.

29. Etienne MC, E Chatelut, X Pivot, et al. Co-variables influencing 5-fluorouracil clearance during continuous venous infusion. A NONMEM analysis. *Eur J Cancer*.1998; 34: 92-97.

30. Denham JW, SP Ackland, B Burmeister, et al. Causes for increased myelosuppression with increasing age in patients with oesophageal cancer treated by chemoradiotherapy. *Eur J Cancer*.1999; 35: 921-927.

31. Gusella M, G Crepaldi, C Barile, et al. Pharmacokinetic and demographic markers of 5-fluorouracil toxicity in 181 patients on adjuvant therapy for colorectal cancer. *Ann Oncol*.2006; 17: 1656-1660.

32. Duffour J, L Roca, F Bressolle, et al. Clinical impact of intesified 5-Fluorouracil-based chemotherapy using a prospective pharmacokinetically-guided dosing approach: comparative study in elderly and non-elderly patients with metastatic colorectal cancer. *J Chemother.*2010; 22: 179-185.

33. Mueller F, B Buchel, D Koberle, et al. Gender-specific elimination of continuous-infusional 5-fluorouracil in patients with gastrointestinal malignancies: results from a prospective population pharmacokinetic study. *Cancer Chemother Pharmacol*.2013; 71: 361-370.

34. Cassidy J, C Twelves, D Cameron, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. *Cancer Chemother Pharmacol.*1999; 44: 453-460.

35. Louie SG, B Ely, HJ Lenz, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). *Br J Cancer*.2013; 109: 1744-1749.

36. Bastian G, A Barrail, S Urien. Population pharmacokinetics of oxaliplatin in patients with metastatic cancer. *Anticancer Drugs.*2003; 14: 817-824.

37. Delord JP, A Umlil, R Guimbaud, et al. Population pharmacokinetics of oxaliplatin. *Cancer Chemother Pharmacol.*2003; 51: 127-131.

38. Panoilia E, E Schindler, E Samantas, et al. A pharmacokinetic binding model for bevacizumab and VEGF165 in colorectal cancer patients. *Cancer Chemother Pharmacol*.2015; 75: 791-803.

39. Tan AR, DF Moore, M Hidalgo, et al. Pharmacokinetics of cetuximab after administration of escalating single dosing and weekly fixed dosing in patients with solid tumors. *Clin Cancer Res.*2006; 12: 6517-6522.

40. Azzopardi N, T Lecomte, D Ternant, et al. Cetuximab pharmacokinetics influences progression-free survival of metastatic colorectal cancer patients. *Clin Cancer Res*.2011; 17: 6329-6337.

41. Ma P, BB Yang, YM Wang, et al. Population pharmacokinetic analysis of panitumumab in patients with advanced solid tumors. *J Clin Pharmacol*.2009; 49: 1142-1156.

42. Klein CE, E Gupta, JM Reid, et al. Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. *Clin Pharmacol Ther*.2002; 72: 638-647.

43. Daher Abdi Z, S Lavau-Denes, A Premaud, et al. Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. *Cancer Chemother Pharmacol.*2014; 73: 1285-1293.

44. Dirks NL, A Nolting, A Kovar, et al. (2008) Population pharmacokinetics of cetuximab in patients with squamous cell carcinoma of the head and neck. *J Clin Pharmacol*.2008; 48: 267-278.

45. Colucci G, V Gebbia, G Paoletti, et al. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol.*2005; 23: 4866-4875.

46. Fuchs CS, MR Moore, G Harker, et al. Phase III comparison of two irinotecan dosing regimens in second-line therapy of metastatic colorectal cancer. *J Clin Oncol.*2003; 21: 807-814.

47. de Gramont A, A Figer, M Seymour, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol.*2000; 18: 2938-2947.

48. Van Cutsem E, PM Hoff, P Harper, et al. Oral capecitabine vs intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomised, phase III trials. *Br J Cancer*.2004; 90: 1190-1197.

49. Price TJ, M Peeters, TW Kim, et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a

randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol*.2014;15: 569-579.

50. Hurwitz H, L Fehrenbacher, W Novotny, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*.2004; 350: 2335-2342.

51. Jonker DJ, CJ O'Callaghan, CS Karapetis, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med*.2007; 357: 2040-2048.

52. Joerger M. Covariate pharmacokinetic model building in oncology and its potential clinical relevance. *AAPS J*.2012; 1: 119-132.

53. Merino-Sanjuan M, JF Monteiro, B Porta-Oltra, et al. Effect of age on systemic exposure and haematological toxicity of carboplatin in advanced non-small cell lung cancer patients. *Basic Clin Pharmacol Toxicol*.2011; 109: 457-464.

54. Robert J, B Hoerni. Age dependence of the early-phase pharmacokinetics of doxorubicin. *Cancer Res*.1983; 43: 4467-4469.

55. Fried LP, CM Tangen, J Walston, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*.2001; 56: M146-156.

56. Hurria A, K Togawa, SG Mohile, et al. Predicting chemotherapy toxicity in older adults with cancer: a prospective multicenter study. *J Clin Oncol*.2011; 29: 3457-3465.

57. Extermann M, I Boler, RR Reich, et al. Predicting the risk of chemotherapy toxicity in older patients: the Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) score. *Cancer*.2012; 118: 3377-3386.

58. Wildiers H, P Heeren, M Puts, et al. International Society of Geriatric Oncology Consensus on Geriatric Assessment in Older Patients With Cancer. *J Clin Oncol*.2014; 32: 2595-2603.

59. Network NCC NCCN Guidelines Version 2.2018 Colon Cancer. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf. Accessed 8.05.2018

60. Labianca R, B Nordlinger, GD Beretta, et al (2013) Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*.2013; 24 (suppl_6):vi64-vi72.

61. EVIQ Colorectal metastatic capecitabine. https://www.eviq.org.au/medical-oncology/colorectal/metastatic/94-colorectal-metastatic-capecitabine. Accessed 8.05.2018

62. Mitry E, P Rougier. Review article: benefits and risks of chemotherapy in elderly patients with metastatic colorectal cancer. *Aliment Pharmacol Ther*.2009; 29: 161-171.

63. Hamaker ME, R Stauder, BC van Munster. Exclusion of Older Patients From Ongoing Clinical Trials for Hematological Malignancies: An Evaluation of the National Institutes of Health Clinical Trial Registry. *Oncologist*.2014; 19: 1069-1075.

Chapter 5 Dried Blood Spot sampling in the monitoring of anticancer therapy of solid tumours: a systematic review

5.1 Overview

This chapter is a published work of a systematic review exploring the potential role of microsampling in improving implementation of therapeutic drug monitoring (TDM) in oncology setting. This review included studies examining utilisation of microsampling to monitor chemotherapy or targeted therapy in the treatment of solid cancers. The review demonstrated that microsampling (DBS and Mitra devices) is a feasible and promising alternative to plasma or venous sampling for the TDM of many anticancer drugs. This work highlighted the need for further research of the clinical utility of microsampling for the measurement of anticancer drugs.

The published manuscript is quoted verbatim. Formatting has been updated for consistency across the thesis.

5.1.1 Publication details

Shafiei M, Mahmood A, Beale P, Galettis P, Martin J, McLachlan AJ, Blinman P. Dried Blood Spot Sampling in the Monitoring of Anticancer Therapy for Solid Tumors: A Systematic Review. *Ther Drug Monit.* 2023; 45(3):293-305. https://doi.org/10.1097/ftd.0000000000001082.

5.1.2 Contribution of authors

Mohsen Shafiei developed the research proposal and research methods, performed the systematic review and data analysis, interpreted the findings, and drafted and revised the

manuscript.

Alina Mahmood performed the systematic review, and contributed to data analysis.

Peter Galettis contributed to the research proposal, and revision of the manuscript.

Philip Beale contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Jennifer Martin contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Andrew McLachlan contributed to the research proposal contributed to the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Prunella Blinman contributed to the research proposal and the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Abstract

Background

Dried blood spot (DBS) sampling offers a convenient alternative to whole blood sampling for therapeutic drug monitoring (TDM) in clinical practice. The aim of this study was to systematically review studies that have examined and utilised DBS sampling for TDM of chemotherapy and targeted therapy agents in the treatment of patients living with solid cancers.

Methods

Using PRISMA guidelines, a systematic literature search of EMBASE and PUBMED was performed to identify eligible clinical studies that used DBS sampling to monitor chemotherapy or targeted therapy in the treatment of solid cancers.

Results

Of the 23 eligible studies, 3 studies measured concordance between drug concentrations determined by DBS and whole bloods, 7 studies developed analytical methods of DBS, and 13 studies included both. DBS sampling investigated TDM of everolimus (3 studies), vemurafenib (2 studies), pazopanib (2 studies), abiraterone (2 studies), and mitotane, imatinib, adavosertib, capecitabine, 5-fluorouracil, gemcitabine, cyclophosphamide, ifosfamide, etoposide, irinotecan, docetaxel, gefitinib, palbociclib/ribociclib and paclitaxel (each 1 study). Studies included a median of 14 participants (range, 6 - 34). Studies used 10 - 50 μL of blood on DBS cards (20) and Mitra® device (3). 17/20 found no significant impact of haematocrit on accuracy and precision of the developed method in the normal haematocrit

ranges (e.g. 29.0–59.0%). DBS and plasma or venous concentrations were highly correlated (correlation coefficient, 0.872 to 0.999) for all drugs except mitotane which did not meet a pre-defined level of significance, $r>0.872$ (correlation coefficient, $r = 0.87$, $p < 0.0001$).

Conclusion

DBS provides an alternative sampling strategy in TDM of many anticancer drugs. More research is required to establish a standardized approach for sampling and processing DBS samples to allow future implementation.

5.2 Introduction

Patients treated with chemotherapy or targeted therapy [e.g. tyrosine kinase inhibitors (TKI's)] have a risk of significant toxicities from over-dosing or compromised treatment due to inadvertent under-dosing. Therapeutic drug monitoring (TDM) is a valuable tool used to avoid treatment failure or treatment-related harms by guiding individualised dosing of anticancer therapy, especially for drugs with a narrow therapeutic index and a wide inter-individual variation in pharmacokinetics (PKs) [1]. TDM uses PK-guided dosing as opposed to body surface area (BSA) -guided dosing or flat dosing, both of which do not account for inter-individual variability in PKs of agents [2].

TDM-based dosing strategies rely on an established relationship between the PKs of anticancer therapies and clinical outcomes (efficacy and toxicity) [3-5]. In clinical practice, however, there are only a small number of anticancer drugs for which TDM-based dosing have been partially implemented according to the randomised control trials (RCT) including carboplatin, methotrexate, busulfan and mitotane. TDM for other agents, such as imatinib, 5-fluorouracil and pazopanib, have not been implemented despite evidence of benefit and feasibility in well-designed RCTs.1, [6-8] Challenges of routine implementation of TDM for cancer include: (i) measured plasma concentrations requiring clinical and pharmacological interpretation to guide decision making; (ii) the need for venepuncture collection of 1-5 mL sample volume over multiple time points and the infrastructure for such collections; (iii) limited availability of TDM assays [1, 3, 9]. To overcome these challenges, new methods and analytical assays for small-volumes using robust and convenient sampling techniques are required.

Novel techniques for micro-sampling and precise analytical investigations for TDM mean that PK-guided individualised dosing is now more feasible in clinical practice [10]. There are several micro-sampling methods used for TDM of drugs. A commonly used method is dried blood spot (DBS) sampling that uses capillary blood from a finger prick with an automatic lancet. A blood drop is collected to fill a pre-marked circle on absorbent paper. The blood drop dries at room temperature and the filter paper is packed for transportation to a laboratory. A disc is punched out from the DBS paper on which the analyte is measured with an analytical technique. Advantages of DBS sampling over venepuncture include its need for only a very small volume of blood, its convenience, simplification of logistics for remote sampling with reduced workforce requirements, increased sample stability and easier storage and shipping [9]. The Food and Drug Administration (FDA) guidelines define the necessary parameters for the validation of quantitative DBS-based methods and on the application of validated methods in routine clinical practice [11]. As such validation should include assessing the effects of storage and handling temperatures, homogeneity of sample spotting, haematocrit, stability, carryover, and reproducibility, including incurred sample reanalysis (ISR).

Another micro-sampling method is volumetric absorptive micro-sampling (VAMS) by the Mitra® device. This device has a relatively simple collection process that can be performed by patients at home. It absorbs a small (10-30 µL) volume of blood from a finger prick into a tip which then is used to extract the analytes, eliminating the need for the sub-punch from a DBS card and problems of homogeneity of the sample. DBS sampling and VAMS have been used to assist in the diagnosis, investigation and measurement of a wide variety of pathogens, including HIV, HBV, HCV, and inherited metabolic disorders drugs [12-14].

Given the potential application of DBS and VAMS in improving barriers for TDM implementation and the interest in individualised dosing of anticancer therapy, the aim of this systematic review was to identify and describe published studies that used DBS sampling and VAMS for TDM of chemotherapy and targeted therapy agents in the treatment of patients with solid cancers. This review also investigates the agreement between conventional venous blood samples and DBS sampling approaches for TDM of anticancer drugs. We hypothesised VAMS and DBS sampling methods are used in the TDM of anticancer therapy and are feasible, less invasive and as effective as venous sampling methods.

5.3 Methods

A systematic literature search of the electronic databases Web of Science, EMBASE and PUBMED was conducted by two independent reviewers (MS, AM), using PRISMA guidelines [15]. A combination of MeSH terminology associated with the Medline database and relevant keywords were used to capture more studies. Studies were included if they assessed the use of DBS sampling or VAMS in the TDM of chemotherapy or biologic anticancer therapies used in the treatment of solid cancers. Only original articles with available full text were eligible to be included in the review: The key words, "DBS", "dried blood spot", "microsampling", "volumetric absorptive microsampling", "finger prick*", AND "metabolism", "pharmaco*", "TDM", "therapeutic drug monitoring", "drug kinetics" and "drug clearance" were used and then the results were combined (AND) with the search results of "cancer", "chemotherapy", "targeted therapy", "tyrosine kinase inhibitor", "solid tumo*" and "cytotoxic". Results were limited to studies in humans, English and publication dates through to July 2022 (see Table 8 and Figure 13). Studies that investigated anticancer therapy used in the treatment of haematological malignancies and hormonal therapies were excluded. The extracted and tabulated data were reviewed together for consensus on each data field for each study. Disagreements were resolved with discussion by which authors repeated review of the relevant study to reach a consensus.

5.4 Results

Twenty-three studies met the eligibility criteria and were included in the review. Of these, 20 studies described analytical method development, 14 of which also investigated the agreement between concentrations determined in DBS and whole blood samples (Table 8). Three studies (concerning vemurafenib, pazopanib and everolimus) only examined the agreement between concentrations determined in DBS and whole blood samples. DBS sampling was used in the TDM of 10 chemotherapy agents including 5-Fluorouracil, capecitabine, gemcitabine, cyclophosphamide, ifosfamide, etoposide, irinotecan, docetaxel, paclitaxel and mitotane (each 1 study). TDM was also explored for the measurement and comparison of 9 targeted therapy agents in 13 studies: everolimus (3 studies), vemurafenib (2 studies), pazopanib (2 studies), gefitinib, abiraterone (2 studies), palbociclib and ribociclib combined (1 study), adavosertib and imatinib. Studies had a median of 14 participants (range, 6 - 34). All studies used 10 - 50 μL of blood on DBS cards (18 studies) or Mitra VAMS device (3 studies).

Most studies (20/23) stated that the assay validation process was conducted and reported according to the U.S. FDA guidelines.11 Ranges of 85%-115% were considered acceptable limits of accuracy and precision. The lowest limit of quantification (LLOQ) was defined as the lowest concentration that could be measured with a precision within 20%, accuracy between 80% and 120% in all studies. Using Passing-Bablok, Demming regression or Bland Altman analysis, DBS and plasma or venous concentrations showed strong agreement (correlation coefficient, ranged 0.872 to 0.999; see Table 8) for all drugs except mitotane by Friedl et al. (2019). This study developed and validated an HPLC-UV assay to measure mitotane concentrations using a Mitra™ VAMS 20 μL micro-sampler.16 The DBS samples were stable at room temperature and at 2–8 °C for 1 week, but unstable at 37 °C when a

significant amount of analytes were lost likely due to evaporation. Mitotane concentration as measured by plasma sampling and DBS by VAMS were not significantly correlated ($r = 0.87$, $p < 0.0001$ where positive correlation was pre-defined as $r > 0.872$). The authors concluded that VAMS for measurement of mitotane in TDM was neither feasible nor reliable.

5.4.1 Utilisation of methods in actual TDM to guide dosing of anticancer agents

Development of an analytic method to measure drug concentrations in DBS was the aim of the majority of the included studies (20/23). The detected concentration ranges by DBS, however, were only compared with the accepted target concentration ranges (therapeutic ranges) in the studies involving paclitaxel and etoposide [17, 18].

Andriguetti et al. (2018) developed and validated a liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) assay using DBS sampling of paclitaxel [17]. The plasma concentration above a threshold of 0.05 μM ($Tc > 0.05$ μM) considered therapeutic range that would represent the relation between exposure to paclitaxel and clinical response [19, 20]. The developed LC-MS/MS assay was validated for concentration range of 2.5-400 ng/mL which would cover the known therapeutic range. The precision (CV %) and accuracy at various concentration were within acceptable ranges (Table 8). The authors concluded paclitaxel could be accurately measured in DBS which could be used for the TDM of paclitaxel.

Kukec et al. (2016) developed and validated a high performance liquid chromatography-fluorescence (HPLC-FL) assay in DBS sampling to establish TDM for etoposide [18]. The therapeutic range was considered from 2000 to 6000 ng/mL and from 8000 to 14000 ng/mL for trough and peak serum concentrations respectively [21]. The developed method covered a

concentration range of 500–20000 ng/mL with a linear relationship (r^2 = 0.9753). Accuracy of ≥ 96.1% and precision (%CV) of ≤ 10.1% were within the accepted criteria (accuracy: 85%-115%, precision; ≤15%). Etoposide measured in plasma and DBS samples were significantly correlated (r^2 = 0.97; p <0.05). The developed method was reported to be a patient friendly and reliable alternative to conventional plasma methods for TDM of etoposide.

The relationship between concentration ranges and toxicity or efficacy were not investigated in any of the included studies in this review.

5.4.2 Physico-chemical factors impacting concentration results

The majority of studies (19/23) evaluated the haematocrit effect, except for those concerning 5-fluorouracil [22], cyclophosphamide [48], gefitinib [26] and abiraterone [47]. Of the 19 studies, most (17/19) found no significant impact (bias >15%) on the determination of drug concentration in the normal haematocrit ranges (e.g. 29.0% –59.0%). Some studies (e.g. etoposide and pazopanib) [18, 26, 44] used an equation: plasma concentration = DBS concentration/ (1−Haematocrit) to adjust for haematocrit impact and reported an acceptable bias of <15%.

A lower range of haematocrit (<25% and <31%) was reported to cause unacceptable bias in two studies concerning everolimus. Knapen et al. (2018) developed and validated an UPLC-MS/MS assay to measure concentrations of everolimus in DBS samples [23]. The effect of blood haematocrit (20%-50%) on the measured concentration was assessed with unacceptable precision of >15% for haematocrit values of <25%. Similarly Verheijen et al. (2019) used a LC-MS/MS to develop and validate a method to quantify everolimus concentrations by

VAMS sampling [24]. Considerable bias (>15%) was observed for haematocrit ranges from 20 to 31% (haematocrit range assessed: 20-50%).

Half of the studies (11/23) examined other factors impacting concentration results including spot homogeneity or spot volume effect [17, 23, 25, 30, 31, 32, 41, 42, 49, 50] [table 8]. For example, Verheijen et al. (2016) developed and validated a DBS assay using LC–MS/MS to measure the concentration of pazopanib by DBS sampling for TDM.25 Inter-individual results variability were observed that could not be explained by haematocrit effect as the samples were taken from the same patients at the same time. Other factors like spot homogeneity and spot volume could have contributed to the variability. In their study by avoiding the use of very large, very small or irregular spots they demonstrated an acceptable bias of within 3.5% for blood spot homogeneity and an acceptable accuracy of within 9.5% for the effect of blood spot volume.

5.4.3 Application to patient samples

Capillary blood sampling and venous DBS sampling were both used in 14/23 studies [17, 22, 25, 26, 27, 29, 30, 40, 41, 42, 43, 45, 46, 50] (Table 8). In 13/14 studies, the DBS sampling was performed by the research team (physicians, nurses, etc) on the participants; only one study had the DBS performed by participants [26]. Kei Irie et al. (2018) used LC-MS/MS to develop and validate a method to quantify gefitinib in DBS sampling [26]. Self-performed capillary samples from 10 patients with NSCLC receiving gefitinib (daily or every other day) were collected for analysis. Participants obtained capillary samples by puncturing their fingertips with a lancet immediately before gefitinib administration (trough concentration). Venous samples were also collected from these participants within 10 minutes of the DBS samples. Good agreement was observed between the gefitinib concentrations measured by the

DBS method and plasma concentrations (r^2=0.99). The feasibility of performing finger-prick testing by patients was not adequately investigated in the included studies.

5.4.4 Sample preparation time

Most studies reported the analytical run time (14/23) [16, 17, 22, 23, 26, 27, 29, 30, 31, 32, 41, 46, 47, 49] ranging from 2.3 minutes to 8.5 minutes, and sample preparation time (20/23), [16, 17, 18, 22, 23, 24, 26, 27, 29, 30, 31, 32, 40, 41, 42, 46, 47, 48, 49, 50] ranging from 80 to 160 minutes. The actual hands on time however was not clarified in any of the included studies. . For example, Raymundo et al. (2018) developed and validated an LC–MS/MS method to measure docetaxel in DBS samples [27]. The reported total analytical run time was 7 minutes, DBS sample dry time was 3 hours and DBS sample preparation was 75 minutes.

5.5 Discussion

The key findings of our systematic review were that all but one included study showed DBS and VAMS sampling methods were feasible with good correlation with plasma sampling methods as per the FDA guidelines.

To our knowledge, there is one other systematic review summarising the use of DBS sampling to measure the concentration of chemotherapy and targeted anticancer therapy for TDM in routine clinical practice [28]. Lacuzzi et al. (2021) reviewed studies that used DBS to measure anticancer drug concentrations from November 2008 until May 2020. Lacuzzi et al investigated the physico-chemical factors of the drugs and impact on blood distribution, and the influence of haematocrit on DBS concentrations, and the reported approach to normalise DBS concentrations to those measured in plasma. The authors found that DBS sampling could replace standard venous blood or plasma sampling without compromising the outcomes when appropriate conversion methods were used. Key differences between our review and Lucuzzi et al. are the inclusion of anticancer agents used in haematological malignancies (e.g. radotinib) and hormonal anticancer therapies (e.g. estrogen receptor modulators – tamoxifen) and a methodological focus on conversion and normalisation approaches to correlate plasma and DBS concentrations.

The primary objective of most of the included studies (20/23) in this review was to develop and validate DBS assays to implement TDM of anticancer therapy. TDM of anticancer therapy has been logistically difficult to implement in clinical practice using venepuncture as presented in the introduction. DBS, however, can be performed by patients, the samples need no processing on site and are dried, stable and easily transported. This review has highlighted other advantages of DBS for TDM for use in clinical practice including: (i) very small

volumes of blood required for DBS (range, 10 to 50μL) compared with plasma sampling (range, 1-5 mL); (ii) stability of samples ranging from 9 days (at -20 to 45°C for docetaxel) 27 to 16 months (at room temperature for imatinib), adequately covering a typical 2 to 4 weeks stability time needed for its use in guiding dose adjustment in routine clinical care [30]. Ideally for TDM, the sampling and analytic method covers the drug therapeutic range if known to propose meaningful dose adjustments to avoid under-dosing and excess toxicity. The evidence of known or accepted therapeutic ranges only exist for several agents like paclitaxel, etoposide, mitotane, imatinib and pazopanib [3-5]. In our review, two studies (e.g. those of paclitaxel and etoposide) covered the known accepted therapeutic range, however, other studies either did not cover the known therapeutic range or the therapeutic range was unknown.

A challenge of DBS is the haematocrit effect as the predominant source of inter-individual variability. Increased haematocrit reflect an increased blood viscosity which can cause less homogenous spread of blood sample on absorbent paper used for DBS [33]. This may impact the measurement of drug concentration by variation in the location of the punch within the heterogeneous spot and subsequent extraction method. International guidelines recommend evaluation of samples from a central or peripheral punch at low and high concentrations of a given drug at low, medium, and high haematocrit. These conditions then need to be analysed in quintuplicate [34, 35]. Studies that evaluated the haematocrit effect in this review (19/23) all adhered to the FDA guidelines, and the majority of those (17/19) found normal haematocrit ranges (e.g. 29.0–59.0%) had no significant impact on accuracy and precision of the developed method. A low haematocrit (<29%) interfered with the accuracy and precision of the developed methods (imatinib). The validation of low haematocrit range (<29%) is very

important as many patients in oncology may have a haematocrit of less than 29% (anaemia) from their cancer and/or systemic anticancer therapy.

VAMS has been introduced as an alternative DBS sampling technique to overcome the challenges of haematocrit effect and punch area bias [36]. Previous studies suggested VAMS could reduce or, for selected analytes, eliminate the influence of haematocrit [37]. Verheijen et al. (2019), however, demonstrated in their study of DBS of everolimus that the VAMS sampling method was strongly influenced by haematocrit in a concentration dependent manner and that VAMS was not superior to DBS methods [24]. VAMS method as a solution to the haematocrit impact remains to be determined in future studies of other drugs used in oncology. The identified articles in this review only used DBS and VAMS, but no other available techniques and therefore only these two techniques were included in the review.

There are several issues with the studies included in this review. Firstly, the research setting. All studies performed micro-sampling in the research environment but not in the at-home environment where micro-sampling occurs for TDM in routine clinical practice. The DBS cards or Mitra® devices were prepared by the research nurse or study staff rather than by participants, and hence do not reflect at-home sampling where patients perform these tasks themselves. Previous studies of DBS use for antiretroviral and immunosuppressive drugs have shown that 87.5% to 98% of the samples obtained by patients were suitable for analysis [38, 39] suggesting that preparation of DBS cards by patients is feasible. Another limitation of the included studies is that lower levels of haematocrit e.g. <30%, were not consistently assessed for its impact on the determination of drug concentrations. Other issues of the included studies consist of publication bias given that all but one included study had positive

results and selection bias in that only certain chemotherapy and targeted therapy agents were investigated in the published studies.

Limitations of this systematic review include the heterogeneity in the methods across studies, different DBS methods e.g. Mitra® and absorbent paper, the small number of studies for each drug included in the review (mostly single study), the small number of patients or samples in the included studies and the variable methods used to adjust for the haematocrit effect. These limitations, particularly the paucity of studies on individual drugs, make it difficult to draw firm conclusions on applicability of the broad methods to individual drugs.

5.6 Conclusion

The reviewed articles mostly support the use of developed micro-sampling methods for the measurement of various chemotherapy and targeted therapy agents using the preselected equivalent concentration range. Given the feasibility and advantage of DBS over venipuncture, further studies are warranted to evaluate the clinical utility of micro-sampling prepared by patients themselves using quality of life measures and comparing clinical outcomes. Clinical research data showing comparative benefit of DBS would ultimately improve the uptake of TDM, dosing of anticancer therapy and patient care.

Table 8. TDM of anticancer agents in patients with solid tumours using microsampling (DBS or VAMS)

Author, Year, Citation	Analyte (name of the drug or metabolite)	Approach/Device (DBS, Capillary sampling) – try and describe this in general terms/sample volume/storage	Evaluation study design (in vitro, ex vivo, in vivo human study) you could add sample size	Working concentration range	Accuracy and precision (compared to concentrations venous sampling)	Influential factors (haematocrit, serum/plasma etc)	Sample and analyte stability (was this assessed)	Comments
Singhal et al 2015[19]	Capecitabine	Venous Blood (plasma kept at -20°C). 10 µL of spiked concentration onto absorbent paper. Dry for 2h at room temperature. Analysed using LC–MS/MS.	1 Ex vivo sample. Design: Extracted blank plasma >ULOQ sample > extracted blank plasma > LLOQ sample.	10-10000 ng/mL	Assessed inter- and intra-assay precision (within 5%), accuracy (within 6%) and linearity r^2 = 0.9995.	Haematocrit 24% and 45% - no impact on accuracy and precision	Bench top stability 8h. Processed sample stability, at 2-8 °C, 69h. Stability under incubation at 50 °C up to 8d. Long-term stability at room temperature 60d.	**Advantage**s: Long term stability Low resource (absorbent paper, not a device) **Disadvantages:** Used venous blood not capillary blood. No comparison of venous and DBS methods. Could be used in TDM

Radovanovic et al 2021[20]	Capecitabine/5-Fluorouracil	Venous capillary Blood, one drop, into MITRA™ (VAMS) devices. Dry for 2h at room temperature. Analysed using LC–MS/MS.	Ex vivo samples of 10 patients receiving capecitabine and 20 patients receiving 5-FU. Aliquots were removed at 4 time points (hour 0,1,2,4)	5-FU:4.24–47.9 mg/L Capecitabine: 11–7712 µg/	The intra and inter-day precision within 8.1% and 13.3 % respectively. Accuracy (within 14%) for all analytes and linearity r2 > 0.990	n/a	Samples were stable up to 9 months at room temperature, 2 years at 30 ℃ and 3 days at 50 ℃.	**Advantage**s: Used capillary samples **Disadvantages:** Needs resources (devices). Could be used in TDM
Kumar et al 2015[21]	Gemcitabine	Venous blood, plasma kept at -20°C. 50 µl of plasma and venous spiked concentration (without THU) spotted onto DBS cards. Dried for 2h at room temperature. Analysed by LC–MS/MS	Ex vivo samples of 6 healthy volunteers. Spiked known concentration at (HQC Level) separately into whole human blood in presence and absence of THU.	5-5000 ng/mL	Demonstrated inter- and intra-assay precision (within 6%), accuracy (within 15%) and linearity r^2 = >0.99	Haematocrit value of 43% (examined 25%-62%) showed a negligible effect on accuracy and precision.	Auto sampler stability 2-8°C: 72h Re-injection 2-8°C: 62h Long term stability: 90d	**Advantage**s: Long term stability Low resource (absorbent paper, not a device) **Disadvantages:** Used venous blood not capillary blood. No comparison of venous and

			Aliquots were removed at multiple time points.					DBS methods. Potential to be used in TDM
Harahap et al 2020[22]	Cyclophospham ide	Venous blood containing analytes spotted (30 µl) on DBS paper left to dry at room temperature for 3h. Analysed by UPLC–MS/MS	Blood samples of 17 patients were collected at 2 and 4 h after drug administratio n.	50–30000 ng/mL	Assessed inter- and intra-assay precision (within 12%) and accuracy (within 20%) and linearity r^2 = >0.99	Haematocri t and plasma effect were not assessed.	Short-term stability (25 °C):24h Long-term stability (-80 °C):14d	**Advantages:** Short extraction time Moderate term stability Low resource (absorbent paper) **Disadvantag es:** Used venous blood not capillary blood. No comparison of venous and DBS methods. Potential for use in TDM

Torres et al 2015 [23]	Ifosfamide	Venous blood (40 µL) and capillary blood on absorbent paper. Dried 6h then stored at -80°C. Analysis by UPLC-MS/MS	Capillary blood samples (28) from 14 patients were taken 12 and 24h after infusion. Median concentration values were compared.	100–10000 ng/mL	Intra-day and inter-day assay at 30% haematocrit, accuracy: within 5% and precision (% CV): within 11% Linearity: $r^2 = 0.97$	Haematocrit between 30-45% (examined 20-50%) had no impact on accuracy and precision.	Stability at 5°C: up to 24h. At room temperature and 40°C: 28d At -80°C: 52d	**Advantages:** Used capillary blood Long term stability **Disadvantages:** No comparison of venous and DBS methods. Requires storage at -80°C before analysis. Potential for use in TDM
Kukec et al 2016 [24]	Etoposide	20 µL of venous blood on absorbent paper. Dried for 1h. Stored at room temperature. Analysed by HPLC-FL	216 samples from 6 patients were collected during 4 chemotherapy cycles on days 1, 2 and 3 of each cycle, 3, 6	500–20000 ng/mL	Intra- and inter-day precision (% CV): within 10.1%. Accuracy: within 3.9% Linearity: $r^2 = 0.9753$.	Haematocrit effect assessed at 30%, 40% and 60%: no impact (deviation <15%).	Stability: At 5 °C: 24h At room temperature and 40 °C: 28d	**Advantage**s: Short extraction time Long term stability Low resource (absorbent paper, not a device)

		Plasma samples were stored at -80°C after centrifugation until analysis.	and 24h after etoposide administration.		Plasma concentration = DBS concentration /1-haematocrit			**Disadvantages:** Used venous blood not capillary blood**.** Did not use capillary blood. No comparison of venous and DBS methods. Could be used in TDM
Hahn et al 2018 [25]	Irinotecan	50 µL of capillary blood and venous blood on absorbent paper. Dried at room temperature for 3h. 50 µL of plasma analysed by HPLC-FL	Blood samples of 19 patients at 1h and 24h after infusion.	10 to 3000 ng/mL	Accuracy: within ≤5.74% Intra- and inter-assay precision (%CV): within ≤4.72% The correlation between DBS and plasma	Haematocrit effect assessed at 25%, 35% and 50%: no impact (deviation <7.5%)	Stability: At room temperature and 42 °C: 14 d	**Advantage**s: Capillary (DBS) and venous methods were compared. Long term stability Low resource (absorbent paper, not a device)

					samples at 1h post-infusion: r = 0.949			**No disadvantages.** Potential for use in TDM
Raymundo et al 2018[26]	Docetaxel	Spiked venous blood (25 µL) and 1 drop of capillary blood on absorbent cards. Dried at room temperature within 3–24h. Analysed by LC-MS/MS	Venous and capillary samples from 31 patients. Venous and DBS methods compared using Passing-Bablok regression analysis.	50 to 3000 ng/mL	Precision (%CV): < 9.8% Accuracy: within 3% r = 93% (High correlation between DBS-derived estimated plasma concentrations and plasma samples, p<0.01).	Haematocrit effect assessed at 30%, 45% and 60%: no impact (deviation <12.1% for 60% and <10.1% for 30%)	Stability: At all temperatures: 9d At 45°C: up to 11d At room temperature: up to 18d	**Advantage**s: Long term stability Low resource (absorbent paper) **No disadvantages.** Potential for use in TDM
Andriguetti et al 2018[27]	Paclitaxel	Non-spiked venous blood (50 µL) and 1 drop of capillary blood on absorbent	Venous and capillary samples from 34 patients, collected between 18h	2.5-400 ng/mL	Intra- and inter-assay precision (% CV): within 6.89% and 8.74%, and	Spotted volume influence: accuracy within 12.7%	Stability: At 25°C and 45°C: 21d	**Advantage**s: Long term stability Low resource (absorbent paper)

		papers. Dried for 3h. Analysis with LC-MS/MS	and 30h after infusion. Venous and DBS methods compared using Passing-Bablok analysis and Bland-Altman comparison.		Accuracy within 9.92%. r = 0.986 (high correlation between DBS and venous blood)	Haematocrit effect between 25-46% assessed: accuracy within 14.8%		Assessed capillary bloods on DBS **No disadvantages.** Potential for use in TDM
Bettina Friedl et al 2019[28]	Mitotane	Spiked (20 μL) whole blood samples kept at 2-8°C into MITRA™ (VAMS) devices. Analysed by HPLC-UV.	51 samples from 6 patients. Venous and VAMS methods compared using Passing-Bablok analysis and Bland-Altman comparison.	1 to 50 mg/L	A nonlinear model may be necessary to relate Mitra™ and plasma concentrations. Poor correlation between mitotane concentration in DBS and venous	Haematocrit effect of adjusted levels 30-55% assessed: accuracy within 13%	Stability: At room temperature: unstable at 7d. At 2-8°C: stable at 7d At -80°C: 6m	**Disadvantages:** Used venous blood not capillary blood. Needs resources (devices). Unstable at room temperature.

			Poor agreement defined: r<0.90		plasma (p<0.0001) r = 0.87 (Concordance correlation coefficient: 0.60)			Should not be used in TDM
Yang Xu et al 2012[29]	Adavosertib	Spiked venous blood and plasma (40 µL) on absorbent papers. Dried overnight. Analysed with HPLC-MS/MS.	Samples from 12 patients on day 1 pre-dose, day 3 pre-dose, 3 and 8h post-dose. Comparison of assay performance between DBS and plasma methods.	2 to 1000 ng/mL	Intra- and inter-day precision (% CV): within 7.2%, and Accuracy within 14%. Mean DBS to plasma ratio of 1.29 indicating good agreement.	Spot size and punch location effect on accuracy: within 5.8%. Haematocrit effect between 16-85% assessed: accuracy within 15%	Stability: At room temperature: 14m At 40 °C: 8d At −20 °C : 6m	**Advantage**s: Long term stability Low resource (absorbent paper, not a device) **Disadvantages:** Used venous blood not capillary blood. Not a commonly used anti-cancer therapy Could be used in TDM

Nijenhuis et al 2014[30]	Vemurafenib	4 drops of capillary bloods and 10 µL of spiked venous blood on absorbent paper. Dried for 3h at room temperature. Analysed by HPLC-MS/MS.	Capillary samples from 8 patients. .	1000 to 100000 ng/mL	Assessed Intra- and inter-assay accuracy: within 13.6% and precision (%CV) within 6.5%, linearity: r2 = 0.997	-Blood spreadability impact: bias within 9.4% and precision within 4.6% DBS volume impact: finger prick volume within 15% Haematocrit 24 and 45 %- The impact on accuracy (<11.4%) and precision (<4.1%)	Stability: At room temperature: 163d At −20 °C : 4m	**Advantage**s: Long term stability. Low resource (absorbent paper, not a device). **Disadvantages:** No comparison of venous and DBS methods. Could be used in TDM
Nijenhuis et al 2016[31]	Vemurafenib	Whole-blood samples centrifuged for 10 minutes at 1700g, stored at −20°C	43 capillary samples and plasma samples from 8 patients.	1000 to 100000 ng/mL	DBS concentrations highly correlated with plasma concentrations (r = 0.964)	Haematocrit effect between 27-49% assessed: accuracy	Stability: On absorbent cards more than 827d	**Advantage**s: Long term stability Low resource (absorbent paper, not a device)

		pending analysis. 4 drops of capillary bloods on absorbent paper. Dried at room temperature for 3h. Analysed by HPLC-MS/MS.	Bland-Altman and Weighted Deming regression analysis used.		but consistently lower than the correspondin g plasma concentration with a slope of 0.64 (95%CI, 0.60 to 0.68), (vemurafenib in plasma = vemurafenib in DBS/0.64)	within 11.4%		Could be used in TDM
Verheijen et al 2016[32]	Pazopanib	Capillary samples and 15 µL of spiked venous blood on absorbent papers. Dried at room temperature for 3 hours. Analysed using LC–MS/MS	329 samples from 30 patients. Venous (plasma) and DBS methods compared using Weighted Deming fit and Bland-	1000–50000 ng/mL	Inter- and intra-run precision (CV) ≤8.6% r^2 = 0.872 (good correlation between DBS and plasma concentration s)	Blood spot homogeneit y: bias within 3.5% Effect of blood spot volume: accuracy within 9.5%	Stability: At room temperature (DBS): 398d At 2–8°C : at least 168d	**Advantage**s: Assessed capillary DBS method and compared with plasma method. Long term stability Low resource (absorbent paper, not a device)

			Altman comparison.		(Slope: 0.709, Intercept: -0.182)	Effect of blood haematocrit (35%-50%): accuracy within 14.2%		Could be used in TDM
de Wit et al 2015[33]	Pazopanib	15 µL of venous blood and capillary blood on absorbent papers. Dried for 2 hours at room temperature. Analysed by HPLC-MS/MS.	12 patients on day 14 of treatment: venepuncture samples at pre-dose and 1, 2, 3, 4, 6, 8, 10, and 24 hours and capillary samples were collected pre-dose, and 3 and 8 hours after dose. Agreement between DBS and plasma methods for TDM examined	100–50000 ng/mL	Within- and between-run precision: within 14.7%. Accuracy within 5.5%. Mean ratio of calculated to measured concentrations was 0.94 (95% CI, 0.65–1.23). 92.6% (88/95) of the data points within the clinical acceptance limits.	-Effect of blood haematocrit (20%-65%): bias within 12.6%	Stability: at room temperature (DBS): 75d	**Advantage**s: Compared capillary DBS method and plasma method. Long term stability. Low resource (absorbent paper, not a device). Could be used in TDM

			using Bland-Altman analysis and Passing-Bablok analysis.		A constant bias between plasma and DBS. (intercept estimate, 4.68; 95% CI, 6.48 to 2.47), (slope estimate, 0.63; 95% CI, 0.57 to 0.68)			
Lotte M. Knapen et al 2018[34]	Everolimus	Spiked 30 µL of venous blood on absorbent papers. Dried overnight at room temperature then kept at 2-8°C. Analysed using UPLC-MS/MS.	Venous blood samples from 6 healthy volunteers.	3-75 ng/mL	Intra- and inter-assay precision (%CV): within10.7% accuracy within 4.4% (haematocrit values of ≥ 0.25 L/L)	Assessed the effect of blood haematocrit (20%-50%): precision within 14.8% but haematocrit <25% not accurate-bias >15% Spot volume effect: precision	Stability: At 15-25°C: 17d At 2–8°C : 80d	**Advantage**s: Long term stability Low resource (absorbent paper, not a device) **Disadvantages:** No comparison of venous and DBS methods. Used venous blood not

						within 3.5%		capillary blood. Potential for use in TDM
Willemsen et al 2018[35]	Everolimus	Two drops of capillary blood on absorbent cards. Analysed using UPLC MS/MS.	Whole blood (plasma, DBS) and finger prick samples from 20 patients on day 7 or after. Examined agreement between DBS and whole blood. Bland-Altman analysis and Passing-Bablok analysis	3.7 to 33.3 ng/mL	Mean ratio of everolimus in WB to DBS concentrations was 0.90 (95% LoA 0.71–1.08). r = 0.97 and r^2 = 0.95 No constant bias (intercept 0.02; 95% CI 0.93–1.35) and a small proportional bias (slope 0.89; 95% CI 0.76–0.99)	-Assessed the effect of blood haematocrit (25%-45%): assumed no impact for >25%	n/a	**Advantage**s: Compared venous and DBS (capillary) methods Low resource (absorbent paper, not a device) **Disadvantages:** Unreported stability period. Potential for use in TDM

Verheijen et al 2019[36]	Everolimus	20 µL of whole blood in Mitra devices. Dried at room temperature and analysed by LC-MS/MS	Whole blood and VAMS samples from 10 Patients collected to compare concentrations obtained by VAMS and DBS.	2.50 to 100 ng/mL	Intra-run precision (%CV): within 14.6% Intra-run accuracy: within 11.1% r > 0.99 Advantage of VAMS over DBS was not demonstrated.	Haematocrit range (30-50%) assessed, a considerable biases from -20 to 31% were observed.	Stability: At 2–8°C :48h At room temperature:36 2d	**No advantages.** **Disadvantages:** Used venous blood not capillary blood. Needs resources (devices) Significant impact of low haematocrit Should not be used in TDM
Kei Irie et al 2018[37]	Gefitinib	One drop of capillary blood (10 µL) and venous blood on absorbent papers. Dried at room temperature for 2h. Analysed using LC-MS/MS	Pre-dose capillary and venous samples of 10 patients. Venous and DBS methods were compared using Bland-Altman analysis and	37.5 to 2400 ng/mL	Intra- and inter-day precision and accuracy of all samples were within 15%. linearity r^2= 0.99	Haematocrit range (31-43%) Impact not assessed.	Stability: at 40°C : 24h at room temperature or -20°C: 5m	**Advantage**s: Compared venous and DBS (capillary) methods Patients self-performed sampling Low resource (absorbent

			Passing-Bablok analysis.					paper, not a device) **Disadvantages:** Unreported haematocrit impact Potential for use in TDM
Valentina Iacuzzi et al 2019[38]	Imatinib	20 µL of venous blood and 2 drops of capillary blood on absorbent paper. Dried for 3h at room temperature. Analysed using LC-MS/MS.	Capillary and venous trough samples of 26 patient (before drug administration). Plasma, venous DBS and finger prick DBS methods compared using Bland-Altman analysis and	50–7500 ng/mL	Intra- and inter-day precision (%CV): within 5.6% Intra- and inter-day accuracy within 11.1%, linearity: r^2 = 0.99	Assessed the effect of haematocrit (29%-59%): accuracy and precision within 4.8% Effect of blood spot volume: Accuracy and precision	Stability: At 4°C or -80°C : 24h At room temperature:16 m	**Advantage**s: Long term stability Low resource (absorbent paper) **No disadvantages.** Potential for use in TDM

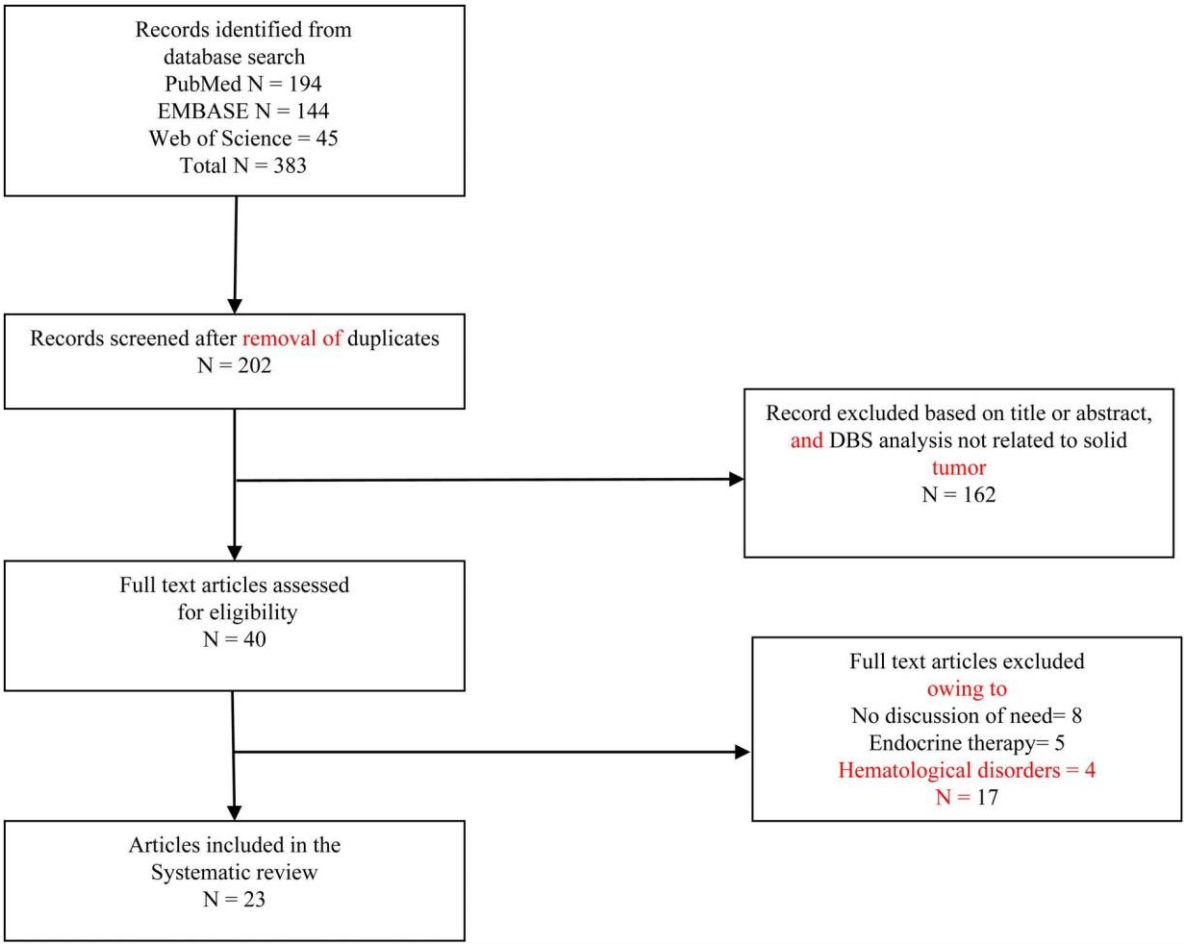
			Passing-Bablok analysis			within 10.1%		
Atul Bhatnagar et al 2019[39]	Abiraterone,	Spotted 15 µL of plasma on absorbent cards. Dried for 2 hours at room temperature. Analysed using UPLC-MS/MS	Venous blood samples from 22 patients. Venous DBS and plasma methods compared using Bland-Altman analysis, Passing-Bablok analysis, Pearson's correlation coefficient and t-test nonparametric	0.132–196.0 ng/mL	Intra- and inter-day accuracy and precision (%CV): within 11.3%, linearity: r = 0.99.	Did not assess the effect of haematocrit or spot volume	Stability: At room temperature: 30d	**Advantage**s: Long term stability Low resource (absorbent paper, not a device) **Disadvantages:** Used venous blood not capillary blood. The haematocrit effect was not reported. Potential for use in TDM

Dillenburg Weiss et al 2021[40]	Abiraterone	Spotted 18 µL of whole blood and capillary samples onto absorbent papers. Dried for 3h at room temperature. Analysed using UPLC-MS/MS.	Capillary, venous and plasma samples from 10 patients. Plasma and finger prick DBS methods compared using Bland-Altman analysis and Passing-Bablok analysis	1−400 ng/mL	Between-run and within-run Precision (%CV): within 9.72% Accuracy: within 7% Linearity r^2=1.0 Concentrations were overestimated using the DBS approach (15%).	Assessed the effect of haematocrit (28%-44%): no significant impact observed. Spot volume effect: precision within 12.1%	Stability: At 2–8°C & room temperature: only 7d	**Advantage**s: Assessed capillary DBS method and compared with plasma. Low resource (absorbent paper, not a device) **Disadvantages:** Short term stability Could be used in TDM
Poetto AS et al 2021[41]	Palbociclib, ribociclib	42 paired venous and finger prick samples (plasma versus DBS) from 18 patients collected. Spotted 20 µL of spiked blood and capillary	38 Capillary blood samples collected by finger prick. Plasma and finger prick DBS methods compared	1 to 250 ng/mL for palbociclib, 40 to 10000 ng/mL for ribociclib	Intra- and inter-day precision (CV (%)) within 11.4% and intra- and inter-day accuracy within10%. Linearity: r^2=0.9979	Assessed the effect of haematocrit (25%-49%): precision within 14.8% Spot size, sample homogeneity precision	Stability: At room temperature :2.5 months	**Advantages:** Assessed capillary DBS method and compared with plasma. Low resource (absorbent paper, not a device)

		samples onto absorbent papers.				within: 15%		Could be used in TDM

DBS; dried blood spot, VAMS; volumetric absorptive micro-sampling , LC–MS/MS; liquid chromatography–mass spectrometry, ULOQ; upper limit of quantification, LLOQ; lower limit of quantification, TDM; therapeutic drug monitoring , THU; tetra-hydro-uridine, HQC; higher quality control, UPLC–MS/MS; ultra-performance liquid chromatography-tandem mass spectrometry, HPLC-FL; high-performance liquid chromatography- fluorescence, LoA; limits of agreement

Figure 13. PRISMA Flow Diagram 2.



5.7 References

1. Kim HY, Martin JH, McLachlan AJ, et al. Precision dosing of targeted anticancer drugs—challenges in the real world. *Transl Cancer Res.* 2017; 6:1500–1511.

2. Gurney H. Dose calculation of anticancer drugs: a review of the current practice and introduction of an alternative. *J Clin Oncol.* 1996;14:2590–2611.

3. Menz BD, Stocker SL, Verougstraete N, et al. Barriers and opportunities for the clinical implementation of therapeutic drug monitoring in oncology. *Br J Clin Pharmacol.* 2021;87:227–236.

4. Mizoguchi K, Nakamura Y, Sano K, et al. Pharmacokinetic parameters of gefitinib predict efficacy and toxicity in patients with advanced non-small cell lung cancer harboring EGFR mutations. *Cancer Chemother Pharmacol.* 2016;78:377–382.

5. Teng JFT, Mabasa VH, Ensom MHH. The role of therapeutic drug monitoring of imatinib in patients with chronic myeloid leukemia and metastatic or unresectable gastrointestinal stromal tumors. *Ther Drug Monit.* 2012;34:85–97.

6. Moeung S, Chevreau C, Broutin S, et al. Therapeutic drug monitoring of carboplatin in high-dose protocol (TI-CE) for advanced germ cell tumors: pharmacokinetic results of a phase II multicenter study. *Clin Cancer Res.* 2017;23:7171–7179.

7. Pignon T, Lacarelle B, Duffaud F, et al. Dosage adjustment of high-dose methotrexate using Bayesian estimation: a comparative study of two different concentrations at the end of 8-h infusions. *Ther Drug Monit.* 1995;17:471–478.

8. Bartelink IH, Bredius RG, Ververs TT, et al. Once-daily intravenous busulfan with therapeutic drug monitoring compared to conventional oral busulfan improves survival and engraftment in

children undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2008;14:88–98.

9. Mauclere-Denost S, Leboulleux S, Borget I, et al. High-dose mitotane strategy in adrenocortical carcinoma: prospective analysis of plasma mitotane measurement during the first 3 months of follow-up. *Eur J Endocrinol.* 2012;166:261–268.

10. Westerdijk K, Desar IM, Steeghs N, et al. Imatinib, sunitinib and pazopanib: from flat-fixed dosing towards a pharmacokinetically guided personalized dose. *Br J Clin Pharmacol*. 2020;86:258–273.

11. Paci A, Veal G, Bardin C, et al. Review of therapeutic drug monitoring of anticancer drugs part 1-cytotoxics. *Eur J Cancer.* 2014;50:2010–2019.

12. Lei BUW, Prow TW. A review of microsampling techniques and their social impact. *Biomed Microdevices*. 2019;21:81.

13. Wilhelm AJ, den Burger JCG, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clin Pharmacokinet.* 2014;53:961–973.

14. Guidance for industry, bioanalytical method validation. Food and drug administration, US department of health and human services web site.

https://www.fda.gov/regulatory-information/search-fda-guidancedocuments/bioanalytical-method-validation-guidance-industry.Published 2018. Accessed May 8, 2022.

15. Lange B, Roberts T, Cohn J, et al. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples - a systematic review and meta-analysis. *BMC Infect Dis.* 2017;17:693.

16. Smit PW, Sollis KA, Fiscus S, et al. Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. *PLoS One.* 2014;9:e86461.

17. Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. *Int J Neonatal Screen*. 2020;6:26.

18. Moher D, Liberati A, Tetzlaff J, et al., GroupPRISMA Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151:264–269.

19. Singhal P, Shah P, Shah J, et al. Determination of capecitabine-an anticancer drug in dried blood spot by LC-ESI-MS/MS. *Int J Pharm Pharm Sci.* 2015;7:238–245.

20. Radovanovic M, Schneider JJ, Shafiei M, et al. Measurement of 5-fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. *J Chromatogr B.* 2022;1188:123075.

21. Kumar DP, Palavan C, Jvln SR. Development and validation of a dried blood spot LC-MS/MS assay to quantify gemcitabine in human whole blood: a comparison with and without cytidine deaminase inhibitor. *Int J Pharm Pharm Sci.* 2015;7:75–81.

22. Harahap Y, Gustiyanti N, Sunanto AE, et al. Method development on analysis cyclophosphamide and 4-hydroxycyclophophamide in dried blood spot and its application in breast cancer patients. *Int J Appl Pharm.* 2020;12:119–125.

23. Torres LM, Rivera-Espinosa L, Chávez-Pacheco JL, et al. A new method to quantify ifosfamide blood levels using dried blood spots and UPLCMS/MS in paediatric patients with embryonic solid tumours. *PLoS One.* 2015;10:e0143421.

24. Rezonja Kukec R, Grabnar I, Mrhar A, et al. A simple dried blood spot method for clinical pharmacological analyses of etoposide in cancer patients using liquid chromatography and fluorescence detection. *Clinica Chim Acta.* 2016;452:99–105.

25. Hahn RZ, Arnhold PC, Andriguetti NB, et al. Determination of irinotecan and its metabolite SN-38 in dried blood spots using high performance liquid-chromatography with fluorescence detection. *J Pharm Biomed Anal*. 2018;150:51–58.

26. Raymundo S, Muller VV, Andriguetti NB, et al. Determination of docetaxel in dried blood spots by LC-MS/MS: method development, validation and clinical application. *J Pharm Biomed Anal*. 2018;157:84–91.

27. Andriguetti NB, Hahn RZ, Lizot LF, et al. Analytical and clinical validation of a dried blood spot assay for the determination of paclitaxel using high-performance liquid chromatography-tandem mass spectrometry. *Clin Biochem.* 2018;54:123–130.

28. Friedl B, Kurlbaum M, Kroiss M, et al. A method for the minimally invasive drug monitoring of mitotane by means of volumetric absorptive microsampling for a home-based therapeutic drug monitoring. *Anal Bioanal Chem*. 2019;411:3951–3962.

29. Xu Y, Fang W, Zeng W, et al. Evaluation of dried blood spot (DBS) technology versus plasma analysis for the determination of MK-1775 by HILIC-MS/MS in support of clinical studies. *Anal Bioanal Chem*. 2012; 404:3037–3048.

30. Nijenhuis CM, Rosing H, Schellens JH, et al. Quantifying vemurafenib in dried blood spots using high-performance LC-MS/MS. *Bioanalysis.* 2014;6:3215–3224.

31. Nijenhuis CM, Huitema AD, Marchetti S, et al. The use of dried blood spots for pharmacokinetic monitoring of vemurafenib treatment in melanoma patients. *J Clin Pharmacol*. 2016;56:1307–1312.

32. Verheijen RB, Bins S, Thijssen B, et al. Development and clinical validation of an LC-MS/MS method for the quantification of pazopanib in DBS. *Bioanalysis.* 2016;8:123–134.

33. de Wit D, den Hartigh J, Gelderblom H, et al. Dried blood spot analysis for therapeutic drug monitoring of pazopanib. *J Clin Pharmacol.* 2015; 55:1344–1350.

34. Knapen LM, Beer Yd, Brüggemann RJM, et al. Development and validation of an analytical method using UPLC-MS/MS to quantify everolimus in dried blood spots in the oncology setting. *J Pharm Biomed Anal.* 2018;149:106–113.

35. Willemsen AECAB, Knapen LM, de Beer YM, et al. Clinical validation study of dried blood spot for determining everolimus concentration in patients with cancer. *Eur J Clin Pharmacol*. 2018;74:465–471.

36. Verheijen RB, Thijssen B, Atrafi F, et al. Validation and clinical application of an LC-MS/MS method for the quantification of everolimus using volumetric absorptive microsampling. *J Chromatogr B*. 2019; 1104:234–239.

37. Irie K, Shobu S, Hiratsuji S, et al. Development and validation of a method for gefitinib quantification in dried blood spots using liquid chromatography-tandem mass spectrometry: application to finger-prick clinical blood samples of patients with non-small cell lung cancer. *J Chromatogr B*. 2018;1087-1088:1–5.

38. Iacuzzi V, Posocco B, Zanchetta M, et al. Development and validation of LC-MS/MS method for imatinib and norimatinib monitoring by fingerprick DBS in gastrointestinal stromal tumor patients. *PLoS One.* 2019; 14:e0225225.

39. Bhatnagar A, McKay MJ, Thaysen-Andersen M, et al. Bioanalytical evaluation of dried plasma spots for monitoring of abiraterone and Δ(4)-abiraterone from cancer patients. *J Chromatogr B*. 2019;1126-1127:121741.

40. Dillenburg Weiss TL, Gössling G, Venzon Antunes M, et al. Evaluation of dried blood spots as an alternative matrix for therapeutic drug monitoring of abiraterone and delta(4)-abiraterone in prostate cancer patients. *J Pharm Biomed Anal*. 2021;195:113861.

41. Poetto AS, Posocco B, Gagno S, et al. A new dried blood spot LC-MS/ MS method for therapeutic drug monitoring of palbociclib, ribociclib, and letrozole in patients with cancer. *J Chromatogr B.* 2021;1185:122985.

42. Krens SD, McLeod HL, Hertz DL. Pharmacogenetics, enzyme probes and therapeutic drug monitoring as potential tools for individualizing taxane therapy. *Pharmacogenomics.* 2013;14:555–574.

43. Joerger M, Kraff S, Huitema ADR, et al. Evaluation of a pharmacology driven dosing algorithm of 3-weekly paclitaxel using therapeutic drug monitoring: a pharmacokinetic-pharmacodynamic simulation study. *Clin Pharmacokinet*. 2012;51:607–617.

44. Moffat AC, Osselton MD, Widdop B, et al. Clarke's analysis of drugs and poisons. Vol 3. London: Pharmaceutical press; 2011. https://www. worldcat.org/title/clarkes-analysis-of-drugs-and-poisons-inpharmaceuticals-body-fluids-and-postmortem-material/oclc/733491270.

Accessed May 8, 2022. Published.

45. Li W, Tse FLS. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. *Biomed Chromatogr.* 2010;24:49–65.

46. Iacuzzi V, Posocco B, Zanchetta M, et al. Dried blood spot technique applied in therapeutic drug monitoring of anticancer drugs: a review on conversion methods to correlate plasma and dried blood spot concentrations. *Pharm Res.* 2021;38:759–778.

47. Denniff P, Spooner N. The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs. *Bioanalysis.* 2010;2:1385–1395.

48. Capiau S, Veenhof H, Koster RA, et al. Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. *Ther Drug Monit*. 2019;41:409–430.

49. Cobb Z, de Vries R, Spooner N, et al. In-depth study of homogeneity in DBS using two different techniques: results from the EBF DBS microsampling consortium. *Bioanalysis.* 2013;5:2161–2169.

50. Denniff P, Spooner N. Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis. *Anal Chem.* 2014;86:8489–8495.

51. De Kesel PMM, Lambert WE, Stove CP. Does volumetric absorptive microsampling eliminate the hematocrit bias for caffeine and paraxanthine in dried blood samples? A comparative study. *Analytica Chim Acta.* 2015;881:65–73.

52. Cheung CY, van der Heijden J, Hoogtanders K, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. *Transpl Int*. 2008;21:140–145.

53. Kromdijk W, Mulder JW, Smit PM, et al. Therapeutic drug monitoring of antiretroviral drugs at home using dried blood spots: a proof-of-concept study. *Antivir Ther.* 2012;18:821–825.

Chapter 6: Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A pilot study

6.1 Overview

This chapter is the manuscript of a prospective observational study (pilot study) aimed to determine the pharmacokinetic (PK) of capecitabine in a real world population of older and younger adults cancers and to explore the correlation between PK of capecitabine and chemotherapy-related toxicity and geriatric assessment domains. Older adults, compared to younger adults, having capecitabine chemotherapy at the standard dose had significantly increased exposure to 5-fluorouracil 5-(FU), but not to the other metabolites of capecitabine. The findings of this study suggests that the increased toxicity in older adults on capecitabine might be due to higher exposure to 5-FU and warrants further study.

This manuscript is accepted for publication in the Cancer Chemotherapy and Pharmacology journal and quoted verbatim. Formatting has been updated for consistency across the thesis.

6.1.1 Contribution of authors

Mohsen Shafiei developed the research proposal and research methods, recruited the patients, collected the specimens, performed the data analysis, interpreted the findings, and drafted and revised the manuscript.

Peter Galettis performed specimen analysis to determine pharmacokinetic data and contributed to the revision of the manuscript.

Stephanie Reuter performed the pharmacokinetic data analysis and their interpretation and revised the manuscript.

Philip Beale contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Jennifer Martin contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Andrew McLachlan contributed to the research proposal contributed to the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Prunella Blinman contributed to the research proposal and the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Abstract

Background:

Capecitabine is an oral chemotherapy prodrug of 5-fluorouracil (5-FU) with unpredictable toxicity, especially in older adults. The aim of this study was to evaluate the pharmacokinetics (PK) of capecitabine and its metabolites in younger adults (<70 years) and older adults (≥70 years) receiving capecitabine for solid cancer.

Methods:

Eligible participants receiving capecitabine had 2 venous samples collected on day 14 of cycle 1 and cycle 2 of their treatment. Capecitabine and metabolite concentrations were determined using liquid chromatography with tandem mass spectrometry. A Bayesian estimation approach was used to generate individual estimates of PK parameters for 5-FU. A linear mixed-effect analysis of variance (ANOVA) model was used to compare dose-normalised log-transformed PK parameters between age groups. Correlations were determined by linear regression and logistic regression analyses.

Results:

Of the total 26 participants, 58% were male with a median age of 67 years (range, 37-85) with 54% aged <70 years and 46% aged ≥70 years. Participants aged ≥70 years, compared to those aged <70 years, had a greater 5-FU exposure based on area under the concentration-time curve (AUC) of 17% (90% CI: 103–134%; 0.893vs0.762mg.h/L) and 14% increase in maximal concentration, Cmax (90%CI: 82.1–159%; 0.343 vs 0.300 mg/L). The 5-FU Cmax was positively associated with time up and go (TUG) (Pearson correlation 0.77, p=0.01), but not other geriatric assessment domains or severe toxicity.

Conclusion:

5-FU exposure was significantly increased in older adults compared to younger adults receiving equivalent doses of capecitabine, and is a possible cause for increased toxicity in older adults.

Key Words: pharmacokinetics, capecitabine, older adults, cancer, toxicity, geriatric tools

6.2 Background

Cancer is predominantly a disease of older adults with an increasing incidence with increasing age [1]. Worldwide, the absolute number of older adults with cancer is expected to increase due to the ageing of the population [2]. The definition of an older adult varies with many studies using age limit of ≥70 years but others using different age limits (e.g. ≥65 years, ≥75 years) [3].

Older adults with cancer are commonly treated with capecitabine, a convenient oral fluoropyrimidine chemotherapy agent [4]. Capecitabine, a prodrug of 5-fluorouracil (5-FU), has common toxicities including fatigue, hand foot syndrome and diarrhoea [4]. Compared with younger adults, older adults on capecitabine require more dose modifications (delays, reductions, omissions), and with, for example, dose reductions required in 51% in those aged ≥70 years versus 39% in those aged <70 years) [5]. Given this, prescribing capecitabine can be challenging in the older, frailer population.

Changes in 5-FU pharmacokinetics (PK) due to physiological changes with ageing may be responsible for the excess toxicity of capecitabine in older adults. Such changes can alter decrease gastric acid secretion, reduce gastric emptying and slow colonic transit times to alter the absorption of orally administered agents. A decline in renal function and changes in fat distribution with ageing can also affect drug disposition [6]. Capecitabine is dosed by body surface area (BSA) dosing but it is unclear if this is the optimal dosing method in older adults. An alternative is PK-guided dosing where measured 5-FU PK parameters are used to refine the dosing of capecitabine in individual patients.

There is limited data on the PK of capecitabine and its metabolites (5-FU, 5-DFCR, 5-DFUR) in older adults with cancer with conflicting results amongst the few published studies. Two studies (Abdi et al, n=60, Louie et al, n=24) [7- 8] investigated the PK of capecitabine in the treatment of a small group of older and younger patients with colorectal cancer. Both studies demonstrated significant differences in capecitabine clearance (CL/F) and volume of distribution (Vd/F) and rate of absorption (=ka) among older adults (aged >70 years). Abdi et al [8] also showed a positive correlation between capecitabine PK parameters and its common toxicity, hand and foot syndrome (HFS) ($p=0.01$). Another study by Cassidy et al [9] showed no impact of age, sex, BSA or creatinine clearance on PK parameters of capecitabine and its metabolites in adult patients (n=25) with solid tumours. The US FDA does not recommend specific dose adjustments of capecitabine for age [10].

The aim of this study was to investigate the PK of capecitabine and its metabolites (5-DFCR, 5-DFUR and 5-FU) in younger (<70 years) and older (≥70 years) adults receiving treatment for breast or gastrointestinal (gastric, pancreas, colorectal, biliary) cancer and to explore the correlation between PK of capecitabine and chemotherapy-related toxicity and geriatric assessment domains.

6.3 Methods

6.3.1 Study design

This was a pilot pharmacokinetic study in adult participants who had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, breast cancer or gastrointestinal cancer (gastric, pancreas, colorectal, biliary) and were planned for treatment with capecitabine (adjuvant or palliative) either as monotherapy or in combination with other anti-cancer drugs. Older adult was defined as age ≥70 years [3]. The study was conducted at three hospitals from November 2017 to February 2020. Ethics committee approval was obtained from the Sydney Local Health District Ethics Committee (CH62/6/2017-133, HREC/17/CRGH/198) and participants provided written, informed consent.

6.3.2 Sample size

Sample size was determined assuming a normal distribution of capecitabine AUC according to the observation by Louie et al. [7], a sample sizes of 12 in each group, with a parallel study design, would achieve 80% power to reject the null hypothesis of equal means with a significance level (alpha) of 0.05 using a two-sided two-sample unequal-variance t-test.

6.3.3 Study procedures

Capecitabine was prescribed and administered as per routine clinical practice protocols. Participants attended clinic on day 14 of cycle one (21 day cycle) of capecitabine (ie at steady state) and day 14 of cycle two for study assessments. Venous blood samples were collected pre-treatment, and 1 h, 2 h and 4 h after dosing for the quantification of plasma concentrations of capecitabine and its primary metabolites (5-DFCR, 5-DFUR and 5-FU)

[11]. Participant's demographic information, inflammatory markers and renal function data were recorded prior to commencement of the study drug. Toxicity data were recorded during treatment and up to 6 months after completion of treatment for all patients.

Geriatric assessment of the included domains [score/ instrument, (range of score)] are as follows: cognition [the OMCT-Score (0-28)], functional ability/frailty [Timed Up and Go test (TUG)- score, the Katz index (0-6), OARS- score (0-14), MOS- score (10-30)], comorbidity and polypharmacy [CIRS-G- score (0-4)], psychosocial function [Geriatric Depression Scale (1-5), the modified MOS social support score (4-20)], nutrition [MNA-score (0-14)] and screening instrument [the G8 score (0-17)] [12].

6.3.4 Assay

Using Mitra® microsampling devices for sample collection, a LC-MS/MS method was developed to simultaneously measure capecitabine, 5-DFCR, 5-DFUR and 5-FU according to Radovanovic et al [13].

6.3.5 Pharmacokinetic analysis

A Bayesian PK estimation approach using observed metabolite concentration-time data and an existing population PK model was employed to estimate individual estimates of PK parameters [14, 15]. Results were then statistically compared using a standard industry approach to determine any impact of age on PK. The selected population pharmacokinetic model [14] is illustrated in (Online Appendix1).

The selected model was then simulated for a typical patient and compared to data presented by Gieschke et al. to confirm model coding [14]. Individual estimates of PK parameters were determined by empiric Bayesian estimation using the PK of the drug (i.e. model), individual

patient factors (i.e. body surface area, estimated creatinine clearance, serum alkaline phosphatase activity) and the measured drug and metabolite concentration(s). Determined model parameters were then used to calculate the following PK parameters on Day 14 for each treatment cycle, these included area under the plasma concentration-time curve over the 12-hour dosing interval (AUCτ), maximum plasma concentration over the 12-hour dosing interval (Cmax) and time of maximum plasma concentration (Tmax). Dose-normalised data (to a dose of 1500 mg) was calculated for AUCτ and Cmax parameters, calculated as: Dose Normalised PK Parameter = (1500 mg)/(Dose Administered (mg)) ×PK Parameter.

6.3.6 Statistical Analysis

A linear mixed-effect analysis of variance (ANOVA) model was used to compare dose-normalised Ln transformed PK parameters between age groups. The residual error (error mean square) was used to construct the 90% confidence intervals for the ratio of treatment means. To construct the 90% confidence intervals, the younger group (i.e. <70 years) was used as the reference. Equivalence was concluded if the 90% confidence intervals were within the standard limits of 80 – 125%. Significance was set at an α-level of 0.05. Linear regression and logistic regression analysis were used to determine the correlation between capecitabine and metabolite PK and domains of geriatric assessment, inflammatory markers and toxicity. Toxicity was graded according to NCI CTCAE version 3.0 during chemotherapy cycles.

6.3.7 Software

Population PK modelling and simulation was conducted using NONMEM® VIII (ICON Development Solutions, Ellicott City, MD, USA) software with an Intel Fortran compiler (Intel Visual Fortran Composer XE 2013) and Wings for NONMEM 7 interface

(http://wfn.sourceforge.net). Data processing was conducted using R® Version 3.3.2 (R Foundation for Statistical Computing). Statistical comparisons were performed using Phoenix® WinNonlin® Version 8.2 (Pharsight®, a Certera™ company). XLSTAT (version 2021.4) software was used for linear regression and logistic regression analysis.

6.4 Results

Of a total 26 participants, the median age was 67 years (range, 37-85 years) and 58% were male. 14/26 (54%) were aged <70 years and 12/26 (46%) were aged ≥70 years. All 26 participants were included in the PK analysis of concentration-time data for capecitabine and its metabolites (Online Appendix 2).

Concentration-time data was collected from 1 treatment cycle for all participants and for 2 treatment cycles for 13/26 (50%) participants. The mean capecitabine dose was 1666 mg twice daily (range, 1000-2000 mg) in the older adult group and 1750 mg twice daily (range, 1500-2000 mg) in the younger adults group. The mean dose-normalised 5-FU concentration-time profiles showed a 17% increase in total exposure (AUCτ 90%CI: 103–134%) and 14% increase in maximal concentrations (Cmax5-FU 90%CI: 82.1–159%) over the dosing interval in the older age group, compared to the younger group (Online Appendix 3)

Individual empiric Bayesian estimates of PK model parameters are presented in (Table 10). The calculated PK parameters for Cycle 1 and Cycle 2 are summarised in Online Appendices 4 and 5, respectively.

Minimal differences between age groups were observed in mean dose-normalised 5-DFUR profiles. The 90% confidence intervals for AUCτ were contained within the limits of 80 – 125%. 5-DFUR Cmax values exhibited great variability such that the 90% confidence intervals were 78.7 – 146%, extending beyond the standard limits; nonetheless, the mean ratio was approximately 100% and no differences found in 5-DFUR PK between older and younger patients. Predicted and observed 5-DFUR and 5-FU concentration-time profiles for each individual are presented in Online Appendices 6 and 7, respectively.

Mean predicted dose-normalised (to a capecitabine dose of 1500 mg) concentration-time profiles on Cycle 1, Day 14 for 5-DFUR and 5-FU, stratified by age group, are presented in Online Appendix 8. The geometric mean ratio of older/younger group PK data and associated 90% confidence intervals are presented in Online Appendix 9.

Logistic regression analysis revealed no significant correlation between PK parameters of capecitabine (AUC5-FU, Cmax5-FU, AUC5-DFUR, Cmax5-DFUR) and capecitabine toxicity [diarrhoea (11/26) (p=0.43)], hand and foot syndrome [(11/26) (p=0.07)], grade 3 & 4 toxicity [(10/26) (p=0.11)], hospitalisation [(4/26) (p=0.56)] and any toxicity [(20/26) (p=0.21)]. No significant association was found between PK parameters of capecitabine and inflammatory markers C-reactive protein (CRP) ≥10 (10/26) (p=0.33) and Neutrophil Lymphocyte Ratio (NLR) ≥5 (5/26) (p=0.19) (Online Appendix 10). 5-FU Cmax and 5-FU AUC were positively associated with the functional ability based on the Timed Up and Go [TUG- score (median=9) (Pearson correlation 0.77, p=0.01 and 0.79, p=0.03 respectively)], but not other domains of geriatric assessment.

6.5 Discussion

In the present study, older adults, compared with younger adults, who had standard dose capecitabine for breast or gastrointestinal cancer had a statistically significant higher exposure to 5-FU. The increased exposure to 5-FU among older adults was positively correlated with the TUG score (a measure of functional ability), but not other geriatric assessment variables, rates of severe chemotherapy-related toxicity or inflammatory markers.

Previous studies determining the effect of age on capecitabine PK have showed differences between older adults and younger adults. Abdi et al. (2014) found the capecitabine absorption rate constant was lower in the older adults (>75years; n=20, 20/60) compared with younger adults (mean ka value of 0.84 h-1 in older adults versus 1.86 h-1 in the younger adults). The elimination rate constant of the 5-FU metabolite (k40) decreased significantly over time (after 2 consecutive weeks), but this time effect was not different between the two age groups [8]. From the second cycle of capecitabine, a significant correlation was found between the higher exposures of capecitabine and its metabolites (5-DFCR, 5-DFUR, 5-FU) and grade 2 or 3 hand-foot syndrome (p=0.01; p=0.03; p=0.006; p=0.008 respectively). Similarly in the present study, a higher Cmax for 5-FU was found in older adults, but there was only a trend among patients (older and younger) with high exposure of 5-FU and hand and foot syndrome toxicity (p=0.07). Other chemotherapy–related toxicity and PK of capecitabine and its metabolites were not correlated, possibly due to low numbers of older adults providing blood samples in cycle 2 (n=3). Louie et al. [7] investigated capecitabine PK in older adults (>70 years; n=24) compared with younger adults (<70 years; n=5). Cmax and AUC of capecitabine were three-fold higher among older adults, compared to younger adults, but there was no difference in the PK parameters of 5-DFCR, 5-DFUR, or 5-FU. Correlation between capecitabine exposure and chemotherapy-related toxicity was not examined in their

study. This greater variation in PK in older adults with cancer is possibly due to a reduction in renal and hepatic clearance and an increase in volume of distribution of lipid soluble drugs with age [16].

To our knowledge, the association between the PK of capecitabine, or any chemotherapy, and geriatric assessment variables has not been previously investigated. Geriatric assessment variables are correlated with a higher risk of chemotherapy-related toxicity, hospitalisation and early death [12, 17]. A systematic review investigated the use of geriatric assessment to predict outcomes in older adults with cancer [12]. Geriatric assessment tools were associated with poor health outcomes such as chemotherapy-related toxicity and mortality [12]. An association between the geriatric assessment variables and PK parameters would enable clinicians, following completion of a geriatric assessment of older adults commencing chemotherapy, to identify older adults at, for example, increased risk of severe chemotherapy toxicity, hospitalisation and/ or mortality due to change in PK parameters (eg exposure, Cmax) of a chemotherapy agent and prescribe appropriate dose modifications to minimise these risks. The American Society of Clinical Oncology (ASCO) guideline (2018) recommends geriatric assessment be performed for all patients with cancer who are older than 65 years [18].

In the present study, functional ability (based on the TUG-score) was the only geriatric assessment variable positively correlated with 5-FU PK. No geriatric assessment variable was associated with chemotherapy related toxicity. Older adults in our study predominantly had adjuvant capecitabine (9/12, 75%) for colorectal cancer (11/12, 92%) possibly reflecting better overall fitness with a great ability to tolerate chemotherapy.

Strengths of the present study include the inclusion of real-world older and younger patients receiving standard chemotherapy, rather than clinical trial participants, to improve the applicability and generalisability of results to day-to-day clinical practice. Prospective collection of toxicities, geriatric assessment variables and inflammatory markers at the point of care strengthened the outcome data. Another strength included determining the relationship between geriatric assessment variables and capecitabine PK and being one of only few reported studies to examine the effect of age on the PK of capecitabine and its metabolites

In addition to previously mentioned limitations of the study, others include a lower participation rate of older adults in the second cycle of the study (3/12), though comparable to other similar studies and fairly typical of PK studies. We had estimated a sample size of at least 12 participants to have 80% power of detecting an effect (p-value<0.05). The low number of participants in the entire study (n=26) also reduced the power to detect a significant association between the variables. Generalisability of the findings are likely limited by the majority of participants having adjuvant chemotherapy and hence of better fitness for chemotherapy, rather than palliative chemotherapy for advanced cancer, and hence not representative of all patients having capecitabine in routine clinical practice.

6.6 Conclusions

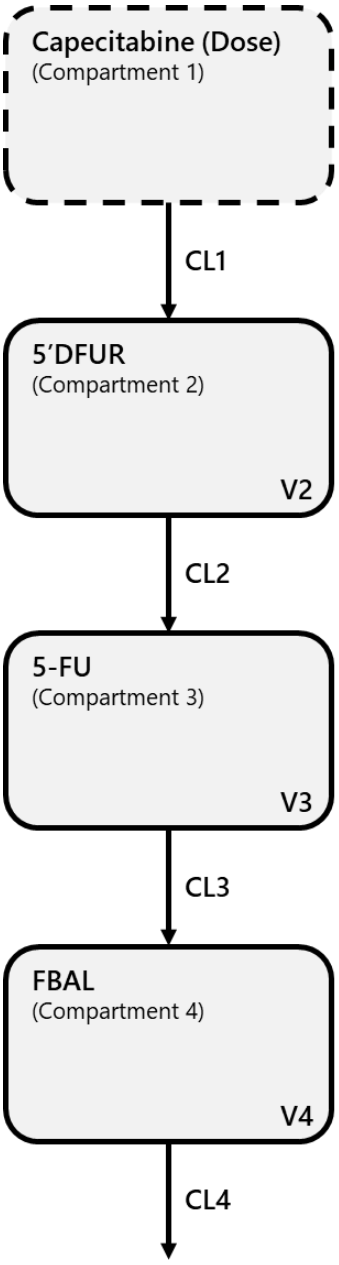
Compared to younger adults, older adults having capecitabine chemotherapy at the standard dose have significantly increased exposure to 5-FU but not to the other metabolites of capecitabine. The clinical significance of these findings requires further investigation in a larger cohort to determine whether it contributes to excess toxicity and/or provides a rationale for dose modifications in older adults receiving capecitabine.

Table 9. Empiric Bayesian estimates of individual model parameters (capecitabine).

Age Group	Younger n=14 Mean (CV %)	Older n=12 Mean (CV %)
Ka (h-1)	1.65 (74.9)	1.52 (59.7)
V2 (L)	88.4 (14.6)	89.4 (9.83)
CL2 (L/h)	92.5 (17.1)	84.1 (11.7)
V3 (L)	17.8 (0.00)	17.8 (0.00)
CL3 (L/h)	2000 (21.1)	1710 (20.3)
V4 (L)	89.0 (17.5)	66.8 (16.2)
CL4 (L/h)	35.4 (18.5)	24.7 (14.8)

CV %- coefficient of variation; ka-elimination rate constant; CL2- apparent 5DFUR clearance; CL3- apparent 5FU clearance; CL4- apparent FBAL clearance; V2- apparent 5DFUR volume; V3- apparent 5FU volume; V4- apparent FBAL volume

Figure 14 (Online Appendix 1): Population PK model of capecitabine and its metabolites [11]



$$Ka_i(hr^{-1}) = \theta_{Ka} \times exp^{(\eta_{Ka} + \kappa_{Ka})}$$

$$Tlag_i(hr) = \theta_{Tlag} \times exp^{(\eta_{Tlag} + \kappa_{Tlag})}$$

$$V2_i(L) = \theta_{V2} \times exp^{\eta_{V2}}$$

$$CL2_i(L/hr) = \theta_{CL2} \times exp^{\eta_{CL2}}$$

$$V3_i(L) = \theta_{V3}$$

$$CL3_i(L/hr) = \theta_{CL3} \times \left(\frac{ALP}{140}\right)^{-0.169} \times exp^{\eta_{CL3}}$$

$$V4_i(L) = \theta_{V4} \times \left(\frac{CrCL}{80}\right)^{0.394} \times \left(\frac{BSA}{1.8}\right)^{0.812} \times exp^{\eta_{V4}}$$

$$CL4_i(L/hr) = \theta_{CL4} \times \left(\frac{CrCL}{80}\right)^{0.618} \times exp^{\eta_{CL4}}$$

Model Parameter Values

θ_{Ka}	1.09	η_{Ka}	70%
		κ_{Ka}	70%
θ_{Tlag}	0.000552	η_{Tlag}	49498%
		κ_{Tlag}	52915%
θ_{V2}	90.6	η_{V2}	30%
θ_{CL2}	75.8	η_{CL2}	24%
θ_{V3}	17.8		
θ_{CL3}	1190	η_{CL3}	33%
θ_{V4}	73.6	η_{V4}	26%
θ_{CL4}	27.5	η_{CL4}	32%

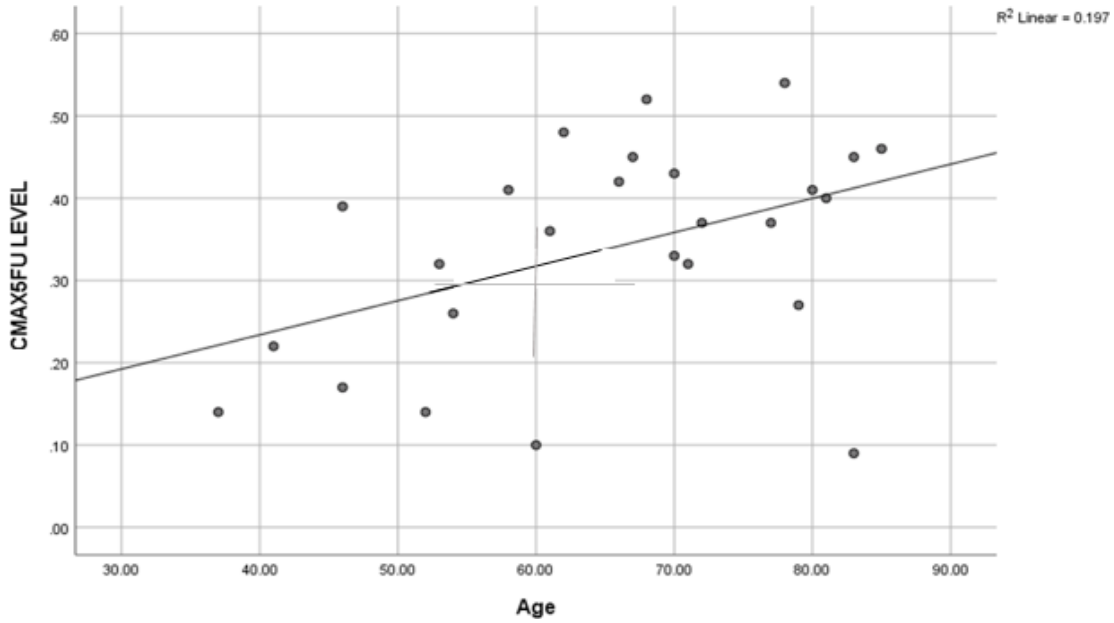
5'FU- 5'-fluorouracil; 5'DFUR- 5'-deoxy-5-fluorouridine; FBAL-alpha-fluoro-beta-alanine; kai-elimination rate constant; Tlag, lag-time (hr); CL2- apparent 5DFUR clearance; CL3- apparent 5FU clearance; CL4- apparent FBAL clearance; V2- apparent 5DFUR volume; V3- apparent 5FU volume; V4- apparent FBAL volume; ALP- alkaline phosphatase; BSA- body surface area; CrCL- creatinine clearance

Table 10 (Online Appendix 2): Study population characteristics.

Age group	Younger (Range 37-68 years) (Median 56 years) Mean (CV %)	Older (Range 70-85 years) (Median 78 years) Mean (CV %)
Sex Number	Male-8, Female-6 n=14	Male-7, Female-5 n=12
Weight (kg)	76.6 (17.7)	67.9 (21.1)
Height (cm)	169 (4.50)	165 (5.92)
SCr (umol/L)	63.0 (15.3)	73.7 (21.0)
ALP (U/L)	101 (46.5)	103 (51.4)
BSA (kg/m^2)	1.86 (8.72)	1.74 (11.1)
CrCL (mL/min)	122 (29.6)	67.8 (23.1)

SCr – serum creatinine concentration; ALP- alkaline phosphatase; BSA- body surface area; CrCL- creatinine clearance (Cockcroft-Gault equation)

Figure 15 (Online Appendix 3): Scatter plot of dnCmax5-FU levels and age



y=0.07+4.15E-3x

Table 11 (Online Appendix 4): Calculated individual 5'DFUR and 5-FU pharmacokinetic parameters after administration of capecitabine on Cycle1, Day 14.

Age Group	Younger n=14 Mean (CV %)	Older n=12 Mean (CV %)
AUCτ 5-DFUR (mg*h/L)	20.2 (22.3)	20.2 (26.7)
C_{max} 5-DFUR (mg/L)	8.67 (42.4)	8.08 (38.2)
T_{max} 5-DFUR (h)	313 (0.182)	313 (0.156)
DN AUCτ 5-DFUR (mg*h/L)	16.5 (15.2)	18.1 (12.1)
DN C_{max} 5-DFUR (mg/L)	7.09 (39.2)	7.30 (27.5)
AUCτ 5-FU (mg*h/L)	0.941 (23.1)	1.00 (27.3)
C_{max} 5-FU (mg/L)	0.405 (43.2)	0.402 (36.9)
T_{max} 5-FU (h)	313 (0.182)	313 (0.156)
DN AUCτ 5-FU (mg*h/L)	0.774 (17.7)	0.910 (20.5)
DN C_{max} 5-FU (mg/L)	0.332 (40.1)	0.370 (31.2)

DN: Dose-normalised to a capecitabine dose of 1500 mg; AUCt- area under the curve; Cmax- maximum concentration; Tmax- time that a drug is present at the maximum concentration; 5-FU- 5- fluorouracil; 5-DFUR- 5-deoxy-5-fluorouridine

Table 12 (Online Appendix 5): Calculated individual 5-DFUR and 5-FU pharmacokinetic parameters after administration of capecitabine on Cycle 2, Day 14.

Age Group	Younger n=10 Mean (CV %)	Olde r n=3 Mean (CV %)
AUCτ 5-DFUR (mg*h/L)	18.2 (21.2)	14.2 (26.5)
C_{max} 5-DFUR (mg/L)	8.70 (59.8)	6.47 (61.7)
T_{max} 5-DFUR (h)	313 (0.153)	313 (0.353)
DN AUCτ 5-DFUR (mg*h/L)	16.3 (18.3)	15.9 (6.22)
DN C_{max} 5-DFUR (mg/L)	7.35 (47.3)	7.76 (65.4)
AUCτ 5-FU (mg*h/L)	0.987 (53.4)	0.627 (12.9)
C_{max} 5-FU (mg/L)	0.461 (64.8)	0.297 (62.5)
T_{max} 5-FU (h)	313 (0.153)	313 (0.353)
DN AUCτ 5-FU (mg*h/L)	0.902 (60.3)	0.718 (13.9)
DN C_{max} 5-FU (mg/L)	0.400 (64.0)	0.368 (75.7)

DN: Dose-normalised to a capecitabine dose of 1500 mg; AUCt- area under the curve; Cmax- maximum concentration; Tmax- time that a drug is present at the maximum concentration; 5'FU- 5'- fluorouracil; 5'DFUR- 5'-deoxy-5-fluorouridine

Figure 16 (Online Appendix 6): Individual predicted (line) and observed (dot) 5-DFUR concentration-time profiles after administration of capecitabine to younger (blue) and older (red) patients on Day 14 of the treatment cycle.

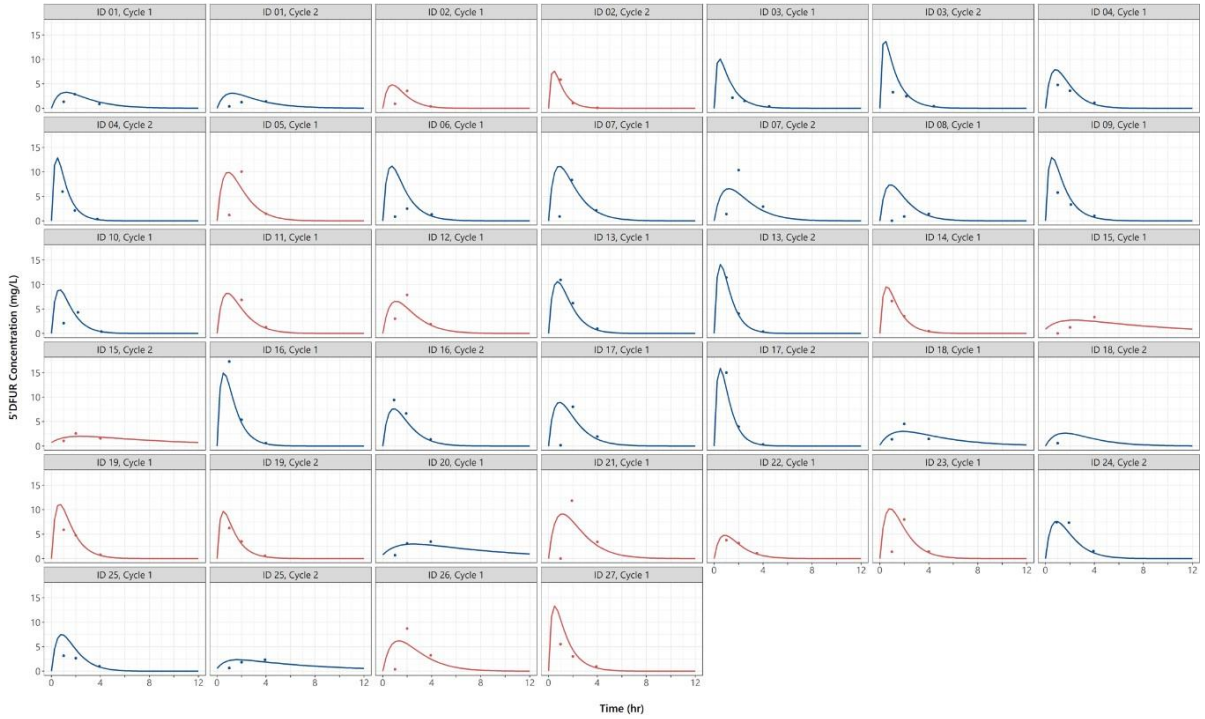


Figure 17 (Online Appendix 7): Individual predicted (line) and observed (dot) 5-FU concentration-time profiles after administration of capecitabine to younger (blue) and older (red) patients on Day 14 of the treatment cycle.

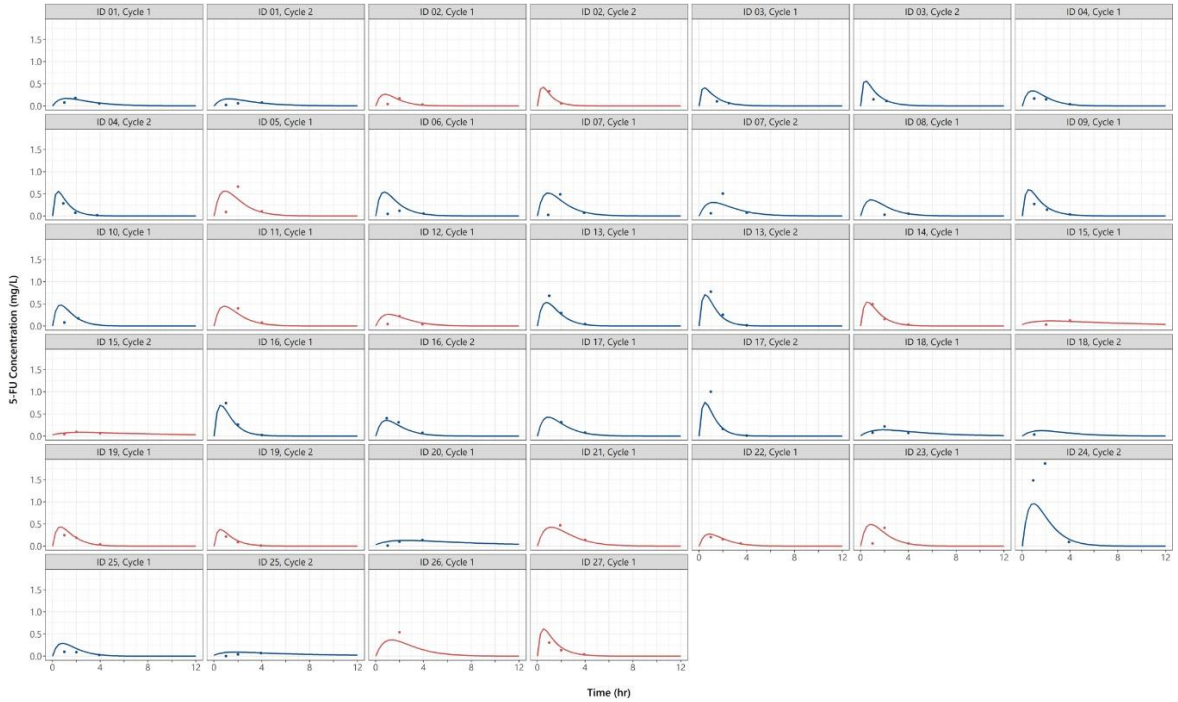
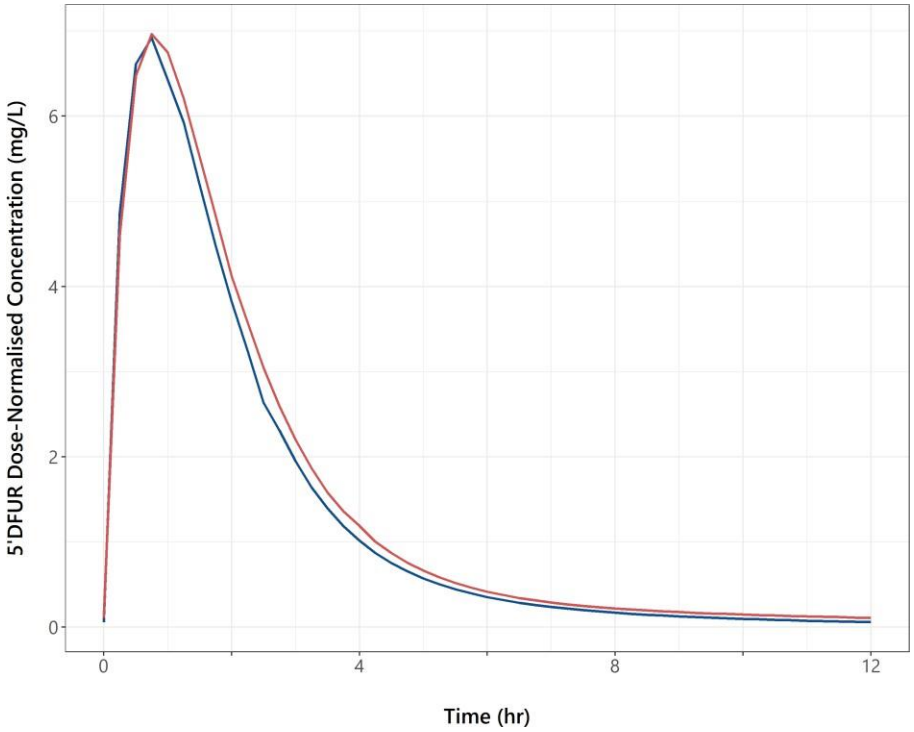


Figure 18 (Online Appendix 8): Mean predicted dose-normalised 5-DFUR (a) and 5-FU (b) concentration-time profiles after administration of capecitabine to younger (blue) and older (red) patients.



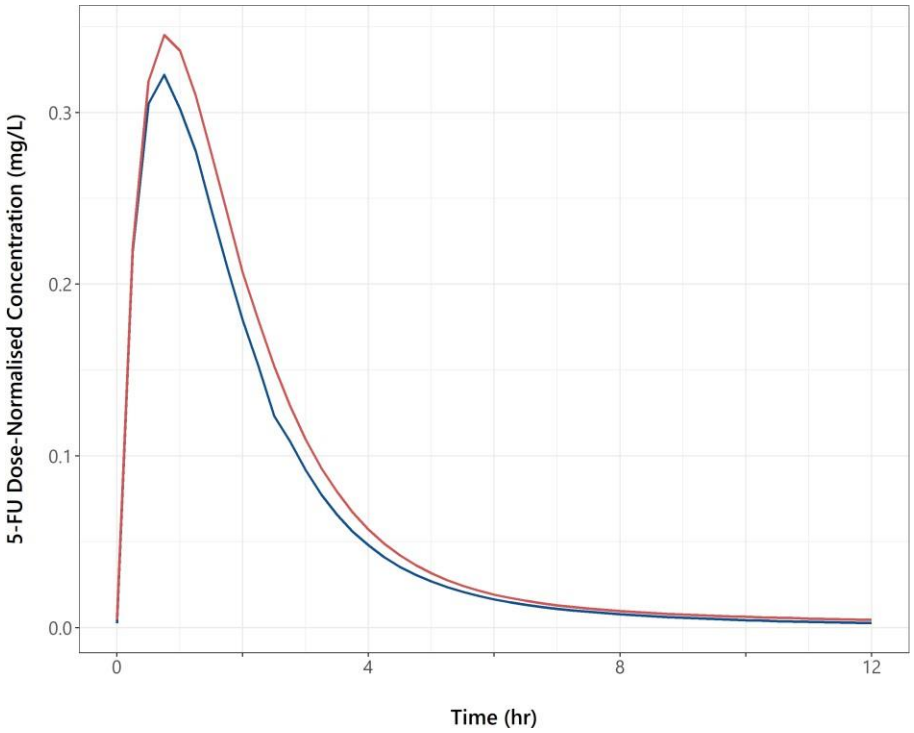


Table 13 (Online Appendix 9): Statistical comparison of 5-DFUR and 5-FU pharmacokinetic parameters between older and younger age groups after administration of capecitabine on Cycle 1, Day 14.

Reference	Younger			
Test	Older			
PK Parameter	Ln(DN $AUC\tau_{5\text{-}DFUR}$)	Ln(DN $AUC\tau_{5\text{-}FU}$)	Ln(DN $C_{max5\text{-}DFUR}$)	Ln(DN $C_{max5\text{-}FU}$)
Units	mg*hr/L	mg*hr/L	mg/L	mg/L
Reference Geo LSM	16.4	0.762	6.44	0.300
Test Geo LSM	18	0.893	6.89	0.343
Ratio (%Test/Ref)	110	117	107	114
90% CI Lower	99.7	103	78.7	82.1
90% CI Upper	121	134	146	159

Table 14 (Online Appendix10): Correlation of inflammatory markers and PK of capecitabine metabolites

	NLR (≥5 vs <5, 5/26 vs 21/26)	CRP (≥10 vs <10, 10/26 vs 16/26)
AUC 5-FU	p=0.1 R^2=0.34	p=0.55 R^2=0.11
C_{max} 5-FU	p=0.15 R^2=0.28	p=0.3 R^2=0.15
AUC 5-DFUR	p=0.15 R^2=0.29	p=0.1 R^2=0.36
C_{max} 5-DFUR	p=0.3 R^2=0.18	p=0.15 R^2=0.28

NLR = Neutrophil Lymphocyte Ratio, CRP = C-reactive protein, p = p value, R^2 = coefficient of determination

6.7 References

1. Society. AC. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Available at: https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21492 Accessed 20 Sep 2022].

2. Australian Institute of Health and Welfare & Australasian Association of Cancer Registries 2012. Cancer in Australia: an overview, 2012. Cancer series no. 74. Cat. no. CAN 70. AIHW.

3. Wildiers H, Heeren P, Puts My, et al. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. *J Clin Oncol*.2014; 32: 2595-603.

4. Stockler, M. R., V. J. Harvey, P. A. Francis, et al. Capecitabine versus classical cyclophosphamide, methotrexate, and fluorouracil as first-line chemotherapy for advanced breast cancer. *J Clin Oncol.*2011; 29:4498-4504.

5. Bajetta E, Procopio G, Celio L, Gattinoni L, Della Torre S, Mariani L, Catena L, Ricotta R, Longarini R, Zilembo N, Buzzoni R. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. *J Clin Oncol*.2005; 23: 2155-61.

6. McLachlan AJ, Hilmer SN, Le Couteur DG. Variability in response to medicines in older people: phenotypic and genotypic factors. *Clin Pharm Therap.*2009; 85:431-3.

7. Louie SG, Ely B, Lenz HJ, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). *Br J Cancer*.2013; 109: 1744-9.

8. Daher Abdi Z, Lavau-Denes S, Prémaud A, et al. Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. *Cancer Chemother Pharmacol*.2014; 73: 1285-93.

9. Cassidy J, Twelves C, Cameron D, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. *Cancer Chemother Pharmacol*.1999; 44: 453-60.

10. Food and Drug Administration Xeloda (Capecitabine), NDA 020896. 2015.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/020896s037lbl.pdf

11. Gieschke R, Burger HU, Reigner B, et al. Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. *Br J Clin Pharmacol*.2003; 55:252-63.

12. M.T. Puts, J. Hardt, J. Monette, et al. Use of geriatric assessment for older adults in the oncology setting: a systematic review. *J Natl Cancer Inst*.2012; 104: 1134-1164.

13. Radovanovic M, Schneider JJ, Shafiei M, et al. Measurement of 5- fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*.2002;1188:123075.

14. Gieschke R, Reigner B, Blesch KS, et al. Population pharmacokinetic analysis of the major metabolites of capecitabine. *J Pharmacokinet Pharmacodyn.*2002; 29: 25-47.

15. Gieschke R, Burger HU, Reigner B, et al. Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. *Br J Clin Pharmacol.*2003; 55: 252-263.

16. Crombag M R, Joerger M, Thürlimann B, et al. Pharmacokinetics of Selected Anticancer Drugs in Elderly Cancer Patients: Focus on Breast Cancer. *Cancers* 2016; 8: 6.

17. Feliu J, Espinosa E, Basterretxea L, et al. Prediction of Chemotoxicity, Unplanned Hospitalizations and Early Death in Older Patients with Colorectal Cancer Treated with Chemotherapy. *Cancers.*2021; 14: 127.

18. Dale W, Williams GR, R MacKenzie A, et al. How Is Geriatric Assessment Used in Clinical Practice for Older Adults With Cancer? A Survey of Cancer Providers by the American Society of Clinical Oncology. *JCO Oncol Pract*.2021; 17: 336-344.

Chapter 7: Comparison of capecitabine concentration determined by capillary sampling versus venous blood sampling for therapeutic drug monitoring: a pilot study

7.1 Overview

This chapter is the manuscript of a pilot study aimed to compare capecitabine concentrations determined by capillary sampling (Mitra) versus venous blood sampling for the purpose of therapeutic drug monitoring (TDM) and participants' preferences for the two sampling methods. Capecitabine concentrations by capillary sampling and venous blood/plasma sampling were highly correlated but were consistently lower than the paired plasma concentration. Microsampling was the preferred method by all patients with minimal pain. The study's findings suggest that microsampling may be a feasible alternative to plasma sampling for TDM of capecitabine in real-world patients.

This manuscript is quoted verbatim. Formatting has been updated for consistency across the thesis.

7.1.1 Contribution of authors

Mohsen Shafiei developed the research proposal and research methods, recruited the patients, collected the specimens, performed the data analysis, interpreted the findings, and drafted and revised the manuscript.

Peter Galettis performed specimen analysis to determine pharmacokinetic data and contributed to the revision of the manuscript.

Philip Beale contributed to the research proposal, interpretation of findings, and revision of

the manuscript.

Jennifer Martin contributed to the research proposal, interpretation of findings, and revision of the manuscript.

Andrew McLachlan contributed to the research proposal contributed to the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Prunella Blinman contributed to the research proposal and the research methods, data analysis, interpretation of findings and drafting and revision of the manuscript.

Abstract

Objectives

Therapeutic drug monitoring allows personalised dosing of chemotherapy, but is not well established for capecitabine. The aim of this study was to compare the concentrations of capecitabine and its metabolites obtained by microsampling with plasma sampling and their acceptability.

Methods

Adults taking capecitabine for cancer had paired (duplicate) microsampling at steady state using Mitra® devices and venous blood samples for analysis. Capecitabine and metabolites were measured using a validated mass spectrometry assay. Correlation between the sampling methods was determined. Patients' preferences were elicited using a Likert numeric rating scale and pain by a Visual Analog Scale (range, 0-10).

Key findings

Capecitabine concentrations from 10 patients (60 paired samples) by microsampling and plasma sampling were highly correlated (Pearson correlation: 0.97, Coefficients of determination: 0.94, $p<0.0001$). Capecitabine concentrations in capillary sampling were consistently lower than the paired plasma concentration (mean capecitabine capillary/ Plasma concentration ratio =2774/3709 μg/L 75%). The agreement between sampling matrices showed a 28% bias (95% Cl, 4.02–52.00). Participant ratings showed microsampling was the preferred method by all 10 patients. Most participants reported no pain with microsampling (median 0, range 0 to 1).

Conclusion

Capecitabine concentration measured by microsampling and plasma sampling were highly correlated, but consistently lower in microsampling. Microsampling was the preferred method with minimal pain.

Keywords

Microsampling, capecitabine, therapeutic drug monitoring, pharmacokinetics

7.2 Introduction

Capecitabine is a convenient oral anti-metabolite chemotherapy in the fluoropyrimidine carbamate class commonly used in the treatment of gastrointestinal cancer and breast cancer. Older adults commonly experience excess toxicity with capecitabine with up to 30% requiring dose modifications (delays, reductions, omissions) and hospitalisations [1].

Capecitabine is dosed based on conventional body surface area (BSA) dosing. It is typically given orally in a dose of 1000-1250 mg/m^2 twice a day for 14 days followed by a 7 day break in a 21 day cycle [2]. An alternative to BSA-based dosing is pharmacokinetic (PK)-guided dosing where measured PK parameters are used to refine the dosing of capecitabine in individual patients. PK-guided dosing is a form of therapeutic drug monitoring (TDM) that uses personalised dosing of chemotherapy to reduce chemotherapy-related toxicity and improve efficacy but adjusting doses within a target concentration range.

There is limited research regarding PK-guided dosing of capecitabine in people with cancer, but promising data is available on the TDM of 5- fluorouracil (5-FU), the active metabolite of capecitabine. Gamelin et al. in two studies compared PK-guided dosing of 5-FU with conventional dosing in the treatment of patients with colorectal cancer [3, 4]. Patients who received PK-guided dosing, compared with patients received conventional dosing, had higher objective response rate (33.7% vs 18.3%, p=0.004) and fewer adverse events [Grade III diarrhoea, 4% vs 14%, (p=0.003)].

Whilst TDM allows personalised chemotherapy dosing, the logistics of TDM by venous blood sampling is challenging. Obtaining and transporting blood samples to the laboratory facilities can be logistically difficult, particularly in regional centres. Furthermore, 5-FU is unstable in whole blood and plasma at room temperature and so venous samples of 5-FU

need to be stored on ice and centrifuged to analyse plasma immediately or keep frozen for later analysis. Such challenges among others mean TDM of 5-FU is not clinically feasible and so is underutilised [5].

Pathways to overcome the limitations of venous blood sampling for TDM include the use of finger prick sampling by blood collection microsampling devices such as dried blood spot (DBS) cards, Mitra® devices and Noviplex cards [6]. These devices use a simple finger prick to produce a small drop of blood that is either drawn up into a device (Mitra®) or placed on a card (DBS and Noviplex). This procedure can be performed either by health practitioners or by adequately trained patients. The devices or cards are then sent to the laboratory for analysis. The feasibility of patients self-performing finger prick testing techniques to sample their blood for TDM has been shown in the measurement of carotenoids and vitamin D, in over 4000 patients with breast cancer, but not with chemotherapy [7]. Microsampling techniques have been examined to measure capecitabine concentrations by DBS cards and Mitra® devices [8, 9].

The aim of this study is to compare the concentrations of capecitabine and its metabolites obtained by capillary volumetric absorptive microsampling (VAMS) microsampling with venous blood VAMS microsampling and plasma sampling for the purpose of TDM, and the acceptability of the sampling methods to participants.

7.3 Methods

7.3.1 Study design and population

This observational pharmacokinetic study collected finger prick blood samples for measurement of capecitabine and its metabolites concentrations and explored their correlation with plasma concentrations.

Eligible participants were aged ≥18 years with a histologically or cytologically confirmed diagnosis of breast or gastrointestinal cancer (gastric, pancreas, colorectal, biliary), who were seen in clinic by an oncologist at the participating sites and planned for treatment with capecitabine (adjuvant or palliative) either as mono-therapy or in combination with other anticancer drugs. The study was approved by the ethics committee (CH 62/6/2017-133-P Blinman HREC/17/CRGH/198, 23 Feb 2018) and all patients gave written informed consent before entering the study. Between February 2018 and January 2020, 10 patients were included in the study.

7.3.2 Sampling and capecitabine concentration

Participants were asked to have a pre-chemotherapy routine venous blood test and then to return to the clinic on day 14 of cycle one (at the time of steady state), hour 2 or day 14 of cycle two for the pharmacokinetic sampling. Venous blood samples were performed by study personnel collecting venepuncture pre-chemotherapy and 1, 2 and 4 hours after capecitabine dosing. At the 2 hour blood collection, two separate Mitra® (devices [Neoteryx (Torrance, CA, USA)] were used to collect a sample from venous blood samples (with etheylenediaminetetraacetic acid (EDTA) anticoagulant). Two other Mitra® devices were used to collect capillary blood samples obtained by finger prick using lancets (BD Microtainer®, Dublin, Ireland) performed by experienced study personnel. After collecting

the Mitra® devices, the venous blood samples were immediately stored on ice (4°C) prior to centrifugation at 2000g for 10 min. Plasma were then harvested before being stored at -80°C until analysis. The 4 Mitra® devices (2 EDTA blood samples and 2 capillary samples) were sealed in the plastic holder and the holder labelled before being stored at room temperature until analysis.

7.3.3 Bioanalysis

Plasma and finger prick samples were analysed for capecitabine, 5-deoxy-fluorocytidine (5-DFCR), 5-deoxy-fluorouridine (5-DFUR) and 5-FU concentration using liquid chromatography with tandem mass spectrometry (LC/MS-MS) using the method of Radovanovic et al. [9] and validated according to the US Food and Drug Administration (FDA) guidelines for bioanalytical method validation [10]. A Shimadzu 8060 LC-MS/MS was employed for sample analysis equipped with electrospray ionisation source interface that operated in positive and negative ion modes with reversed-phase chromatographic separation. Using acetonitrile containing stable isotope-labelled internal standards, the samples were extracted from Mitra® devices. Samples were then sonicated, evaporated under vacuum and resuspended in 0.1 % formic acid before injection into the LC-MS/MS. The injection volume was 1 µL with a total run time of 5 min [9].

7.3.4 Data collection and Statistical analysis

The concentration data for each sampling method (capillary VAMS, venous VAMS and plasma samples) were analysed and investigated using Pearson correlation, a Bland-Altman bias plot and Passing-Bablok analyses, in line with standard practice for comparison of assays [11]. Patient satisfaction and preferences between finger prick sampling and venous blood

sampling were elicited by using Likert numeric rating scale and Visual Analog Scale (range, 0-10, 0-no pain; 10-very painful) for the assessment of pain.

7.4 Results

7.4.1 Patients and capecitabine concentrations

Of the 10 participants included in this microsampling study (Table 15), 40% were female and the median age was 69 years (range, 41-85 years). Microsampling concentration-time data was collected from treatment cycle 1, 2 hour after the dose for all participants. Capecitabine concentrations ranged from 42.1 to 7712 μg/L (mean 2774 μg/L) in capillary VAMS, 10 to 6596 μg/L (mean 2800 μg/L) in venous VAMS and 30 to 4770 μg/L (mean 3709 μg/L) in plasma sampling (Table 16). The capecitabine concentrations after capillary VAMS and venous VAMS collection were highly correlated (Pearson correlation: 0.935, Coefficients of determination: 0.94, $p<0.0001$). The capecitabine concentrations in capillary VAMS and plasma samples were also highly correlated (Pearson correlation: 0.973, Coefficients of determination: 0.94, $p<0.0001$), with the concentration in capillary VAMS samples consistently lower than the paired plasma concentrations (mean capecitabine VAMS/Plasma ratio, 2774/3709 μg/L, 75%). The 5-FU concentrations obtained by capillary VAMS and venous VAMS were correlated (Pearson correlation: 0.90, Coefficients of determination: 0.83, $p<0.0001$). This correlation was slightly weaker between concentrations obtained by capillary VAMS and plasma sampling (Pearson correlation: 0.89, Coefficients of determination: 0.69, $p=0.05$). Inter-patient variability was observed for 5-DFCR and 5-DFUR metabolites. Concentrations ranged from 257 to 6554 μg/L and 96 to 7286 μg/L for 5-DFCR and 5-DFUR, respectively.

Table 15. Patient Baseline Characteristics

Characteristic	
Numbers	10
Age (y)	69 (41-85)
Sex (n)	
Male	6 (60%)
Female	4 (40%)
Weight (kg)	70.4 (56-88)
ECOG PS (n)	
0	4 (40%)
1	4 (40%)
2	2 (20%)
Capecitabine dose (BSA-guided, mg twice daily)	1850 (1500-2250)
Cancer type (n)	
Breast	1
Colon	5
Rectum	4

Data are presented as mean (range).
ECOG, Eastern Cooperative Oncology Group performance score; BSA, body surface area;

Table 16. Capecitabine, 5-FU, 5-DFCR and 5-DFUR concentrations taken by capillary VAMS, venous VAMS and plasma sampling from 10 patients (60 samples)

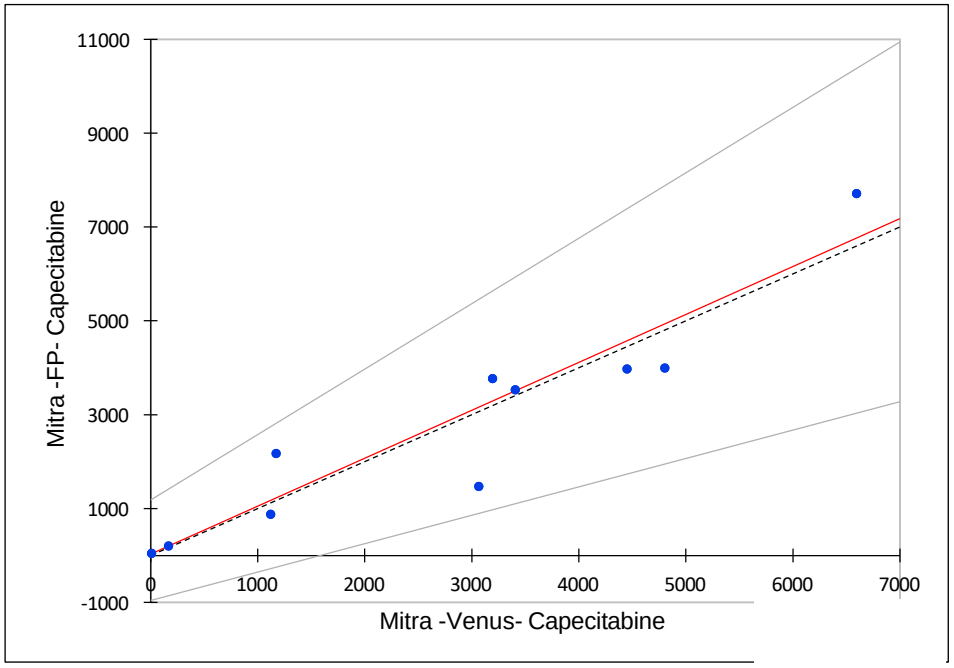
Analyte	Plasma	Venous VAMS	Capillary VAMS
Capecitabine	3709.2 (30.7-9550)	2800.2 (10.9-6596.0)	2774.3 (42.1-7712.1)
5-FU	270 (0-1286.4)	138.1 (0-496.6)	183.6 (0-492.4)
5-DFCR	1954.6 (97.4-4500.2)	2985.8 (267.9-6553.7)	2471.1 (256.7-5770.5)
5-DFUR	4166.1 (317.3-10891)	2817.6 (95.7-6990.5)	2577.4 (146.1-7286.1)

*Mean concentrations (range), μg/L.
5-FU- 5-fluorouracil; 5-DFUR- 5-deoxy-5-fluorouridine; 5-DFCR-deoxy-5-fluorocytidine

7.4.2 Agreement between analyte concentrations from capillary VAMS and venous VAMS sampling

Passing-Bablok regression analysis showed good agreement between concentrations from the capillary VAMS and venous VAMS sampling for capecitabine [y = 1.02x +30.75] (Figure 20) and its metabolites with a slightly weaker agreement for 5-DFCR (y = 0.628x + 194).

Figure 19: Passing-Bablok analysis of concentrations obtained by capillary VAMS (Mitra finger prick, FP) samples and venous blood VAMS samples for capecitabine (y = 1.02x +30.75). Plot shows the line of unity (black dotted), the slope (red) and confidence interval (grey).



7.4.3 Agreement between analytes concentrations from capillary VAMS and venous plasma sampling

Bland-Altman bias plot showed a bias of 28% (95% Cl, 4.02 – 52.00) between sampling matrices of capecitabine concentrations prepared by capillary VAMS and plasma samples (Figure 21). The Passing- Bablok regression between capecitabine concentrations in the two

methods was [y = 0.810x +0.862] (Figure 22). The analysis of agreement between the capecitabine metabolites (5-FU) concentrations collected by capillary VAMS and venous plasma sampling showed a slope coefficient of 0.749; (95% CI 0.345-1.774) and intercept of – 8.645; (95% CI, -134–56) µg/L (Figures 23 and 24).

Figure 20. Bland-Altman bias plot between concentrations collected from capillary (Mitra finger prick, FP) samples and venous plasma samples for capecitabine.

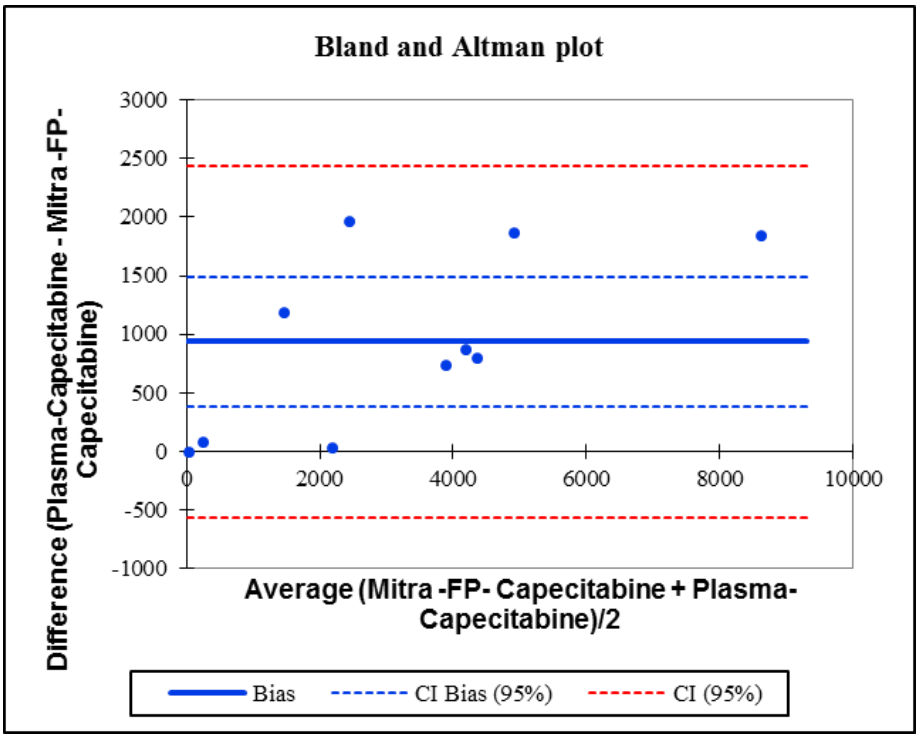


Figure 21. Passing-Bablok analysis of capecitabine concentrations obtained by capillary (Mitra finger prick, FP) samples and plasma samples for capecitabine (y = 0.810x +0.862). Plot shows the line of unity (black dotted), the slope (red) and confidence interval (grey).

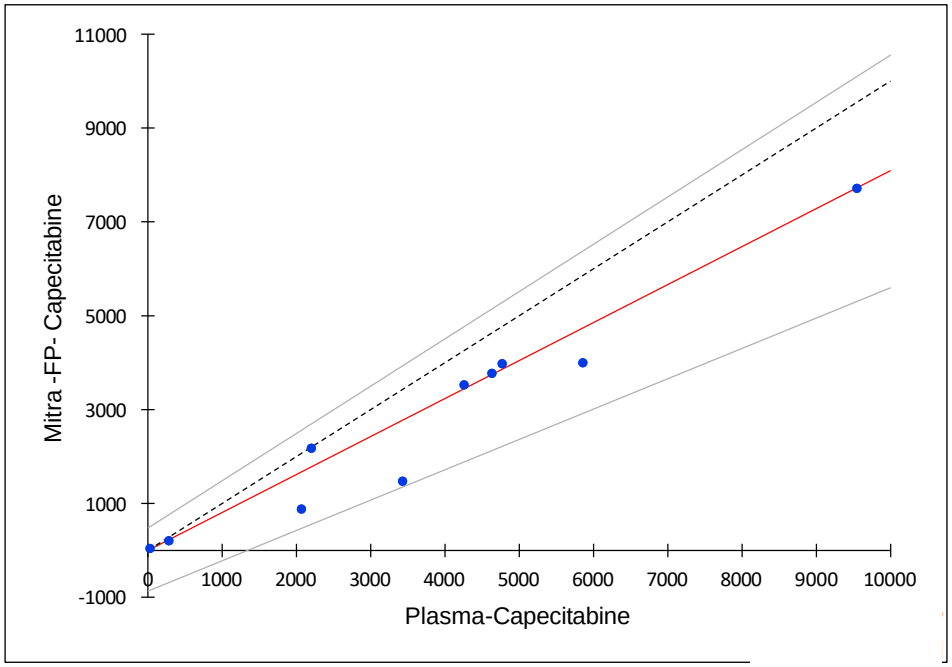


Figure 22. Bland-Altman bias plot between concentrations collected from capillary (Mitra finger prick, FP) samples and plasma samples for 5-FU.

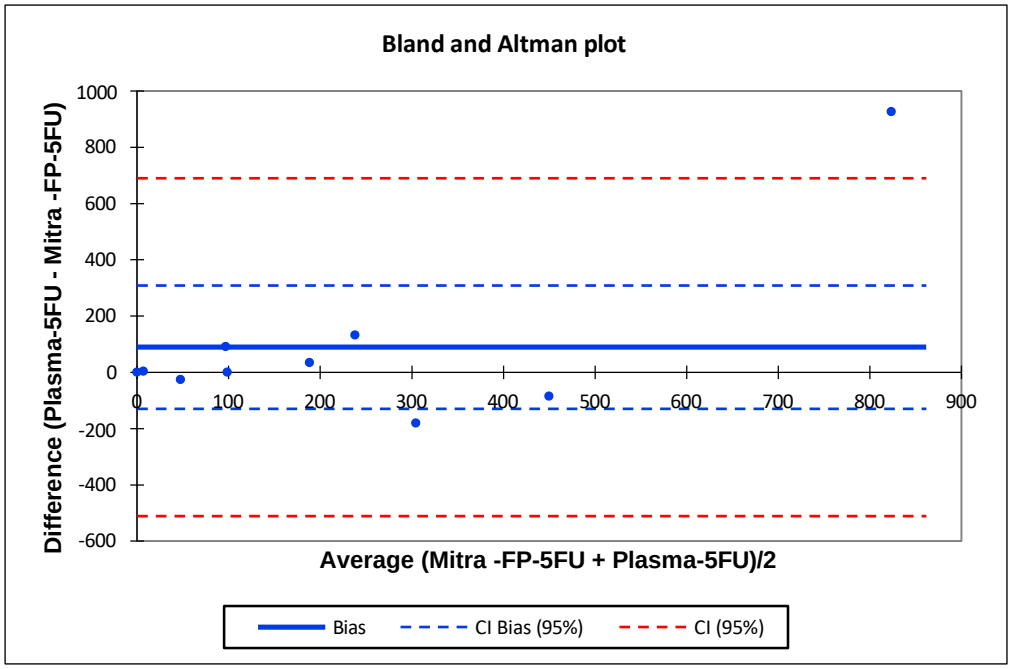
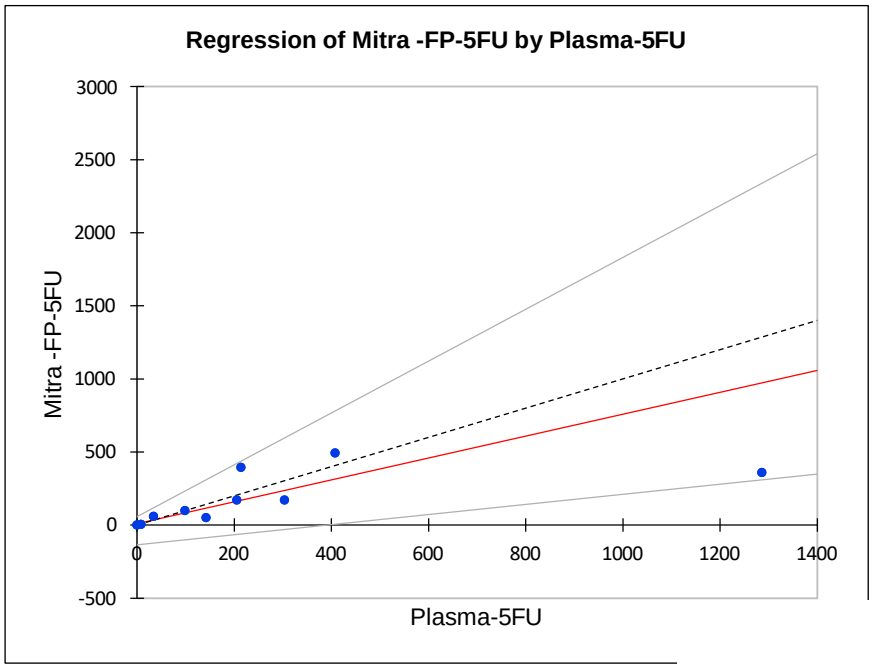


Figure 23. Passing-Bablok analysis of concentrations obtained by capillary (Mitra finger prick, FP) samples and plasma samples for 5-FU (y = 0.749x - 8.654). Plot shows the line of unity (black dotted), the slope (red) and confidence interval (grey).



7.4.4 Patient preferences between the blood collection methods

All 10 participants reported that they preferred the microsampling method over venous blood sampling (microsampling *strongly* preferred: 9/10, microsampling *slightly* preferred: 1/10). Participants' ratings of pain showed most reported no pain (median 0, range 0 to 1).

7.5 Discussion

This study found that capecitabine concentrations obtained by capillary VAMS sampling and to a lesser extent those of 5-FU, were correlated with the concentrations determined by venous blood VAMS and plasma sampling. Comparison analysis showed a high bias (28%). Microsampling was preferred over venous blood sampling by 100% of the participants.

There is little published literature on microsampling of capecitabine. Singhal et al. used microsampling to measure capecitabine concentration for TDM [8]. These authors used DBS and LC-MS/MS and developed and validated a method using 10μL of whole blood on Whatman® cards. The method was established for a concentration range of 10-10000 ng/ml with acceptable accuracy (within 95.0 to 105.9 %) and imprecision (within 1.3-4.6 %). Radovanovic et al. using capillary VAMS and venous blood VAMS samples from 20 patients with cancer, developed and validated a method to determine capecitabine and its metabolites concentrations. Correlation between capillary VAMS and paired venous blood VAMS was examined [9]. The developed method was linear from 10–10,000 μg/L for capecitabine. Similar to our findings, in their study, a good relationship between capillary VAMS concentrations and venous VAMS concentrations was observed [for capecitabine, (y=30.75+1.021x)]. To our knowledge, the current study is the first to correlate capillary VAMS sampling and plasma sampling concentrations of capecitabine and its metabolites in real-world patients living with cancer undergoing capecitabine treatment.

In this study participants' preferred microsampling method reporting no or minimal pain, endorsing further study of microsampling methods as an alternative to venous sampling for TDM. We could find only one other similar study that determined patients' satisfaction and ratings of pain. Woods et al. used a 10-point visual analogue scale to quantify patient satisfaction (0-very satisfied; 10-very dissatisfied) and pain (0-no pain; 10-very painful) with microsampling versus venous blood sampling in a randomised study of patients attending anticoagulant clinic (n=60) to determine INR (international normalized ratio) [12]. Microsampling was the preferred method over venous blood sampling (1.64 vs. 4.45; $P<0.001$) and a strong preference for microsampling was also observed (0.83 vs. 2.23; $P\leq0.004$).

Results of this study support further research and potential use of microsampling of capecitabine and its metabolite 5-FU for dose adjustment according to their concentrations determined by capillary VAMS [13, 14]. The feasibility, reliability and effectiveness of microsampling need to be assessed in larger studies with consideration of real time dose adjustments. Proposed TDM target ranges for 5-FU associated with reduced toxicity and improved efficacy are an area under the curve (AUC) of 20-25 mg · h/l [3] and 20-30 mg · h/l [15]. If microsampling methods proved effective for the measurement of capecitabine and its metabolites (measured on day 14 of treatment at steady state), then could potentially be used for consideration of rapid dose adoption into clinical practice.

Strengths of the current study are the inclusion of real-world participants taking capecitabine treatment for cancer in routine clinical practice. Another strength is the comparison of the concentration of capecitabine and its metabolites in capillary and plasma sampling which, to our knowledge, has not been done previously. Determining participants' preferences for the sampling method and a pain rating scale provides participants' views of the methods to complement and strengthen the PK results.

Limitations of the current study include microsampling being performed by study personnel at the study sites rather than point of care sampling as would occur in a real-world setting. Point of care sampling is feasible when clear instructions and training are provided with studies showing 86 to 98% of finger prick samples performed at home are suitable for analysis [16, 17]. Another limitation is, as a pilot study, the small number of participants and samples meaning wider confidence intervals and decreased power to detect a difference between sampling methods.

7.6 Conclusion

Capecitabine concentration measured by capillary VAMS and plasma sampling were highly correlated, but consistently lower in capillary VAMS sampling. Poor agreement was likely due to small number of samples and great variation of differences. Microsampling method was the preferred method with minimal pain. Further research with higher number of participants is required to determine the effectiveness of microsampling as a substitute for plasma sampling of capecitabine.

7.7 References

1- Bajetta E, Procopio G, Celio L, et al. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. *J Clin Oncol.*2005; 23: 2155-61.

2- Van Cutsem, E., P. M. Hoff, P. Harper, et al. Oral capecitabine vs intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomised, phase III trials. *Br J Cancer*.2004; 90:1190-1197.

3- Gamelin E, Delva R, Jacob J, et al. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *J Clin Oncol.*2008; 26: 2099-105.

4- Capitain O, Asevoaia A, Boisdron-Celle M, et al. Individual fluorouracil dose adjustment in FOLFOX based on pharmacokinetic follow-up compared with conventional body-area-surface dosing: a phase II, proof-of-concept study. *Clin Colorectal Cancer*.2012; 11: 263-7.

5- Murphy RF, Balis FM, Poplack DG. Stability of 5-fluorouracil in whole blood and plasma. *Clin Chem.*1987; 33: 2299-300.

6- Deprez S, Stove CP. Dried blood microsampling-assisted therapeutic drug monitoring of immunosuppressants: An overview. *J Chromatogr A*.2023; 1689: 463724.

7- Sakhi AK, Bastani NE, Ellingjord-Dale M, et al. Feasibility of self-sampled dried blood spot and saliva samples sent by mail in a population-based study. *BMC Cancer.*2015; 15: 265.

8- Singhal, P., P. A. Shah, J. V. Shah, et al. Determination of capecitabine-an anticancer drug in dried blood spot by LC-ESI-MS/MS. *Int J Pharma Pharmaceut Sci.*2015; 7: 238-45.

9- Radovanovic M, Schneider JJ, Shafiei M, et al. Measurement of 5- fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci.*2022; 1188: 123075.

10- Food and Drug Administration Xeloda (Capecitbine), NDA 020896.2015.

11- Capiau S, Veenhof H, Koster RA, et al. Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology Guideline: Development and Validation of Dried Blood Spot-Based Methods for Therapeutic Drug Monitoring. *Ther Drug Monit.*2019; 41:409-430.

12- Woods K, Douketis JD, Schnurr T, et al. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. *Thromb Res*.2004; 114:161-5.

13- Santini J, Milano G, Thyss A, et al. 5-FU therapeutic monitoring with dose adjustment leads to an improved therapeutic index in head and neck cancer. *Br J Cancer.*1989; 59: 287-290.

14- Deng R, Shi L, Zhu W, et al. Pharmacokinetics-based dose management of 5-fluorouracil clinical research in advanced colorectal cancer treatment. *Mini Rev Med Chem.*2020; 20: 161-167.

15- Farkouh A, Ettlinger D, Schueller J, et al. A rapid and simple HPLC assay for quantification of capecitabine for drug monitoring purposes. *Anticancer Res.*2010; 30: 5207-5211.

16- Jager NG, Rosing H, Linn SC, et al. Dried blood spot self-sampling at home for the individualization of tamoxifen treatment: a feasibility study. *Ther Drug Monit.*2015; 37: 833–836.

17- Cheung CY, van der Heijden J, Hoogtanders K, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. *Transpl Int*.2008; 21:140–145.

Chapter 8: Discussion, future directions, and conclusion

8.1 Overview

This chapter brings together the work in this thesis considered as a whole and begins by summarising the principal findings in section 8.2, clinical implications in section 8.3, and research implications in section 8.4, then discussing the strengths of the research in section 8.5, limitations of the research reported in this thesis in section 8.6, and concluding remarks in section 8.7.

8.2 Summary of principal findings

8.2.1 Older adults with colorectal cancer (CRC) compared with younger adults with CRC have worse overall survival (OS) and cancer specific survival (CSS)

The retrospective studies of real-world data presented in Chapters 2 and 3 reported on the survival outcomes and utilisation of adjuvant chemotherapy of older adults, compared with younger adults, with potentially curable colon cancer and rectal cancer. These studies showed older adults with stage III CRC had worse OS and CSS, more comorbidity, and lower utilisation of adjuvant chemotherapy. Adjuvant chemotherapy independently predicted improved OS in older adults with stage III CRC.

8.2.2 Age influences the pharmacokinetics (PK) of some chemotherapy agents such as irinotecan, 5-fluorouracil (5-FU) and capecitabine

A systematic literature review presented as Chapter 4 identified 21 studies examining the impact of age on the PK of chemotherapy agents used for the treatment of patients with colorectal cancer (CRC). The most consistent findings related to the impact of older age were in the studies on irinotecan [1-3] and, to a lesser extent, the studies investigating

panitumumab [4], 5-FU [5] and capecitabine [6, 7]. Overall, however, there were few studies that determined the effect of age as a primary variable on the outcomes of people with cancer and therefore a clear impact of age on the PK of anticancer therapies used in the management of patient with CRC is unknown.

8.2.3 Microsampling may be used in the monitoring of anticancer therapy including capecitabine in the treatment of adults with solid cancers

A systematic literature review of studies using microsampling methods [dried blood spot (DBS), Mitra device] of capillary or venous samples in the measurement of anticancer therapy for the treatment of solid cancer is presented as Chapter 5. Most studies in the review showed microsampling methods were reliable and feasible with good correlation with analyte concentration determined from plasma sampling methods as per the Food and Drug Administration (FDA) guidelines.

8.2.4 Older age was associated with increased exposure of 5-fluorouracil (5-FU) a metabolite of capecitabine

A prospective observational study (n=26) investigating the pharmacokinetic (PK) of capecitabine in a real-world population of older adults and younger adults with cancer is presented as Chapter 6. This study explored changes in PK of capecitabine and the association with excess toxicity in older adults. A significantly increased exposure to 5-FU (17%), but not to the other metabolites of capecitabine, was observed in older adults compared with their younger counterparts. The increased 5-FU exposure was positively associated with *timed up and go* (TUG) score [8] but not with other geriatric assessment variables, severe chemotherapy-related toxicity, or inflammatory markers.

8.2.5 Capecitabine concentrations determined by microsampling were highly correlated with concentrations obtained by plasma sampling but consistently lower in microsampling

The pilot study presented in Chapter 7 examined the feasibility, acceptability, and reliability of microsampling method compared with plasma sampling method (n=10, samples=60) in the measurement of capecitabine concentrations for therapeutic drug monitoring (TDM). Capecitabine concentrations obtained by microsampling were highly correlated with concentrations obtained from plasma sampling, but consistently lower with an unacceptable bias. Microsampling performed by study personnel was feasible and acceptable to all participants with minimal associated pain.

8.3 Clinical implications

The real-world studies of people with colorectal cancer (CRC) highlight the broad need of improving cancer outcomes in older adults, which was the central aim of this thesis. The demonstrated poorer outcomes are possibly due to the underutilisation of adjuvant chemotherapy, an independent predictor of improved overall survival (OS). Oncologists should carefully consider adjuvant chemotherapy in older adults with CRC given the low rates of utilisation of adjuvant chemotherapy in this population [9].

Ways to improve prescribing of chemotherapy in all people with cancer, including older adults, are needed. One method is pharmacokinetic (PK)-guided dosing to optimise efficacy, minimise toxicity and increase confidence in the use of the treatment [10]. PK-guided dosing has not been fully implemented into routine oncology clinical practice due to challenges of this method including the need for multiple venepunctures, unknown appropriate concentration target ranges, and analytical difficulties with pro-drugs like capecitabine [11]. These challenges require further study, as discussed in research implications.

The excess toxicity observed in older adults with CRC receiving chemotherapy such as capecitabine is likely due to the influence of ageing on PK parameters (changes in systemic exposure) of chemotherapy. Oncologists ideally should be able to identify older adults at increased risk of severe chemotherapy-induced toxicity due to age-related changes in PK and consider dose adjustments pre-treatment or during treatment or use a less toxic alternative chemotherapy.

Microsampling, a more convenient and acceptable alternative to venous blood sampling, can overcome some of the challenges associated with implementation of PK-guided dosing, moving drug monitoring closer to the point of care. Key advantages of microsampling include the need for a minimal quantity of blood, the method able to be performed by patients, no need for on-site processing of the sample, stability of samples at room temperature and samples in a readily transported format to testing facilities. If proved as effective as venous blood sampling, microsampling could potentially increase the use of PK-guided dosing for dose adjustment and monitoring of anticancer therapy in routine clinical practice.

8.4 Research implications

Research implications of the work in this thesis include exploration of the barriers of the utilisation of anticancer therapy for older adults with colorectal cancer (CRC).

Further research areas with regards to microsampling and PK-guided dosing include the feasibility and correlation between the venous (plasma) and microsampling concentrations and dose adjustments made in real time. Ultimately, microsampling for PK-guided dosing should be tested in randomised trials investigating PK-guided dosing of anticancer therapy

[chemotherapy, tyrosine kinase inhibitors (TKIs)] using microsampling methods compared with standard of care conventional body surface area (BSA) dosing in adults with cancer.

A possible clinical benefit of further research of microsampling is the potential increased uptake of chemotherapy in older adults due to improved safety of prescribing chemotherapy in this population. The PREDICT study is a non-randomised, open label study to determine the feasibility of therapeutic drug monitoring (TDM) of 5-fluorouracil (5-FU) and capecitabine in patients with breast, gastrointestinal and head and neck cancer with the recruitment aim of 50 patients [12]. The outcome of the PREDICT study will determine if TDM of 5-FU and capecitabine improves the patient's outcomes such as toxicity.

Qualitative and observational studies could be conducted to explore patient's views about the perceived patient centeredness of PK-guided dosing, and ways to improve the TDM process. Such study could investigate if tailored dose of anticancer therapy to the patient's individual needs, according to the individual's PK profile, has helped to improve patient's satisfaction and quality of life.

Further research investigating the association of aspects of geriatric assessment with PK of anticancer therapy and related toxicity in older adults with cancer is of interest. Positive factors would enable oncologists to identify older adults at increased risk of excessive chemotherapy toxicity by clinical assessment rather than PK analysis, and allow oncologists to modify chemotherapy doses accordingly. Exploratory work in this thesis found a significant correlation between *time up and go* (TUG score) [8], a measure of functional ability, and the PK of capecitabine's metabolite (5-FU). Further evaluation of the association between geriatric assessment tools and PK of anticancer therapy in a wider setting (adjuvant and palliative chemotherapy) and larger cohort of older adults with cancer would complement this work.

The work in this thesis identified knowledge gaps including limited studies determining the effect of age on the PK of anticancer therapy, limited clinical validation of the available PK data on anticancer therapy and limited integration of new technologies such as real-time PK profiling of anticancer therapy. The identified PK models of anticancer therapy in the presented literature reviews were mostly based on the data from early-phase clinical trials and therefore require extensive validation in large real-world studies including older adults. The uptake and implementation of microsampling for PK profiling of anticancer therapy including capecitabine is an area of future research.

8.5 Strengths of this thesis

The strengths of the thesis are discussed in this section. The strengths of the individual studies are presented in Chapters 2, 3, 4, 5, 6 and 7.

The main strength of this thesis arises from its investigation of a common and relatable clinical problem in oncology with study of real-world patients. This thesis investigates outcomes of older adults in a common solid cancer using a real-world database, chemotherapy-related toxicity in people with cancer receiving capecitabine in routine care, and the feasibility of microsampling for the purpose of pharmacokinetic (PK)-guided dosing in the same population. The inclusion of real-world older adults on chemotherapy, usually a heterogeneous population, rather than clinical trial participants, in this thesis, improves the applicability and generalisability of results to day-to-day clinical practice.

Another strength of this thesis is the inclusion of two systematic reviews with broad search terms to find, analyse and summarise the available evidence with regards to the association of

older age on the PK of anticancer therapy used in patients with colorectal cancer (CRC) (Chapter 4) and the use of microsampling in therapeutic drug monitoring in oncology setting (Chapter 5) as a potential strategy for better dosing of anticancer therapy.

A novel aspect and strength of the research in this thesis is the exploration of patients' preferences for blood sampling methods (microsampling versus venous blood sampling) in the oncology setting. The acceptance rate of microsampling, an emerging research methodology, was evaluated (Chapter 7) in real-world older adults with CRC, 40% of whom aged >70 years, that is pertinent to microsampling implementation. The incorporation of the acceptability aspect, encourages evaluation of microsampling as the future method of choice for clinical or research purposes.

Another strength of this thesis is that being the first study that investigates correlation of geriatric assessment variables with PK of capecitabine and its metabolites in real-world patients with cancer (prospective observational study reported in Chapter 6), contributing to evidence relevant to external validation of the geriatric assessment tools.

8.6 Limitations of this thesis

Limitations of the individual studies are discussed within each chapter, but the more general limitations of the thesis as a whole are discussed here for emphasis, and include selection bias, small sample size, and methodological heterogeneity.

A predominant and recurring limitation of this thesis is the selection bias and reduced generalisability of the results to the wider population. The two retrospective studies and the two prospective studies in this thesis were observational research and therefore inherently

subject to selection bias based on predetermined study design, definition of older adults, inclusion of participants with certain cancers types, treatment setting (adjuvant and/or palliative) and treatment site/s. For example, older adult in the prospective studies of this thesis was defined as aged 70 years and older according to many previous geriatric oncology studies. There is no universal definition of older adult. Age limit of 65 or 75 years, however, has been considered as the cut off for "older adults" elsewhere. Therefore, the studied population may not best represent the group of patients for whom clinicians perceive to be older adults.

Another example of selection bias is that of the pharmacokinetic (PK) study (Chapter 6) where most participants received adjuvant chemotherapy and hence had better fitness for chemotherapy, rather than palliative chemotherapy for advanced cancer, and therefore were not entirely representative of all patients having capecitabine in routine clinical practice. This limitation, however, had only a small impact due to the overall small number of participants.

The participants included in this work were limited to those adults with cancer who had sufficient English language to participate in the study and does not include adults from Culturally and Linguistically Diverse (CALD) background with insufficient English language. This work therefore may not be representative of all adults with cancer of other linguistic backgrounds who are unable to participate in such research and future studies should endeavour to include the CALD population where feasible.

Another limitation of the work in this thesis was the small sample size in the two prospective studies. The key consequence of the small sample size was reduced power to detect clinically significant correlation between covariates, for example, the effect of age on the PK of

anticancer therapy. Additionally, the small number of participants in the two prospective studies reduced the generalisability of the results to the wider population of older adults with colorectal cancer (CRC). These findings therefore requires further investigation in a larger cohort of patients for increased power and to better define the role of PK-guided dosing of capecitabine in older adults with cancer.

Methodological heterogeneity across studies included in the two systematic reviews is another limitation of this thesis. The included studies used different methods to determine haematocrit effect, haematocrit conversion methods, and microsampling techniques. Whilst the different methods used in the included studies may have better sensitivities to detecting certain effects, the heterogeneity of methods made it difficult to draw firm conclusions from the systematic reviews and reduces the generalisability of the results. More inclusive studies with higher number of participants and homogenous methodology would provide more robust evidence and increase the generalisability of the results.

8.7 Conclusion

Older adults with colorectal cancer, compared with the younger adults with colorectal cancer, have worse survival outcomes, and received less adjuvant chemotherapy. Improving the safety and prescribing of chemotherapy such as capecitabine would likely lead to increased utilisation of adjuvant chemotherapy and improved cancer outcomes in older adults. Pharmacokinetic (PK)-guided dosing of chemotherapy agents allows individualised dosing of anticancer therapy, and hence has the potential to improve the safety and efficacy of chemotherapy in older adults. Currently, the uptake of PK-guided dosing is limited due to logistical and technical challenges, many of which can be overcome by microsampling, an

acceptable method to adults with cancer but for which there is limited data for its use for therapeutic drug monitoring (TDM) in routine clinical practice.

Future directions include conducting studies using PK- guided dosing to determine the optimal dosing of chemotherapy in older adults with cancer, typical of those seen in routine clinical practice. Additional study of microsampling with different anticancer therapies and its implementation, and randomised trials of PK-guided dosing versus standard of care BSA-guided dosing of anticancer therapies is also warranted.

8.8 References

1. Klein CE, Gupta E, Reid JM, et al. Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. *Clin Pharmacol Ther*.2002; 72:638-647.

2. Miya T, Goya T, Fujii H, et al. Factors affecting the pharmacokinetics of CPT-11: the body mass index, age and sex are independent predictors of pharmacokinetic parameters of CPT-11. *Invest New Drugs*.2001; 19:61-67.

3. Poujol S, Bressolle F, Duffour J, et al. Pharmacokinetics and pharmacodynamics of irinotecan and its metabolites from plasma and saliva data in patients with metastatic digestive cancer receiving Folfiri regimen. *Cancer Chemother Pharmacol*.2006; 58:292-305.

4. Ma P, Yang BB, Wang YM, et al. Population pharmacokinetic analysis of panitumumab in patients with advanced solid tumors. *J Clin Pharmacol*.2009; 49:1142-1156.

5. Etienne MC, Chatelut E, Pivot X, et al. Co-variables influencing 5-fluorouracil clearance during continuous venous infusion. A NONMEM analysis. *Eur J Cancer*. 1998; 34:92-97.

6- Louie SG, Ely B, Lenz HJ, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). *Br J Cancer*. 2013; 109:1744-1749.

7- Cassidy J, Twelves C, Cameron D, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. *Cancer Chemother Pharmacol*.1999; 44:453-460.

8- Barry E, Galvin R, Keogh C, et al. Is the Timed Up and Go test a useful predictor of risk of falls in community dwelling older adults: a systematic review and meta-analysis. *BMC Geriatr*.2014; 14:14.

9. Kahn KL, Adams JL, Weeks JC, et al. Adjuvant chemotherapy use and adverse events among older patients with stage III colon cancer. *JAMA*.2010; 303:1037-1045.

10. Lucas CJ, Martin JH. Pharmacokinetic-Guided Dosing of New Oral Cancer Agents. *J Clin Pharmacol*.2017; 57 Suppl 10:S78-S98.

11. Salman B, Al-Khabori M. Applications and challenges in therapeutic drug monitoring of cancer treatment: A review. *J Oncol Pharm Pract*.2021; 27:693-701.

12. PREDICT Fluorouracil (5-FU): A Study to Describe the Feasibility of Therapeutic Drug Monitoring of 5-FU in Cancer Treatment. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=380450&isRev

Appendices

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Our Ref: *2017-133 Amendment*

CONCORD
REPATRIATION GENERAL
HOSPITAL

22 February 2019 – revised 15 March 2019

Dr Prunella Blinman
c/- Dr Mohsen Shafiei
Medical Oncology
Building 76
CONCORD RGH

Dear Dr Blinman

Re: CH62/6/2017-133
HREC/17/CRGH/198
Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: a prospective observational study

Thank you for submitting the following document which was approved by the Deputy Chair on behalf of the Sydney Local Health District Human Research Ethics Committee – Concord Repatriation General Hospital on 21/02/2019:

- Revised Protocol, including Participant Information Sheet (Section 17.2) & Consent Form (Section 17.1) Version 6.0 dated 19/02/2019

This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and the *CPMP/ICH Note for Guidance on Good Medical Practice.*

A copy of this letter must be forwarded to Principal Investigators at each site for submission to the Research Governance Officer.

Research governance review
This amendment has also been reviewed by the Research Governance Officer at Concord Hospital. Implementation of this amendment can now proceed.

Please quote the above Concord Hospital File No. in all correspondence.

Yours sincerely,

Virginia Turner
Executive Officer
Sydney Local Health District Human Research Ethics Committee -
Concord Repatriation General Hospital
Research Governance Officer

Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: a prospective observational study

Short title: Pharmacokinetics of capecitabine in older adults with cancer

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Table of Contents

Title

1.1 Scientific title

Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

1.2 Simplified title

Pharmacokinetics of capecitabine in older adults with cancer

2. Synopsis

Capecitabine is an oral fluoropyrimidine chemotherapy agent commonly used in the management of patients with gastrointestinal cancer and metastatic breast cancer. As mono-therapy, it is a suitable agent for older adults and is used frequently in this population. Some older adults experience excess toxicity with capecitabine requiring dose modifications (delays, reductions, omissions), hospitalisations and other use of health care resources (1). Another challenge of toxicity from capecitabine is that its occurrence can be unpredictable meaning that prescribing capecitabine can be very challenging in the older, frailer population. Capecitabine is currently dosed based on conventional body surface area (BSA) dosing. It is typically given twice a day for 14 days followed by a 7 day break for a 21 day cycle. An alternative to BSA dosing is pharmacokinetic (PK)-guided dosing where measured PK parameters are used to refine the dosing of capecitabine in individual patients. There is limited research regarding PK-guided dosing of capecitabine in patients with cancer, and the results of the few published studies are conflicting. One study (n = 60) showed a positive correlation between capecitabine PK parameters and its common toxicity, hand and foot syndrome (HFS) ($p = 0.01$) (2), whereas a pooled data analysis from two larger trials (n = 481) showed no correlation between the capecitabine drug exposure and safety or efficacy (3). This project overall intends to improve the treatment outcomes of older patients with cancer receiving capecitabine by developing individualised dosing methods to reduce inter-patient variability, significant chemotherapy-related toxicity, and under-dosing from this agent.

3. Background and Scientific Rationale

Cancer is predominantly a disease of older adults with an increasing incidence with increasing age. In Australia and worldwide, the absolute numbers of older adults with cancer is expected to increase due to the ageing of the population. In New South Wales in 2012, the age specific rate of cancer (per 100,000) increased from 929 for persons aged 50 to 64 years to 2074 for persons aged 65 to 79 years and to 2641 for persons aged 80 years and over (4). The definition of an older adult with cancer varies; many cancer studies have used a lower age limit of ≥ 70 years to define an older adult, however, other lower age limits are also used (≥65, ≥75 years) (5). Older adults with cancer, compared with younger adults with cancer, generally obtain similar benefit from chemotherapy but experience higher rates of chemotherapy toxicity (6). Reasons for this are multifactorial including age-related physiological changes (eg reduced renal clearance), increasing frailty and other geriatric syndromes, more medical co-morbidities, and fewer social supports (7, 8).

Conventional dosing of chemotherapy is based on body surface area (BSA) or fixed-dose. Whilst BSA dosing aims to reduce PK variability compared with fixed dosing, PK variability is still seen with some agents such as capecitabine. This means that some patients are unintentionally under-dosed with potentially compromised treatment, whilst others are unintentionally over-dosed resulting in severe chemotherapy-related toxicity. Hence alternative methods to BSA dosing of capecitabine are needed. One alternative to BSA dosing is PK-guided dosing (9) that uses measured PK parameters to refine the dosing of a drug such as capecitabine in individual patients. PK-guided dosing of chemotherapy agents has not been feasible in the past due to the need for multiple blood sampling over many consecutive hours from patients already burdened by the commitment of their anti-cancer therapy, inadequate turn-around times of test results to enable dose adjustments within a chemotherapy cycle, the lack of established therapeutic concentration ranges and analytical challenges particularly with pro-drugs like capecitabine (10, 11). Recent technological and analytic advances, however, mean PK-guided dosing is now more feasible for use in the clinical setting such as the availability of liquid chromatography-tandem mass spectrometry (LC/MS) that shortens analysis time with higher sensitivity and specificity (12, 13).

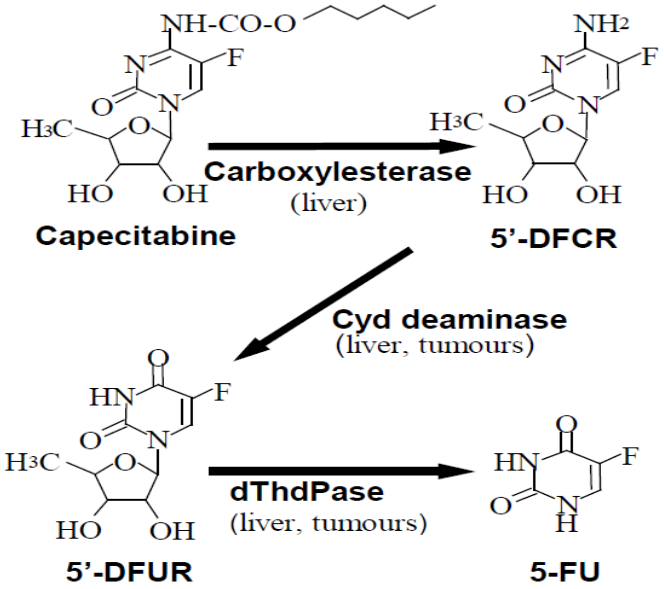
3.1 Ageing and its effect on drug metabolism

The PKs of chemotherapy drugs in older adults with cancer vary widely due to the complexity and heterogeneity of ageing (14, 15). Factors for consideration include age-related physiological changes (eg homeostasis impairment, organ dysfunction), the presence of geriatric syndromes (eg frailty, poly-pharmacy, falls) and pharmacodynamics of the drug (16-18). There is significant variability in the response to drugs in general and their adverse effects in older adults (19). The many physiological changes associated with ageing that influence the PK of drugs including changes in distribution (eg body composition), metabolism (eg ageing liver, hepatic blood flow) and elimination parameters (eg decline in renal function). Although altered absorption and bioavailability in older adults have not been documented to lead to significant or relevant clinical changes in PK of drugs, there are concerns regarding impaired gastric emptying, nutrition and adherence to treatment (16).

One of the most important factors associated with ageing affecting the PK of drugs is the decline in renal function. Glomerular filtration rate (GFR) decreases by about 10 mL/minute/1.73 m^2 with each decade of life, leading to an average 50% decline in GFR between the third and ninth decades of life (20, 21). A significant dose reduction is therefore required for drugs with predominant renal excretion. Changes in liver function in older adults are also important in affecting drug clearance with consequent variability in response (22), for example, age-related decline in hepatic blood flow leading to a decrease in total clearance of high and low extraction ratio drugs (22). The US FDA does not currently suggest specific dose adjustments based on older age due to insufficient data (23).

3.2 Capecitabine in the management of older adults with breast and gastrointestinal cancer

Capecitabine is an oral, anti-metabolite in the fluoropyrimidine carbamate class that is a convenient alternative to intravenous 5FU. Capecitabine is commonly used in the management of patients with gastrointestinal cancers (eg colorectal, gastric, pancreatic, biliary) and metastatic breast cancer. As a pro-drug, it is absorbed rapidly and unaltered through the small intestine and then metabolised primarily in the liver by carboxyl-esterase to 5'-deoxy-5-fluorocytidine (5'-DFCR). 5- DFCR is then converted to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine-deaminase, principally located in the liver and tumour tissue. Further metabolism of 5'-DFUR to the pharmacologically active agent 5-FU occurs mainly at the tumour site by thymidine phosphorylase, present in high levels in tumour tissues (24). (Figure



1)

In colorectal cancer, capecitabine is a suitable chemotherapy agent for older adults with cancer and is used frequently in this population. It has equivalent efficacy with 5FU/ leucovorin (LV) in the adjuvant and palliative settings. In the adjuvant setting, capecitabine was compared with bolus 5FU/LV in patients (n=1987) with stage III colon cancer (396 patients aged >70 years) with similar disease free survival (DFS) and overall survival (OS) across all age groups (HR for DFS: 0.88, 95% CI: 0.77-1.01, p-value for non-inferiority <0.0001; HR for OS: 0.86, 95% CI: 0.74–1.01, p-value for non-inferiority <0.001) (25). Capecitabine had an acceptable toxicity profile, however, older patients required more dose reductions compare with the younger

patients (51% in those aged ≥70 years and 39% in those aged <70 years). Common toxicities of capecitabine include fatigue, hand foot syndrome and diarrhoea. In the palliative setting, multiple phase III randomised control trials have shown equivalent OS and time to disease progression for capecitabine and 5FU/LV in the management of metastatic colorectal cancer (26-29).

In breast cancer, capecitabine is used in metastatic disease rather than as adjuvant treatment, predominantly as first-line palliative chemotherapy in patients with hormone sensitive metastatic breast cancer (30, 31). A pooled data analysis from multiple phase II/III trials of capecitabine mono-therapy from 1996 to 2008 demonstrated acceptable response rate and higher objective response rates in the first-line compared with the later lines (ORR: 25.0 vs. 19.0 %, respectively, odds ratio 0.70; 95 % CI: 0.5-1.0) (32) with higher rates of OS and PFS in patients with HFS ($p < 0.0001$ PFS/OS) or diarrhoea ($p = 0.004$ OS; $p = 0.0045$ PFS) compare with patients without these toxicities.

3.3 Influence of ageing on pharmacokinetics of capecitabine and its metabolites

There is limited data on the PKs of capecitabine and its metabolites in the older adults with cancer. The results of the few published studies are conflicting. Louie et al investigated the PKs of single agent capecitabine in the treatment of a small group of older adults with colorectal cancer (n=29). They showed a large variability in capecitabine clearance (CL/F) and volume of distribution (Vd/F) among older patients (>70 years), compared with younger patients (<60 years), but no difference in the PK parameters of 5'DFCR, 5'DFUR, or 5-FU between the two age groups (33). Abdi et al compared the capecitabine PK data of 20 older patients with breast or colorectal cancer (aged >75 years) with 40 younger patients (aged <60 years) from two previous clinical trials (2, 34). This study showed a slower rate of absorption of capecitabine in older patients, but no difference in the clearance of capecitabine and its metabolites between older and younger patients. Higher PK parameters were correlated with a higher toxicity rate and a lower absorption rate constant (ka) of capecitabine in older versus younger patients (2). Cassidy et al showed no impact of age, gender, BSA or creatinine clearance on PK parameters of capecitabine and its metabolites in adult patients (n=25) with solid tumours (35). The sample size was, however, small and this study was not designed to compare the PKs of capecitabine between older and younger patients. The US FDA conducted a pooled data analysis of 505 patients with metastatic colorectal cancer, which demonstrated a 15% increase in AUC of FBAL, (a-fluoro-b-alanine) the major renally-excreted metabolite, per 20% increase in age. There was no impact of age on the PK of 5-FU or other metabolites, and so the FDA does not recommend specific dose adjustments of capecitabine based on older age (23).

3.4 Enzymatic marker activity in plasma and their correlation with capecitabine toxicity

3.4.1 Cytidine deaminase

Cytidine deaminase converts 5'DFCR to 5'DFUR. A genetic polymorphism in the promoter region of the cytidine deaminase gene, leading to increased expression of the enzyme, has been associated with increased susceptibility to the common toxicity of hand-foot syndrome from capecitabine (36). In a young patient with metastatic corticosurrenaloma (a rare adrenocortical cancer), cytidine deaminase extensive metabolizer phenotype was associated with early severe toxicities from capecitabine (37). Cytidine deaminase activity is easily measured in serum or plasma, where a deficiency has been correlated with

significant toxicities from gemcitabine, a drug that relies on the enzyme for its elimination (38, 39). Whilst these data suggest cytidine deaminase expression and activity has a significant role in the quantity of 5-FU production leading to susceptibility to capecitabine toxicity, the actual effects of enzyme phenotype on the PK parameters of capecitabine and its metabolites have not been shown. Moreover, the impacts of ageing on cytidine deaminase activity have not as yet been examined, nor the assay's utility in predicting capecitabine toxicity in the older adults with cancer.

3.4.2 Dihydropyrimidine dehydrogenase (DPD)

Deficiency in DPD, the key 5-FU metabolic enzyme, is the most well-known biochemical cause of intolerance to fluoropyrimidines (40) . Dose individualisation based on polymorphisms in DPYD, the gene encoding DPD, by upfront screening for the most well-known variant, IVS14þ1G4A (DPYD*2A), improves the safety of 5-FU in patients (41, 42). DPYD genotyping, however, has suboptimal sensitivity and positive predictive value (PPV) (40-80%) (43). Upfront measurement of DPD phenotype has therefore been evaluated and shown the potential to identify patients at risk of severe and potentially fatal fluoropyrimidine-associated toxicity. These measurements on a routine basis have not been implemented as they are technically and logistically challenging, labour intensive, and expensive (44). Meulendijks et al recently investigated the predictive value of serum uracil concentration compared with dihydrouracil/uracil ratio and pharmacogenetic variants of DPYD in predicting severe toxicity in 550 patients treated with fluoropyrimidines. They demonstrated that the simple pre-treatment serum uracil concentration was superior to other markers as a predictor of severe toxicity. Uracil concentrations (>16 ng/ ml) were strongly correlated with global severe toxicity (OR 5.3, P=0.009). They concluded that pre-treatment measurement of uracil concentration is a simple, reliable and highly promising phenotypic marker to identify patients at risk of severe fluoropyrimidine-associated toxicity (45).

3.5 Therapeutic drug monitoring of fluoropyrimidines

There is no data in the literature on PK-guided dosing of capecitabine in patients with cancer. There is, however, promising data on the therapeutic drug monitoring (TDM) of 5FU/LV (the active metabolite of capecitabine). Gamelin et al evaluated PK-guided dosing of 5FU/LV in a phase III, multi-centre, randomized trial of patients with metastatic colorectal cancer (n = 208) compared with conventional dosing. The objective response rate was 33.7% in the PK-guided arm versus 18.3% in the conventional dosing arm (P = 0.004). There was a trend to a higher median OS rate of 22 months compare with 16 months in the conventional arm (P = 0.08). Adverse events were significantly less frequent and severe in the PK-guided arm compared with the conventional dosing arm (P = 0.003) (13). A subsequent phase II study by the same group evaluated PK-guided dosing of 5FU compared with body surface area (BSA) dosing in patients with colorectal cancer. Again, the PK-guided dosing arm had a significantly higher objective response rate of 70% versus 46% in the BSA dosing arm (P <0.0001). The median OS and median PFS in PK–guided arm were 28 and 16 months, respectively, compared with 22 and 10 months In the BSA-guided dosing arm. Grade 3 or 4 toxicity was significantly lower in the PK-adjusted arm (46).

TDM is an excellent way to personalise dosing and reduce adverse effects, the logistics of obtaining and transporting blood samples to the laboratory facilities can be difficult, particularly in regional centres. Further, 5-fluorouracil is unstable in whole blood and plasma at room temperature and so venous samples of 5-fluorouracil need to be placed on ice and centrifuged to analyse plasma immediately or kept frozen for delayed analysis. This process is not clinically feasible and meaning TDM is underutilised.

Means of overcoming the limitations of venous blood sampling for TDM include the use of fingerpick sampling and blood collection devices such as dried blood spot (DBS) cards, Mitra devices and Noviplex cards. These devices use a simple finger prick to produce a small drop of blood that is either drawn up into the device (Mitra) or placed on a card (DBS and Noviplex). The sample can then be easily sent to the laboratory for analysis. The feasibility of patients using finger prick sampling techniques to sample their blood for analysis has been demonstrated in the measurement of carotenoids and vitamin D in over 4000 patients with breast cancer but not with chemotherapy (47).

3.6 Prediction of treatment toxicity with frailty markers in older adults with cancer

In addition to PK-guided dosing, minimising chemotherapy-toxicity experienced by older adults with cancer can be achieved by improvement in the selection of older adults for treatment. Geriatric assessment (GA) tools provide an overview of older adults' general health and their physiological (versus chronological) age and frailty. Available tools include a Mini Nutritional Assessment (MNA)(48), the six-item Orientation-Memory-Concentration-test (OMC)(49) a complex geriatric assessment (CGA) (50), screening tools to identify patients in need of a CGA [eg the G8 (15)] and prediction tools that identify patients at risk of frailty [eg CSHA score (51)]. The main issue with the CGA is that it is resource intensive and time consuming and not feasible for routine, clinical practice. There are no published data about the association between GA tools and the PKs of chemotherapy including capecitabine. Such an association would mean oncologists could use GA tools on older adults at baseline (before chemotherapy) to identify individuals at risk of PK variability leading to excess toxicity and so warranting individualised dosing. This study will use an abbreviated geriatric assessment with demonstrated feasibility in a recent study of 127 older adults with cancer at Concord Hospital and the Chris O'Brien Lifehouse (52).

4. Aims

The aims of this research project are to:

determine and compare the PK of capecitabine and its metabolites in older and younger adults with cancer, and the correlation between enzymatic activity and PKs of capecitabine and its metabolites with chemotherapy-related toxicity, inflammatory markers, hospitalisations, dose modifications, health-related quality of life and GA tools.

1. investigate whether capecitabine can be measured in a fingerprick blood sample, and the stability of this.

5. Hypotheses

The hypotheses are that the:

1. PK profile of capecitabine is likely different in older adults with cancer compared with their younger counterparts, the PK profile of its metabolites are similar between older and younger adults with cancer

   the PK parameters of capecitabine are closely correlated with chemotherapy-related toxicity and GA tools, and
2. blood concentrations of capecitabine can be determined in capillary blood samples collected using minimally invasive finger prick techniques.

6. Objectives

To determine the:

i. PKs of capecitabine and its metabolites (5'DFCR, 5'DFUR, 5-FU and FBAL) in adults with cancer
ii. correlation of the PKs of capecitabine and its metabolites with enzymatic markers (cytidine deaminase, uracil)
iii. differences, if any, in PK parameters of capecitabine and its metabolites between older and younger adults with cancer
iv. association between PK parameters of capecitabine and its metabolites and chemotherapy-related toxicity and GA tools.
v. the relationship between finger prick blood samples and venous blood samples in patients receiving capecitabine
vi. acceptability of finger prick sampling to patients with cancer having capecitabine chemotherapy

6.1 Primary Endpoint

PK parameters [eg Area Under the Curve (AUC), Volume of distribution (Vd), clearance (CL), maximum concentration (Cmax), and half-life (T1/2)] of capecitabine and its metabolites (5'DFCR, 5'DFUR, 5-FU and FBAL) and their covariance with age

6.2 Secondary Endpoints

i. Plasma cytidine deaminase activity
ii. Plasma uracil activity
iii. Grade 3 or 4 chemotherapy-related toxicity
iv. Dose modifications, hospitalisations, palliation (treatment cessation) or death
v. Health-related quality of life
vi. GA tools (G8, MNA, short OMC and polypharmacy)
vii. Efficacy outcomes eg overall survival (in patients with metastatic cancer)
viii Inflammatory markers [C-reactive protein (CRP), absolute neutrophil count, absolute lymphocyte count, neutrophil/ lymphocyte ratio (NLR)]
ix Correlation between concentrations of capecitabine measured in finger prick blood samples and venous blood concentrations
x satisfaction with and preferences for finger prick sampling (over venous blood sampling)

7. Study Design

7.1 Study setting

This is a prospective observational study to be conducted at the Concord Repatriation General Hospital, Bankstown hospital and the Dubbo Hospital. Extra sites may be added to assist recruitment.

7.2 Study Population

The sample population is new patients aged ≥18 years with a histologically or cytologically confirmed diagnosis of breast or gastrointestinal cancer (gastric, pancreas, colorectal, biliary), who are seen in clinic by an oncologist at the participating site(s) and planned for treatment with capecitabine (adjuvant or palliative) either as mono-therapy or in combination with other anticancer drugs and consent to the study. The definition of an older adult for this study is age ≥ 70 years (5).

7.2.1 Inclusion criteria

- Patients aged 18 years and over with histologic or cytologic diagnosis of breast or gastrointestinal cancer planned for treatment with capecitabine (adjuvant or palliative) either as mono-therapy or in combination with other anticancer drugs.
- Estimated life expectancy of greater than 3 months
- ECOG performance status of 0 to 2

7.2.2 Exclusion criteria

- Pregnant or breastfeeding
- Insufficient English language
- Under 18 years of age
- A significant pre-existing hepatic or renal disease
- Any condition or disease that might affect oral absorption of medications, including:
    - Crohn's disease
    - Ulcerative colitis
    - Major gastric or small bowel resection

7.3 Sample size determination

Assuming a normal distribution of capecitabine AUCs, according to the observation by Louie et al. (33), group sample sizes of 12 and 12 achieve 80% power to reject the null hypothesis of equal means when the population mean difference is μ1 - μ2 = 4098.0 - 10238.0 = -6140.0 with standard deviations of 2852.0 for group 1 and 6355.0 for group 2, and with a significance level (alpha) of 0.050 using a two-sided two-sample unequal-variance t-test. To account for dropouts and incomplete data sets, 18 patients less than 70 years old and 18 patients 70 years or older (a total of 36 patients) will be recruited.

7.4 Recruitment

Participants will be recruited via personal communication with either their treating oncologist or a research team member if they are prescribed capecitabine for their cancer as part of their usual care.

7.5 Reimbursement

Patients will not be reimbursed for taking part in this study.

7.6 Treatment regimen

The clinical management of the patients will not be altered or modified in anyway by this observational study. Participants will be taking capecitabine as part of their usual care prescribed by their treating oncologist.

7.7 Materials and supplies

The patient's own supplies of capecitabine will be used. Vacutainers and laboratory materials needed for the pharmacokinetic assays will be provided by the Concord Cancer Centre, and then will be transferred to the pharmacology lab at the University of Newcastle, where the samples will be assayed.

7.8 Duration and discontinuation of therapy

This study will have no impact on treatment decisions about capecitabine. Capecitabine will be continued and discontinued as per the treating oncologist according to usual clinical practice.

7.9 Ethical considerations

The study will comply with legal requirements and the Declaration of Helsinki and will seek approval from the regional ethics committee.

8. Study procedures

Participants will be asked to have a pre-treatment blood test to measure their plasma uracil level and inflammatory markers and then to return to the clinic on day 14 of cycle one (when they will be in steady state) and day 14 of cycle two for the study session. They will be asked to not take their morning dose of capecitabine at home on the day of the study session, but to bring their capecitabine tablets with them to take at the study session. Patients are allowed to take their regular medication as per their doctor's recommendations. On the morning of the study session, a cannula will be placed and a time zero blood sample will be drawn into an EDTA tube after which the patient will take their morning dose of capecitabine. Venous blood samples will then be taken 1 hour, 2 hours and 4 hours after dosing (3). . Additionally at 1 hour, finger prick blood samples will be obtained using lancet and 2 Mitra devices which draw up 10uL of capillary each. The 2 Mitra devices will be sealed in the plastic holder and the holder labelled with MRN, date, time and the words "finger prick samples". The concentration of capecitabine and its primary metabolites, 5'DFCR, 5'DFUR, 5-FU and FBAL, will be measured in the samples after plasma has been separated. During the initial pre-treatment study session, the study personnel will administer the GA tools (patients aged over 70 years will have G8, MNA and short OMC) (see Appendix 16.5). The investigator will complete a concise data collection form from the medical records of the patient including medication list

(poly-pharmacy) on this day of sampling. The patients will be followed for chemotherapy-related toxicity, dose modifications and hospitalisations at the start of every cycle for up to 6 months as described below.

8.1 Sample Collection and preparation

All venous blood samples will be gently mixed by study personnel. Venous blood samples will be used to complete/fill two Mitra devices with the container of the Mitra devices labelled with MRN, date, time and "venous blood". The venous blood samples then will be immediately stored on ice (4°C) prior to centrifugation at 2000g for 10 min and plasma will be harvested before being stored at -80°C until analysis (2, 35). The exact time that each sample was taken will be recorded on the sampling sheet (See Appendix 16.4)

In addition, residual blood samples for routine FBC (blood counts) and EUC (blood electrolyte) assessment of patients participating in this study will be collected for analysis.

8.2 Sample analysis

Plasma samples will be analysed for capecitabine, 5-DFCR, 5-DFUR, 5-FU and FBAL using LC/MS-MS as described by Dennen et al 2013 (53). Two individual assays will be used: one for the simultaneous quantification of capecitabine, 5-DFCR and 5-DFUR using reverse phase chromatography and gradient elution, and one assay for 5-FU and FBAL using hydrophilic interaction chromatography and isocratic elution. The PK parameters of capecitabine, 5'DFCR, 5'DFUR, 5-FU and FBAL will be measured by non-compartmental analysis and means compared between subjects less than 70 years old and 70 years old and older by a 2-sided t test (if parameters are normally distributed) or by nonparametric analysis (if not normally distributed).

A population pharmacokinetic analysis will be undertaken using nonlinear mixed effects modeling to evaluate the effects of covariates including age, creatinine clearance, and liver function on the clearance, absorption parameters, and overall PKs of capecitabine and its metabolites. Upon development of the initial model, covariates will be added and removed in a stepwise fashion and assessed for a significant effect on the model by the effect of their addition and removal on the objective function value. The final model will be assessed using a bootstrap resampling method.

Exploratory multivariate analyses will be undertaken to examine the correlation between the chronological age and drug toxicity.

Up to 10 sample pair sets (fingerprick and venous blood sample) will be collected for the fingerprick sampling validation. The concentration data for each set will be analysed using a Bland-Altman plot to investigate correlation in line with standard practice for comparison of assays.

9. Data collection form

A data collection form will be used to extract key data from the patient's medical records including demographics, medical and medication history, the management of cancer including chemotherapy protocols (see Appendix 12.3). The information regarding any subsequent changes to capecitabine dose and reason(s) for change, hospitalisation or death due to disease progression or disease-related complications and

toxicities due to capecitabine and grade of toxicity will be recorded regularly after the study session as part of follow-up.

10. End of study

The study ends when the follow up assessments have been completed on all subjects.

11. Withdrawal from the study

In obtaining informed consent, the study investigator will provide the potential participant with information about the purposes, methods, possible risks and benefits of participating in the study (See appendix 16.2). All potential participants will have an opportunity to discuss the study with the investigator. The participant and the person obtaining informed consent will each sign and date two copies of the consent form, one copy of which will be provided to the participant and the other copy will be kept by the study site. Participation in the study is voluntary and all participants are free to withdraw at any time, without consequence to their future care.

12. Confidentiality

Participants will be assigned a random number and thus de-identified. Hard copy consent forms and data collection forms will be stored in a locked cabinet in a secured room. All information obtained from participants will be coded in spread sheets and password protected. Data will be kept for 15 years and then disposed of through deletion (in the case of computer files) or appropriately disposed of in waste (for clinical samples).

13. Retention of study documentation

The Investigator at each study site will retain study essential documents, including subject clinical source documents, for a required period of time after completion or discontinuation of the study according to local regulations. The study documents will be archived at the end of the study in accordance with local standard operating procedures. After this period of time the documents will be destroyed.

14. Expected side effects of the blood sampling

There is a small risk of infection and a risk of bruising and/or bleeding as a result of venepuncture required for the additional blood samples.

15. Authorship

The results of this research will be published in peer-reviewed journals and included in Dr Shafiei's PhD thesis. Authorship will include all principal investigators of this research and others who have made a significant contribution (based on the Vancouver statement by the International Committee of Medical Journal Editors).

16. References

1. Bajetta E, et al. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(10):2155-61.
2. Daher Abdi Z, et al. Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. Cancer chemotherapy and pharmacology. 2014;73(6):1285-93.
3. Gieschke R, et al. Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. British journal of clinical pharmacology. 2003;55(3):252-63.
4. Australian Institute of Health and Welfare & Australasian Association of Cancer Registries 2012.Cancer in Australia: an overview, 2012. Cancer series no. 74. Cat. no. CAN 70. AIHW.
5. Wildiers H, et al. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(24):2595-603.
6. Crombag MR, et al. Pharmacokinetics of Selected Anticancer Drugs in Elderly Cancer Patients: Focus on Breast Cancer. Cancers. 2016;8(1).
7. Korc-Grodzicki B, et al. Prescribing for older patients with cancer. Clinical advances in hematology & oncology : H&O. 2014;12(5):309-18.
8. Korc-Grodzicki B, et al. Geriatric assessment for oncologists. Cancer biology & medicine. 2015;12(4):261-74.
9. Griggs JJ, et al. Appropriate chemotherapy dosing for obese adult patients with cancer: American Society of Clinical Oncology clinical practice guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(13):1553-61.
10. Alnaim L. Therapeutic drug monitoring of cancer chemotherapy. Journal of oncology pharmacy practice : official publication of the International Society of Oncology Pharmacy Practitioners. 2007;13(4):207-21.
11. Paci A, et al. Review of therapeutic drug monitoring of anticancer drugs part 1--cytotoxics. European journal of cancer (Oxford, England : 1990). 2014;50(12):2010-9.
12. Engels FK, et al. Therapeutic drug monitoring for the individualization of docetaxel dosing: a randomized pharmacokinetic study. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(2):353-62.
13. Gamelin E, et al. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(13):2099-105.
14. Wasil T, Lichtman SM. Clinical pharmacology issues relevant to the dosing and toxicity of chemotherapy drugs in the elderly. The oncologist. 2005;10(8):602-12.
15. Ferrucci L, et al. The frailty syndrome: a critical issue in geriatric oncology. Critical reviews in oncology/hematology. 2003;46(2):127-37.
16. Klotz U. Pharmacokinetics and drug metabolism in the elderly. Drug metabolism reviews. 2009;41(2):67-76.
17. Wynne H. Drug metabolism and ageing. The journal of the British Menopause Society. 2005;11(2):51-6.
18. Hilmer SN, et al. Clinical pharmacology in the geriatric patient. Fundamental & clinical pharmacology. 2007;21(3):217-30.
19. McLachlan AJ, et al. Variability in response to medicines in older people: phenotypic and genotypic factors. Clinical pharmacology and therapeutics. 2009;85(4):431-3.
20. Gault MH, Cockcroft DW. Letter: Creatinine clearance and age. Lancet (London, England). 1975;2(7935):612-3.
21. Siersbaek-Nielsen K, et al. Rapid evaluation of creatinine clearance. Lancet (London, England). 1971;1(7709):1133-4.

22. Wilkinson GR. Drug metabolism and variability among patients in drug response. The New England journal of medicine. 2005;352(21):2211-21.
23. Miles DR, et al. Population pharmacokinetics of motexafin gadolinium in adults with brain metastases or glioblastoma multiforme. Journal of clinical pharmacology. 2005;45(3):299-312.
24. Maroun JA. Capecitabine in the management of colorectal cancer. Expert review of anticancer therapy. 2001;1(3):327-33.
25. Twelves C, et al. Capecitabine versus 5-fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: final results from the X-ACT trial with analysis by age and preliminary evidence of a pharmacodynamic marker of efficacy. Annals of oncology : official journal of the European Society for Medical Oncology. 2012;23(5):1190-7.
26. Twelves C. Capecitabine as first-line treatment in colorectal cancer. Pooled data from two large, phase III trials. European journal of cancer (Oxford, England : 1990). 2002;38 Suppl 2:15-20.
27. Van Cutsem E, et al. Oral capecitabine vs intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomised, phase III trials. British journal of cancer. 2004;90(6):1190-7.
28. Van Cutsem E, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(21):4097-106.
29. Hoff PM, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(8):2282-92.
30. Minea L, et al. Capecitabine monotherapy for elderly patients with metastatic breast cancer. Journal of Clinical Oncology. 2004;22(14_suppl):797-.
31. O'shaughnessy J, et al. Randomized, open-label, phase II trial of oral capecitabine (Xeloda®) vs. a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. Annals of Oncology. 2001;12(9):1247-54.
32. Blum JL, et al. Pooled analysis of individual patient data from capecitabine monotherapy clinical trials in locally advanced or metastatic breast cancer. Breast cancer research and treatment. 2012;136(3):777-88.
33. Louie SG, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). British journal of cancer. 2013;109(7):1744-9.
34. Urien S, et al. Pharmacokinetic modelling of 5-FU production from capecitabine--a population study in 40 adult patients with metastatic cancer. Journal of pharmacokinetics and pharmacodynamics. 2005;32(5-6):817-33.
35. Cassidy J, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. Cancer chemotherapy and pharmacology. 1999;44(6):453-60.
36. Caronia D, et al. A polymorphism in the cytidine deaminase promoter predicts severe capecitabine-induced hand-foot syndrome. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(7):2006-13.
37. Mercier C, et al. Early severe toxicities after capecitabine intake: possible implication of a cytidine deaminase extensive metabolizer profile. Cancer chemotherapy and pharmacology. 2009;63(6):1177-80.
38. Ciccolini J, et al. Cytidine deaminase residual activity in serum is a predictive marker of early severe toxicities in adults after gemcitabine-based chemotherapies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(1):160-5.
39. Mercier C, et al. Toxic death case in a patient undergoing gemcitabine-based chemotherapy in relation with cytidine deaminase downregulation. Pharmacogenetics and genomics. 2007;17(10):841-4.
40. van Kuilenburg AB. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. European journal of cancer (Oxford, England : 1990). 2004;40(7):939-50.

41. Caudle KE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics. 2013;94(6):640-5.
42. Deenen MJ, et al. Upfront Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34(3):227-34.
43. Terrazzino S, et al. DPYD IVS14+1G>A and 2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. Pharmacogenomics. 2013;14(11):1255-72.
44. Van Kuilenburg AB, et al. Pitfalls in the diagnosis of patients with a partial dihydropyrimidine dehydrogenase deficiency. Clinical chemistry. 2000;46(1):9-17.
45. Meulendijks D, et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. British journal of cancer. 2017;116(11):1415-24.
46. Capitain O, et al. Individual fluorouracil dose adjustment in FOLFOX based on pharmacokinetic follow-up compared with conventional body-area-surface dosing: a phase II, proof-of-concept study. Clinical colorectal cancer. 2012;11(4):263-7.
47. Sakhi AK, et al. Feasibility of self-sampled dried blood spot and saliva samples sent by mail in a population-based study. BMC cancer. 2015;15(1):265.
48. Vellas B, et al. The Mini Nutritional Assessment (MNA) and its use in grading the nutritional state of elderly patients. Nutrition (Burbank, Los Angeles County, Calif). 1999;15(2):116-22.
49. Goring H, et al. Validation of short screening tests for depression and cognitive impairment in older medically ill inpatients. International journal of geriatric psychiatry. 2004;19(5):465-71.
50. Ellis G, et al. Comprehensive geriatric assessment for older adults admitted to hospital: meta-analysis of randomised controlled trials. BMJ (Clinical research ed). 2011;343:d6553.
51. Rockwood K, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2005;173(5):489-95.
52. Deenen MJ, et al. Quantitative determination of capecitabine and its six metabolites in human plasma using liquid chromatography coupled to electrospray tandem mass spectrometry. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2013;913-914:30-40.

53. Moth EB, et al. Predicting chemotherapy toxicity in older adults: Comparing the predictive value of the CARG Toxicity Score with oncologists' estimates of toxicity based on clinical judgement. Journal of geriatric oncology. 2018.

17.1 Consent form

Faculty of Medicine
The University of Sydney

Study Title: Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

Principal Investigator: Dr Mohsen Shafiei
Location: ☐ Concord ☐ Dubbo Hospital ☐ Bankstown Hospital

PARTICIPANT CONSENT FORM

I, ……[name] of

……[address]

have read and understood the Information for Participants for the above named research study and have discussed the study with Dr Mohsen Shafiei/study investigator(s).

- I have been made aware of the procedures involved in the study, including any known or expected inconvenience, risk, discomfort or potential side effect and of their implications as far as they are currently known by the researchers.
- I consent to giving access to blood samples and medical records.
- I freely choose to participate in this observational study and understand that I can withdraw at any time.
- I also understand that the research study is strictly confidential.
- I hereby agree to participate in this research study.

Name (Please Print):…….

Signature:…… **Date:** ……

Declaration by Study Doctor/Senior Researcher:

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of person who conducted informed consent discussion (Please Print):…….

Signature:…… **Date:** ……

17.2 Participant Information Sheet

Faculty of Medicine
The University of Sydney

Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

PARTICIPANTS INFORMATION SHEET

You are invited to take part in a research study that will investigate the possible effect of age on how the body breaks down (metabolises) the anticancer medicine capecitabine in people living with breast or gastrointestinal cancer.

What is this study about?

adults with cancer receiving capecitabine (Xeloda) chemotherapy by better understanding how age affects how the body breaks down (metabolism) this drug. This will also help doctors in the future to choose the best dose for a patient having capecitabine to reduce the risk of under-dosing and possible less effective treatment and over-dosing and possible serious side effects. This study will measure concentrations of capecitabine in blood samples from participants to study the metabolism (breakdown) of capecitabine. The study will include both younger adults (less than 70 years) and older adults (70 years or older) to see how age affects the metabolism of capecitabine.

You have been asked to take part because you have been prescribed capecitabine chemotherapy for your cancer treatment and are above the age of 18 years. In this study, there will be no alteration to your usual treatment with regards to dosing or chemotherapy treatment. Extra blood samples will be collected to measure capecitabine levels in your body.

The study is being conducted by Dr Mohsen Shafiei, Prof Alan Boddy, Prof. Andrew McLachlan, A/Prof. Phillip Beale and Dr Prunella Blinman from the Concord Cancer Centre, Concord Repatriation General Hospital and the University of Sydney and Dr Peter Galettis from the University of Newcastle.

This study is supported by research funds from the Concord Cancer Centre.

Who can enter this study (inclusion and exclusion criteria)?
If you are over the age of 18 years, have breast cancer or gastrointestinal cancer and planned for treatment with capecitabine, you are potentially eligible for this study (inclusion criteria). If you have known liver or kidney disease, are pregnant or nursing a baby, or are unable to provide written consent then you cannot enter this study (exclusion criteria).

What does this study involve?

This study does not involve any change to your treatment plan, nor does it involve adding or removing any medications from your medication regimen. If you agree to participate in this study after discussion with your usual oncologist, a face to face interview will take place. During the course of this interview you will have the opportunity to discuss all aspects of the study and to have the remaining questions you may have answered. If you agree, consent will need to be given prior to the study and, then the date and time of conducting the study will be discussed. You will be asked not to take any herbal or complementary medicines during the study. You can bring your regular medications to the study session and take them as per your doctor's recommendation. Light refreshment will be provided to you during the study session.

Before you start any capecitabine, you will be asked to have a blood sample (10mL) to check the level of uracil (an enzyme that helps break down drugs). **On the two occasions that you visit the ☐ Concord ☐ Dubbo Cancer ☐ Bankstown Hospital Centres for the study session [on the 14th day after you start capecitabine (of cycle 1 where 1 cycle is 21 days) and on the 14th day of cycle 2]** you are asked to;

- attend the Concord or Dubbo Cancer Centres (for approximately 5 hours)
- bring the capecitabine tablets provided as part of your chemotherapy treatment to the study session
- have a blood sample (10 mL) taken before taking your capecitabine morning dose
- have a cannula (small flexible tube) inserted into your vein so that 3 more blood samples (10 mL) could be taken over 4 hours.
- have a finger prick blood sample using a lancet at hour one

This study will take approximately 5 hours and it is important that NO capecitabine other than that provided by the hospital is consumed during this period either before or after taking the capecitabine tablets. The study investigators will collect medical information from your records for details about your cancer. The study investigators will record any chemotherapy-related toxicity, changes in the dose of your capecitabine, admissions to hospital, and palliation (treatment cessation) at the start of every cycle for up to 6 months.

What are the risks associated with this study?

You are already taking capecitabine as part of your cancer treatment. There will be no alteration to your usual treatment with regards to dosing or chemotherapy treatment. This study involves the collection of blood samples which does have some minor risks such as pain, discomfort and possible bruising at the site of sampling. For convenience, this can be at the same time that you have your follow up blood collections.

What are the benefits of this study?

to you. This study will help doctors in selecting the most appropriate dosing for capecitabine in people living with cancer.

What happens to my samples?

is. Drug analysis would include measurement of levels of capecitabine and its metabolites (break down products) and some natural enzymes necessary for the breakdown (metabolism) of capecitabine. The samples will be destroyed of through the clinical waste stream at the end of the study (after data publication, approximately 2 years) according to the national regulations.

Can I have other treatments during this research project?

Whilst you are participating in this research project, you may be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. If you experience any side effects from capecitabine you should contact your oncologist as soon as possible.

Can I obtain the results of tests on my sample?

The results of any tests done on your sample will not be made known to you or your family members only the research team members.

Can I withdraw from the study?
Participation in this study is entirely voluntary. You are in no way obliged to participate and - if you do participate - you can withdraw at any time. Whatever your decision, please be assured that it will not affect your relationship with medical staff. If you decide to withdraw from this research project, please notify a member of the research team before you withdraw.

Confidentiality
All details obtained by those named will remain confidential. A report of this study may be submitted for publication, but individual participants will not be identifiable in such a report.

Compensation
Every reasonable precaution will be taken to ensure your safety during the course of the study. In the event that you suffer any injury as a result of participating in this research project, hospital care and treatment will be provided at no extra cost to you.

Further Information
When you have read this information, the study investigator will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact Dr Mohsen Shafiei, research investigator from the University of Sydney on 97675000 or 68096809. This information sheet is for you to keep.

This study has been approved by the Human Research Ethics Committee - CRGH Zone of the Sydney Local Health District. If you have any concerns or complaints about the conduct of the research study, you may contact the Manager of the Concord Hospital Human Research Ethics Committee, on (02) 9767 5622. Alternatively, if you wish to speak with an independent person within the Hospital about any problems or queries about the way in which the study was conducted, you may contact the Patient Representative on (02) 9767 7488.

Faculty of Medicine
The University Of Sydney

17.3 Data collection form

Study Title: Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

Data Collection Form

Data Collection Site: Concord Repatriation General Hospital (CRGH)

Investigators:

Dr Mohsen Shafiei	**PhD candidate, Faculty of Medicine, University of Sydney** **Concord Cancer Centre, Concord Hospital, NSW, 2139 Australia**
A/Prof. Phillip Beale	**Faculty of Medicine, The University of Sydney, NSW, 2006 Australia** **Concord Cancer Centre, Concord Hospital, NSW, 2139 Australia**
Prof. Andrew McLachlan	**Professor of Pharmacy (Aged Care), Faculty of Pharmacy, University of Sydney and Centre for Education and Research on Ageing (CERA) at Concord Hospital, Australia.**
Dr Peter Galettis	**Head of Clinical Pharmacology Lab, School of Medicine, the University of Newcastle Australia**
Prof Alan Boddy	**Faculty of Pharmacy, the University of Sydney, NSW, 2006 Australia**
Dr Prunella Blinman	**Faculty of Medicine, The University of Sydney, NSW, 2006 Australia** **Concord Cancer Centre, Concord Hospital, NSW, 2139 Australia**

Data entry completed (tick box) [__]

Part 1 : PATIENT DEMOGRAPHICS

1(a) Patient's Code :

1(b) Age (years) :

1(c) Gender : 1 Male
2 Female

1(d) Weight (kg): 1(e) Height(cm):

1(f) BSA 1(g) eGFR

1(h) Capecitabine dose 1(j) Diagnosis

Part 2: MEDICAL HISTORY- Presence of Coexisting Medical Conditions

● Part 3 : MEDICATIONS Currently being administered including complementary and herbal medicines

Medication	Dosage regimen

Additional Comments:

The following information will be recorded after the follow-up session:

1) Any subsequent changes to capecitabine dose and reason(s) for change:

2) Toxicities due to capecitabine and grade of toxicity including hospitalisation or palliation or death:

17.4 Sampling Sheet

Faculty of Medicine

The University Of Sydney

Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

Dosage Information

Patient's code_____ Study Code: ____ (Filled by investigator)

Date: ____

Sample Number	Actual Sample Time
Pre dose	
1 h	
2 h	
4h	

Comments

(Study staff use only) *Study ID:* *Number of Finger Prick sampling:*

Please choose a number from 0 to 10 that best describes your current pain caused by finger prick sampling.

The far left end indicates "no pain" and the far right end indicates "worst possible pain".

0	1	2	3	4	5	6	7	8	9	10
No Pain										Worst possible pain

We would like to ask you some questions about your experience with finger prick sampling and venous blood sampling done as part of this study.

Please tick one box per line to indicate whether you prefer finger prick sampling or venous blood sampling or you are neutral for each given factor.

	Finger prick strongly preferred	Finger prick slightly preferred	Neutral (neither finger prick nor venous sampling preferred)	Venous sampling slightly preferred	Venous sampling strongly preferred	Did not apply to me
Pain from the sampling	☐	☐	☐	☐	☐	☐
Bleeding after the sampling	☐	☐	☐	☐	☐	☐
Convenience of the sampling	☐	☐	☐	☐	☐	☐
Distress caused by the sampling	☐	☐	☐	☐	☐	☐
Overall preference	☐	☐	☐	☐	☐	☐

Would you be willing to perform finger prick sampling at home?

Yes ☐ No ☐ Don't know ☐

Please write any comments you have about finger prick sampling and/ or venous blood sampling:

Faculty of Medicine
The University of Sydney

17.5. The Geriatric Assessment

Study Title: Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: A prospective observational study

Patient's code_____

Study Code: ____ (Filled by investigator) Date: ____

INVESTIGATOR ASSESSMENT – GERIATRIC ASSESSMENT

Participant number: _____

This Geriatric Assessment form is to be completed by the Principal Investigator. Some sections of the form are completed through direct questioning of the participant; other sections of the form are completed through direct observation and measurement, or through review of the clinical record. Explanatory notes are provided with each section. Text in italics and quotation marks is the suggested wording presented to participants (patients).

INTRODUCTION TO PATIENT

"Part of this study is to better describe the level of independence and function of the patients 65 years of age and older that we treat with chemotherapy. In order to do this, I will be taking you through a series of questions about you, including specific questions about your level of independence and available supports, your nutrition, your memory, and your physical abilities."

Section 1: DEMOGRAPHICS

"The first series of questions is to gather basic information about yourself."

How old are you? Age: _____ years

Sex: ☐ male ☐ female

Are you currently working (paid employment)?

☐ Yes ☐ no

Are you currently married, widowed, separated / divorced, or single?

□ Married □ widowed □ divorced / separated □ single

Are you presently living alone, or with others (partner, family, or friends)?

□ Lives alone □ lives with others

What language do you mainly speak at home?

□ English □ non-English

Do you receive any community services to help you at home?

□ Yes □ no

Section 2: GENERAL HEALTH

"In general, how would you rate your health today? Would you give it a rating of excellent, very good, good, fair, or poor?"

□ Excellent □ very good □ good □ fair □ poor

"In comparison with other people of the same age, how would you rate your health status?"

□ Not as good □ as good □ better □ does not know

Section 3: FUNCTIONAL STATUS

The Timed Up and Go

The Timed Up and Go is performed by the patient and observed by the Principal Investigator. It may be performed at the end of the assessment if logistically easier.

"One way of looking at a person's general health is to watch them walk. Would it be okay if I watched you walking up and down the corridor?"

Test instructions:

The participant is asked to sit in an armed chair. A mark on the floor is made 3 metres away.

The participant is instructed: *"On the word GO, you will stand up, walk to the line on the floor, turn around and walk back to the chair and sit down. Please walk at your own pace, there is no hurry."* (The patient's usual mobility aids should be used.)

The time to complete the task is recorded in seconds.

Timed Up and Go: _____ seconds

Timed Up and Go ≥ 14 seconds?

☐ Yes ☐ No

Activities of Daily Living – The Katz Index

This section is completed after direct questioning of the patient about their activities of daily living. The Katz Index of ADLs is provided as a guide for clarifying the level of assistance needed.

"Now I would like to ask you some questions about things we all need to do every day."

Are you able to bathe (wash / shower) without help, or do you need some help?

☐ Independent ☐ Dependent

Are you able to get dressed without help, or do you need some help?

☐ Independent ☐ Dependent

Are you able to go to the toilet without help, or do you need some help?

☐ Independent ☐ Dependent

Are you able to get in and out of bed by yourself, or do you need help?

☐ Independent ☐ Dependent

Do you have any trouble with losing control of your bladder or bowels, or need to use catheters or regular enemas?

☐ Independent ☐ Dependent

If you have a meal in front of you, are you able to feed yourself, or do you need some help?

☐ Independent ☐ Dependent

ACTIVITIES	INDEPENDENCE (0 point) No supervision, direction or personal assistance	DEPENDENCE (1 points) With supervision, direction, personal assistance or total care
Bathing	Assistance only in bathing a single body part or bathes self completely	Assistance in bathing more than one part, assistance getting in or out of tub, does not bathe self
Dressing	Gets clothes, puts them on, manages buttons etc; help with shoe laces allowed	Does not dress self or remains partly undressed
Toileting	Gets to toilet, gets on and off, cleans, manages clothing	Uses bedpan or commode or receives assistance getting to and using toilet
Transferring	Moves in and out of bed independently and in and out of chair independently (may have aids)	Needs assistance
Continence	Urination/defaecation entirely self-controlled	Partial or total incontinence, partial or total control by enemas/pans/catheters

Feeding	Gets food from plate into mouth (assistance with cutting up food allowed)	Assistance in act of feeding

Total points (0 - 6) = _____ *(0 = independent in all tasks 6 = dependent in all tasks)*

Instrumental Activities of Daily Living – The OARS Multidimensional Functional Assessment

"Now I'd like to ask you about some of the activities of daily living, things that we all need to do as part of our daily lives. I would like to know if you can do these activities without any help at all, or if you need some help to do them, or if you can't do them at all." (May use answer prompt sheet 1)

1. Can you use the telephone…
   - ☐ Without help, including looking up numbers and dialling
   - ☐ With some help (can answer phone or dial in an emergency, but need a special phone or help getting the number or dialling)
   - ☐ Or are you completely unable to use the telephone?

2. Can you get to places out of walking distance…
   - ☐ Without help (can travel alone on buses, taxis, or drive your own car)
   - ☐ With some help (need someone to help you or go with you when travelling)
   - ☐ Or are you unable to travel unless emergency arrangements are made like in an ambulance?

3. Can you go shopping for groceries or clothes (assuming you have transport)…
   - ☐ Without help (taking care of all shopping needs yourself, assuming you had transportation)
   - ☐ With some help (need someone to go with you on all shopping trips)
   - ☐ Or are you completely unable to do any shopping?

4. Can you prepare your own meals…
   - ☐ Without help (plan and cook full meals yourself)
   - ☐ With some help (can prepare some things but unable to cook full meals yourself)
   - ☐ Or are you completely unable to prepare any meals?

5. Can you do your housework…
   - ☐ Without help
   - ☐ With some help (can do light housework but need help with heavy work)
   - ☐ Or are you completely unable to do any housework?

6. Can you take your own medicine…
   - ☐ Without help (in the right doses at the right time)
   - ☐ With some help (able to take medicine if someone prepares it and / or reminds you)
   - ☐ Or are you completely unable to take your medicine?

7. Can you handle your own money…
   - ☐ Without help (write cheques, pay bills)
   - ☐ With some help (manage day to day buying but need help with bills)

- Or are you completely unable to handle money?

Score 2 for without help; Score 1 for with some help; Score 0 for answers with unable

Total score = _____ (range 0 to 14)

MOS-physical functioning measure

"Now I would like to ask you a few more questions about daily activities. The following are activities you might do during a typical day. I would like to know if your health limits you a lot, a little or not at all in doing these activities.

Does your health limit you in…" (May use answer prompt sheet 2)

1. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports?
Yes, limited a lot Yes, limited a little No, not limited at all

2. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf?
Yes, limited a lot Yes, limited a little No, not limited at all

3. Lifting or carrying groceries?
Yes, limited a lot Yes, limited a little No, not limited at all

4. Climbing several flights of stairs?
Yes, limited a lot Yes, limited a little No, not limited at all

5. Climbing one flight of stairs?
Yes, limited a lot Yes, limited a little No, not limited at all

6. Bending, kneeling or stooping?
Yes, limited a lot Yes, limited a little No, not limited at all

7. Walking more than one mile?
Yes, limited a lot Yes, limited a little No, not limited at all

8. Walking several blocks?
Yes, limited a lot Yes, limited a little No, not limited at all

9. Walking one block?
Yes, limited a lot Yes, limited a little No, not limited at all

10. Bathing or dressing yourself?
Yes, limited a lot Yes, limited a little No, not limited at all

Score 1 for limited a lot; Score 2 for limited a little; Score 3 for not limited at all

Total Score: _____ (range 10 to 30)

Section 4: COMORBIDITY AND POLYPHARMACY

"Do you have any medical problems in addition to a diagnosis of cancer?"

_____
_____
_____
_____

The Cumulative Illness Rating Scale – Geriatrics (CIRS-G)

The Investigator will complete the CIRS-G through review of the EMR and clarification, where necessary, with the participant based on the above question.

Refer to the CIRS-G manual for further instructions	
Rating of comorbidities: 0 – No problem 1 – Current mild problem or past significant problem 2 – Moderate disability or morbidity / requires "first line" therapy 3 – Severe / constant significant disability / "uncontrollable" chronic problems 4 – Extremely severe / immediate treatment required / end organ failure / severe impairment in function	
	Score
Heart	
Vascular	
Haematopoietic	
Respiratory	
Eyes, ears, nose and throat	
Upper gastrointestinal	
Lower gastrointestinal	
Liver	
Renal	
Genitourinary	
Musculoskeletal	
Neurological	
Endocrine / metabolic and breast	
Psychiatric illness	

Total number categories endorsed = _____

Total score = _____

CIRS-G Severity Index Score (range 0-4) = _____
(total score / number of categories endorsed)

Polypharmacy

"How many medications are you taking at home?" (Includes prescription and over-the-counter)
Medication count: _____

Section 5: COGNITION

The Short Blessed Test (Orientation Memory Concentration)

"Now I would like to ask you some questions to check your memory and concentration. Some of them may be easy and some of them may be hard."

1. What year is it now? _____
   □ Correct (0) □ Incorrect (1)

2. What month is it now? _____
   □ Correct (0) □ Incorrect (1)

Please repeat this name and address after me (repeat until learnt):
John Brown, 42 Market Street, Chicago

Good, now remember that name and address for a few minutes.

3. Without looking at your watch or clock, tell me about what time it is. (within one hour)
   □ Correct (0) □ Incorrect (1)

4. Count aloud backwards from 20 to 1
   20 19 18 17 16 15 14 13 12 11

   10 9 8 7 6 5 4 3 2 1

   (Mark correctly sequenced numbers)

   □ 0 errors □ 1 error □ 2 errors

5. Say the months of the year in reverse order. Start with the last month of the year. The last month of the year is…
   D N O S A JL JN MY AP MR F J

   □ 0 errors □ 1 error □ 2 errors

6. Repeat the name and address I asked you to remember. (John, Brown, 42, Market, Chicago)
   □ 0 errors □ 1 error □ 2 errors □ 3 errors □ 4 errors □ 5 errors

Item	Errors (0 - 5)	Weighting factor	Item score
1		X 4	
2		X 3	
3		X 3	
4		X 2	
5		X 2	
6		X 2	

TOTAL SCORE = **_____** (range 0 to 28)

Section 6: PSYCHOSOCIAL FUNCTION

"The next few questions relate to how you are feeling."

The Geriatric Depression Scale 5-Item Short Form

Item	Score	
Are you basically satisfied with your life?	Yes	No
Do you often get bored?	Yes	No
Do you often feel helpless?	Yes	No
Do you prefer to stay home rather than going out and doing new things?	Yes	No
Do you feel pretty worthless the way you are now?	Yes	No
"No" to question 1 is scored as 1. *"Yes" to questions 2,3,4,5 are scored as 1.*		

Total score = _____ *(A score of 2 or higher indicates possible depression.)*

The Modified MOS Social Support Survey – tangible subscale

"People sometimes look to others for companionship, assistance, or other types of support. How often is each of the following kinds of support available to you if you need it?" (May use answer prompt sheet 3)

1. Someone to help you if you were confined to bed?

None of the time	A little of the time	Some of the time	Most of the time	All of the time
1	2	3	4	5

2. Someone to take you to the doctor if you need it?

None of the time	A little of the time	Some of the time	Most of the time	All of the time
1	2	3	4	5

3. Someone to prepare your meals if you are unable to do it yourself?

None of the time	A little of the time	Some of the time	Most of the time	All of the time
1	2	3	4	5

4. Someone to help with daily chores if you were sick?

None of the time	A little of the time	Some of the time	Most of the time	All of the time
1	2	3	4	5

Total score: _____ (range 4 to 20)

Section 7: NUTRITION

Height:	_____m
Weight:	_____kg
Body Mass Index:	_____kg/m^2

"Have you lost any weight (without trying) in the last 6 months?"

☐ YES ☐ NO

Mini Nutritional Assessment – Short Form

	Item	Scoring	Score
A	Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?	0 = severe decrease in food intake 1 = moderate decrease in food intake 2 = no decrease in food intake	
B	Weight loss during the last 3 months	0 = weight loss greater than 3kg 1 = does not know 2 = weight loss between 1 and 3kg 3 = no weight loss	
C	Mobility	0 = bed or chair bound 1 = able to get out of bed / chair but does not go out 2 = goes out	
D	Has suffered psychological stress or acute disease in the past 3 months?	0 = yes 2 = no	
E	Neuropsychological problems	0 = severe dementia or depression 1 = mild dementia 2 = no psychological problems	
F	Body mass index	0 = BMI <19 1 = BMI 19 to < 21 2 = BMI 21 to < 23 3 = BMI 23 or greater	

Screening score = _____ (maximum 14 points)

12 to 14 points: normal nutritional status

8 to 11 points: at risk of malnutrition

0 to 7 points: malnourished

Section 8: SCREENING INSTRUMENTS

THE G8 SCREENING QUESTIONNAIRE

The G8 Screening Questionnaire can be completed in its entirety using data obtained within the Geriatric Assessment. Location of data is indicated in parentheses under each Item description.

Item	Answer	Score
Has food intake declined over the past 3 months due to loss of appetite, digestive problems, or chewing or swallowing difficulties? **(answer within MNA short form)**	Severe decrease in food intake	0
	Moderate decrease in food intake	1
	No decrease in food intake	2
Weight loss during the last 3 months **(answer within MNA short form)**	Weight loss > 3kg	0
	Does not know	1
	Weight loss between 1 and 3kg	2
	No weight loss	3
Mobility **(answer within MNA short form)**	Bed or chair bound	0
	Able to get out of bed/chair but does not go out	1
	Goes out	2
Neuropsychological problems **(from Comorbidity section of GA)**	Severe dementia or depression	0
	Mild dementia or depression	1
	No psychological problems	2
Body Mass Index **(calculated by investigator)**	BMI < 18.5	0
	BMI 18.5 to <21	1
	BMI 21 to <23	2
	BMI 23 to >23	3
Takes more than 3 prescription drugs per day **(from Comorbidity section of GA)**	Yes	0
	No	1
In comparison with other people of the same age, how do they consider their health status? **(from General Health section of GA)**	Not as good	0
	Does not know	0.5
	As good	1
	Better	2
Age	>85yrs	0
	80-85yrs	1
	<80yrs	2

Total Score: _____ (range 0 – 17)

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ORIGINAL RESEARCH

Utilisation of Adjuvant Chemotherapy and 5-Year Survival Analysis of Prospectively Recorded Cohort Data for Older Adults Versus Younger Adults with Resected Primary Colon Cancer

Mohsen Shafiei[1,2,3] · Philip Beale[1,2] · Prunella Blinman[1,2]

Abstract

Purpose Colon cancer is predominantly a disease of older adults. Studies determining the influence of age on outcomes of colon cancer have conflicting results. We aim to determine the long-term outcomes and utilisation of adjuvant chemotherapy of older adults compared with younger adults who had had a resection of a primary colon cancer.

Methods Consecutive patients who had resection of a primary colon cancer between January 1, 2000 and December 31, 2010 were identified from a prospective database and stratified into three age groups: ≤ 69 years, 70 to 79 years, and ≥ 80 years. Age-related differences in patients, cancer, and treatment characteristics were determined by chi-square tests. Five-year overall survival and cancer-specific survival were determined by Kaplan-Meier method and by multivariable Cox regression analysis to adjust for potential confounding factors.

Results Of 1135 included patients, 469 (41%) patients were aged ≤ 69 years, 382 (34%) were 70–79 years, and 284 (25%) were ≥ 80 years. Increasing age group predicted more comorbidity ($p < 0.001$), cardiac comorbidity ($p < 0.001$), right-sided cancers ($p < 0.001$), and less adjuvant chemotherapy (stage III only; $p < 0.001$). Increasing age group was associated with worse overall survival by stage ($p < 0.001$) but not cancer-specific survival by stage ($p = 0.83$). Adjuvant chemotherapy in patients with stage III colon cancer independently predicted improved overall survival ($p < 0.001$) and cancer-specific survival ($p = 0.01$).

Conclusions Compared with younger adults, older adults with colon cancer had worse survival outcomes and received less adjuvant chemotherapy.

Keywords Colon cancer · Adjuvant chemotherapy · Older adults · Overall survival · Cancer-specific survival

Introduction

Colon cancer is a common cancer predominantly affecting older adults. In 2018 worldwide, 1,096,601 individuals were diagnosed with colon cancer with the highest incidence in developed countries [1]. In Australia, the median age of diagnosis of colon cancer is 69 years, with more than 57% of the 10,000 effected individuals aged ≥ 70 years [2]. Given the ageing of the population, this means that an increasing number of older adults will be diagnosed with colon cancer and require treatment for this condition [3, 4].

Older adults with colon cancer present distinct challenges to cancer clinicians involved in their care. Ageing is a heterogeneous process with chronological age not always reflecting physiological age resulting in a wide range of suitability and fitness for cancer treatments. Noncancer-related factors that affect decisions about cancer treatments in older adults include frailty, geriatric syndromes (e.g. polypharmacy, falls, malnutrition, cognitive impairment), and multiple comorbidities. In colon cancer, outcomes of older adults are also influenced by adverse tumour characteristics and differences in tolerance of treatment. Older adults with colon cancer are more likely to have right-sided cancers [5, 6] and present at a more advanced cancer stage at the time of diagnosis [7], both of which are poor prognostics factors [7, 8]. Older adults also have a higher

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risk of perioperative morbidity and mortality [9–11] and more frequent and severe chemotherapy toxicity [12] which may lead to underutilisation of the treatments.

Older adults with cancer are typically underrepresented in clinical trials [13] meaning there is little available randomised data from trials including older adults to help guide their cancer care. Survival outcomes for older adults hence are often derived from subset analyses from clinical trials in which older adults only make up a small proportion of the trial population, rather than from specific trials of older adults [14]. Older adults who participate in clinical trials are generally fitter and less frail than older adults with cancer seen in routine clinical practice, where most decisions about chemotherapy are made. Outcome studies can help fill this gap.

We aimed to determine the long-term outcomes of older adults who had resection for primary colon cancer compared with their younger counterparts and their utilisation of adjuvant chemotherapy at our local institution. We hypothesised that older adults, compared with younger adults, have worse long-term outcomes and lower rates of utilisation of adjuvant chemotherapy.

Methods

Study Design

The study was a retrospective observational study of a prospectively maintained database of consecutive patients aged ≥ 18 years who had undergone curative or palliative resection of a primary colon cancer at Concord Repatriation General Hospital, Sydney, Australia. Patients registered between January 1, 2000 and December 31, 2010 were included in this study to allow at least 5 years of follow-up on all patients who had not died by December 31, 2016 for observed rather than estimated 5-year outcomes. The prospectively maintained database commenced in 1971 and includes details of patient characteristics, presentation, comorbidity, investigations, pathology, surgical management, complications, receipt of adjuvant therapy, and follow-up data. Patients were excluded from the database if their tumour was not an invasive carcinoma or if they had inflammatory bowel disease or familial adenomatous polyposis coli. The database has ethics committee approval from the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206), and patients gave written consent for the use of their data and tumour specimens for research.

Patients were assigned to one of three age groups according to their age at the time of diagnosis with colon cancer: ≤ 69 years, 70 to 79 years, and ≥ 80 years. This study included and explored the following variables: patient gender, previous history of colorectal cancer, number of comorbidities, cardiac comorbidity (New York Heart Association, NYHA), resection at urgent operation, histological type, staging TNM classification, tumour location, maximum surface dimension, number of nodes examined, distant metastasis, lymphatic vessel invasion, venous invasion, positive margin, and adjuvant chemotherapy. Right-sided tumour was defined as tumour confined to caecum, ascending colon, hepatic flexure, and transverse colon, and left-sided tumour was defined as tumour that involved splenic flexure, descending colon, and sigmoid colon. Stage III was defined as colonic tumours with regional or apical nodal involvement and with no identifiable systemic metastatic disease (pTNM stage III). The rationale for the focus on stage III colon cancer was this being the stage with a clear indication for adjuvant chemotherapy. The confounding factors of interest were chosen as evidence-based factors affecting the OS of patients with colorectal cancer (as described in the introduction).

Statistical Analysis

Patient demographic, tumour, and treatment characteristics between the three age groups (≤ 69 years, 70–79 years, and ≥ 80 years) were compared by the chi-square test. The failure event for overall survival (OS) was death from any cause, and the failure event for cancer-specific survival (CSS) was death due to colon cancer, other cases being censored. The *p*-values were determined across the three age groups, and values < 0.05 were considered statistically significant.

Five-year OS and CSS were assessed using Kaplan-Meier method and Cox regression. Patients were reviewed at 6-monthly intervals for the first 2 years after resection and yearly thereafter until death or December 31, 2016. Analyses were conducted on the basis of intention to treat. Results are presented as 5-year OS and 5-year CSS curves by age group for the overall population and for the subset of patients with stage III colon cancer. In patients with stage III colon cancer, associations between dichotomised patient demographics, tumour, and treatment characteristic and OS were determined using bivariate and multivariable Cox regression analysis. Statistical analysis was performed using SPSS Version 24 (IBM Australia Limited, 2016). Two-sided tests were used with the level for significance set at 0.05.

Results

Of the 1135 included patients, the mean age was 70.5 years (range, 31–97 years), and just over half were male (604/1135, 53.2%). Patients predominantly had a resection for stage II (439/1135, 38.7%) or stage III (302, 26.6%) colon cancer. The primary site was more frequently right-sided (622/1135, 54.8%) than left-sided (513/1135, 45.2%).

Demographic, tumour, and treatment characteristics are presented in Table 1. The total population by age group was

Table 1 Clinical, tumour and treatment characteristics stratified by age

Characteristics	Age group years				
	Total N=1135 (%) Mean 70.5	≤69 N=469 (%) Mean 58.7	70-79 N= 382 (%) Mean 74.7	≥80 N= 284 (%) Mean 84.4	P for 3 age groups
Male	604 (3.2)	257 (54.8)	209 (54.7)	138 (48.6)	0.20
Female	531 (46.8)	212 (45.2)	173 (45.3)	146 (51.4)	
No Previous CRC resected	1073 (94.5)	447 (95.3)	357 (93.5)	269 (94.7)	0.49
Previous CRC resected	62 (5.5)	22 (4.7)	25 (6.5)	15 (5.3)	
≤1 comorbidity	784 (69.1)	385 (82.1)	238 (62.3)	161(56.7)	<0.001
>1 comorbidities	351(30.9)	84 (17.9)	144 (37.7)	123 (43.3)	
No Cardiac comorbidity [a]	737 (71.1)	398 (87.3)	234 (67.2)	105 (45.1)	<0.001
Cardiac comorbidity	300 (28.9)	58 (12.7)	114 (32.8)	128 (54.9)	
No Resection at urgent operation	1050 (92.5)	442 (94.2)	353 (92.4)	255 (89.8)	0.08
Resection at urgent operation	85 (7.5)	27 (5.8)	29 (7.6)	29 (10.2)	
Right side primary tumour	622 (54.8)	209 (44.6)	217 (56.8)	196 (69.0)	<0.001
Left side primary tumour	513 (45.2)	260 (55.4)	165 (43.2)	88 (31.0)	
Adenocarcinoma	1007 (88.7)	418 (89.1)	346 (90.6)	243 (85.6)	0.12
Mucinous Adenocarcinoma/ Signet ring	128 (11.3)	51 (10.9)	36 (9.4)	41(14.4)	
≤ 5 cm Maximum surface dimension	755 (66.5)	326 (69.5)	255 (66.8)	174 (61.3)	0.07
>5 cm Maximum surface dimension	380 (33.5)	143 (30.5)	127 (33.2)	110 (38.7)	
≤ 11 nodes examined	307 (27)	113 (24.1)	121 (31.7)	73 (25.7)	0.04
12+ nodes examined	828 (73)	356 (75.9)	261(68.3)	211(74.3)	
No Distant metastasis	985 (86.8)	403 (85.9)	337 (88.2)	245 (86.3)	0.60
Distant metastasis	150 (13.2)	66 (14.1)	45 (11.8)	39 (13.7)	
No Lymphatic vessel permeation	904 (79.6)	366 (78.0)	319 (83.5)	219 (77.1)	0.07
Lymphatic vessel permeation	231 (20.4)	103 (22.0)	63 (16.5)	65 (22.9)	
No Venous invasion	999 (88.0)	408 (87.0)	343 (89.8)	248 (87.3)	0.79
Venous invasion	136 (12.0)	61 (13.0)	39 (10.2)	36 (12.7)	
No Positive Margin	1108 (97.6)	458 (97.7)	372 (97.4)	278 (97.9)	0.91
Positive Margin	27 (2.4)	11 (2.3)	10 (2.6)	6 (2.1)	
No Adjuvant chemotherapy [b]	130 (43)	24 (17.4)	39 (41.5)	67 (95.7)	<0.001
Adjuvant chemotherapy	172 (57)	114 (82.6)	55 (58.5)	3 (4.3)	
Stage I (TNM stage)	244 (21.5)	95 (20.3)	98 (25.7)	51 (18.0)	0.09
Stage II	439 (38.7)	170 (36.2)	145 (38.0)	124 (43.7)	
Stage III	302 (26.6)	13 8(29.4)	94 (24.6)	70 (24.6)	
Stage IV	150 (13.2)	66 (14.1)	45 (11.8)	39 (13.7)	

[a] 98 missing cases were missing data for New York Heart Association evaluation.

[b] Adjuvant chemotherapy for patients with stage III colon cancer only.

41% (469/1135) aged ≤ 69 years, 34% (382/1135) aged 70–79 years, and 25% (284/1135) aged ≥ 80 years. Increasing age group was significantly associated with higher comorbidity ($p < 0.001$), cardiac comorbidity ($p < 0.001$), right-sided cancers ($p < 0.001$), and less adjuvant chemotherapy (stage III only; $p < 0.001$). In stage III colon cancer, 83% (114/138) of patients aged ≤ 69 years had adjuvant chemotherapy compared to 58% (55/94) for patients aged 70–80 years and 4% (3/70) for patients aged ≥ 80 years (Table 1). There was a trend towards a higher rate of larger tumours (> 5 cm) ($p = 0.07$), resection at urgent operation ($p = 0.08$), and lymphatic vessel invasion ($p = 0.07$) among older patients compared with younger patients.

The OS and CSS by cancer stage and age group are presented in Table 2. Kaplan-Meier survival curves are presented in Figs. 1, 2, 3 and 4. OS decreased significantly with increasing age group for all stages considered in total ($p < 0.001$) (Table 2, Fig. 1). For the smallest stage category of stage IV (150/1135, 13.2%), due to small numbers, OS was similar at 9% for patients aged 70–79 years (4/45), 8% for patients aged ≤ 69 years (5/66), and 5% for patients aged ≥ 80 years (2/39). OS for patients with stage III colon cancer, considered alone, also decreased for increasing age group (Fig. 3). For CSS, there was no significant difference across age groups by stage considered in total ($p = 0.83$) (Table 2, Fig. 2). For stage III,

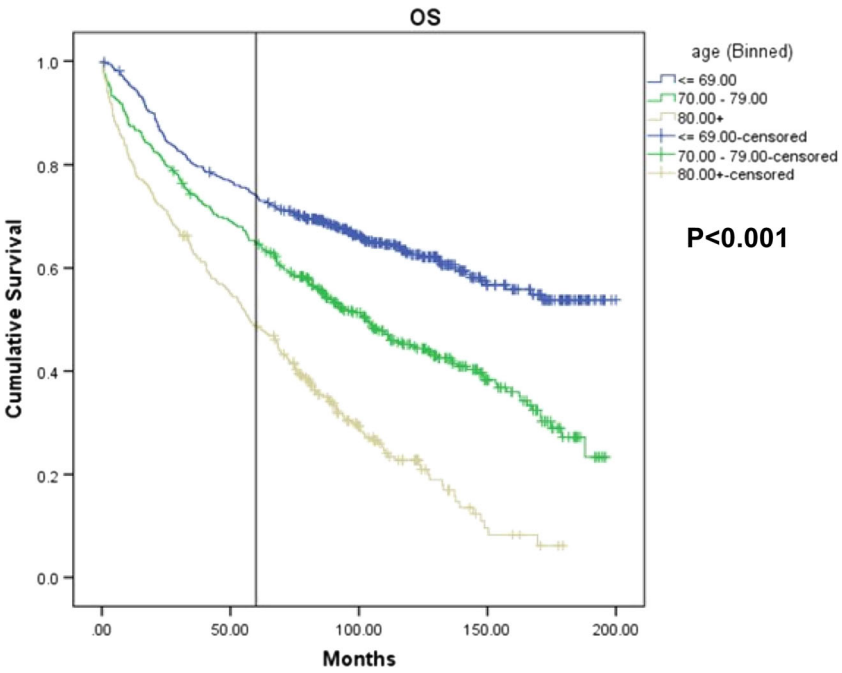

Fig. 1 OS curve by age group for all stages

however, CSS for patients aged ≤ 69 years was significantly longer than CSS for patients ≥ 70 years ($p = 0.01$) (Fig. 4).

Predictors of OS in stage III colon cancer are presented in Table 3. Bivariate predictors of better OS were age ≤ 69 years ($p < 0.001$), resection at nonurgent operation ($p < 0.001$), no lymphatic vessel invasion ($p = 0.001$), no positive margin ($p = 0.004$), receipt of adjuvant chemotherapy ($p < 0.001$) (Fig. 5), maximum surface dimension of ≤ 5 cm, number of comorbidities of ≤ 1(< 0.001), left-sided tumour ($p = 0.003$), and no venous invasion ($p = 0.04$). Adjuvant chemotherapy was also a predictor of better CSS in stage III colon cancer (HR 0.59, $p = 0.01$) (Fig. 6). Independent predictors of improved OS were age ≤ 69 years [HR (hazard ratio) 0.46, $p = 0.002$], no resection at urgent operation (HR 0.30, $p < 0.001$), no lymphatic vessel invasion (HR 0.70, $p = 0.03$), no venous invasion (HR 0.65, $p = 0.04$), and receipt of adjuvant chemotherapy (HR 0.52, $p = 0.001$).

Discussion

The key findings of our study were that older adults, compared with younger adults, who had had a resection of a primary colon cancer of stage I to IV had higher comorbidity and

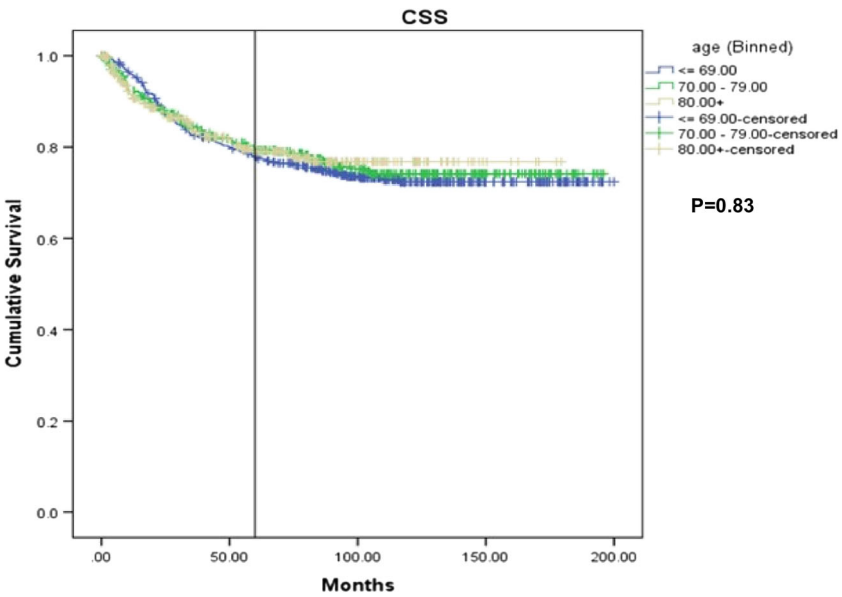

Fig. 2 CSS curve by age group for all stages

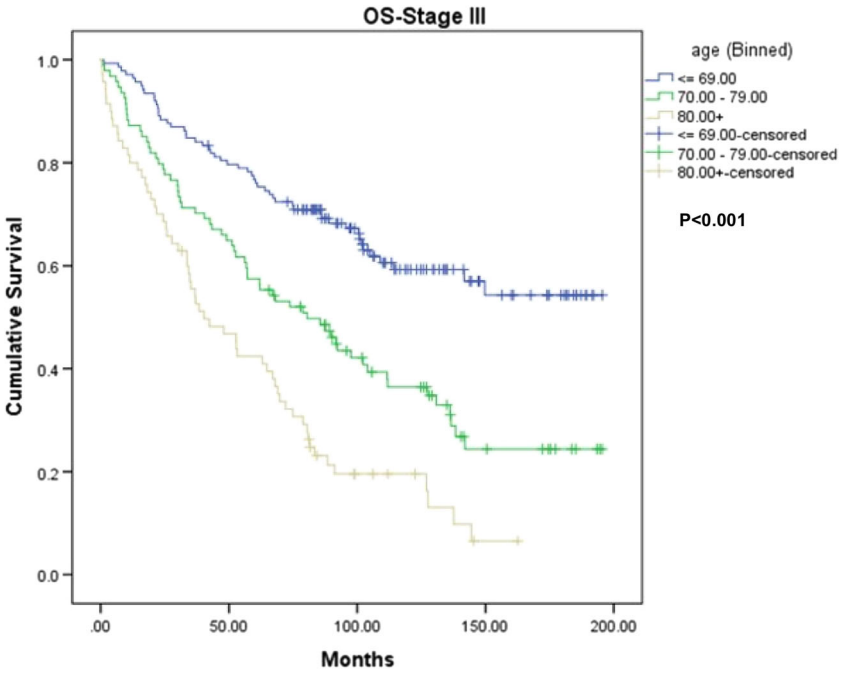

Fig. 3 OS curve by age group for stage III

more frequent right-sided cancers and received less adjuvant chemotherapy. OS worsened with increasing age. CSS was similar across age groups other than in stage III where older adults had worse CSS.

Studies determining the effect of age on outcomes of colon cancer have conflicting results. Some studies show that older adults with colon cancer, compared with younger adults with colon cancer, have worse OS and CSS [4, 17]. Other studies show similar survival outcomes between older adults with colon cancer and younger adults with colon cancer especially in patients undergoing curative surgery for their colon cancer [18–20]. We had similar results with previous studies with regard to increasing age being associated with worse OS [4, 21, 22] more right-sidedness [4, 8, 23, 24], comorbidity [8, 19], and receipt of less adjuvant chemotherapy [4, 8, 18, 19] but heterogeneous results with regard to CSS [20].

The largest two comparable outcome studies include Kotake et al. who studied over 40,000 patients with colorectal

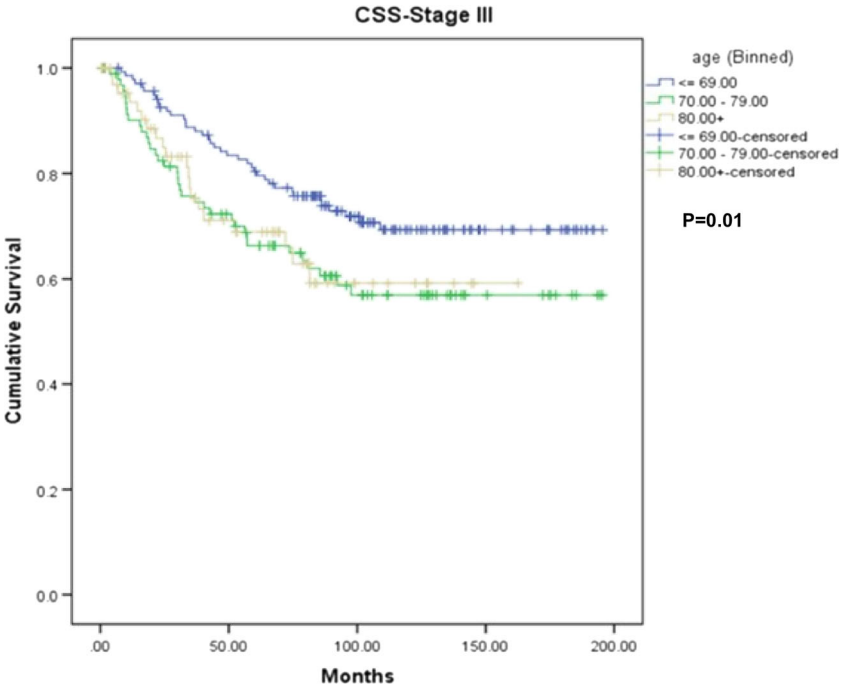

Fig. 4 CSS curve by age group in stage III

Table 2 5-year OS and CSS by age group and pathological stage

Stage	Age group	Cases (n)	5 Year OS rate (%)	P value	5 Year CSS rate	P value
Stage I	≤69	95	92%	<0.001	99%	0.78
	70-79	98	79%		99%	
	≥ 80	51	74%		97%	
	All	244				
Stage II	≤69	170	87%	<0.001	91%	0.39
	70-79	145	78%		93%	
	≥ 80	124	55%		93%	
	All	439				
Stage III	≤69	138	77%	<0.001	80%	0.053
	70-79	94	57%		66%	
	≥ 80	70	42%		69%	
	All	302				
Stage IV	≤69	66	8%	0.007	9%	0.45
	70-79	45	9%		12%	
	≥ 80	39	5%		11%	
	All	150				

cancer from the Japanese cancer registry [8]. This study showed increasing age was associated with worse 5-year OS (50% in ≥ 80 years age group vs 73% in 50–64 years age group, $p < 0.001$) and worse CSS (65% in ≥ 80 years age group vs 76% in 50–64 years age group, $p < 0.001$) in patients with stage III disease. Similarly, Patel et al. in a study of nearly 33,000 patients with colon cancer also found increasing age was associated with lower 5-year OS (26% in ≥ 80 years age group vs 61% in 50–64 years age group, $p < 0.001$) and lower 5-year CSS (50% in ≥ 80 years age group vs 69% in 50–64 years age group, $p < 0.001$) in patients with stage III disease [4]. Two smaller studies by Devon et al. (n = 623) and

Table 3 Bivariate and multivariable OS analysis for stage III colon cancer

Variable	Number	Bivariate hazard ratio (95% CI)	p	Multivariable hazard ratio (95% CI)	p
Female	135	1.01 (0.75-1.37)	0.93		
Male	167				
Age ≤69 years	138	0.26 (0.18-0.38)	<0.001	0.46 (0.29-0.75)	0.002
Age ≥ 70 years	164				
No Previous CRC	289	1.04 (0.49-2.21)	0.92		
Previous CRC	13				
No Resection at urgent operation	283	0.21 (0.13-0.34)	<0.001	0.30 (0.17-0.51)	<0.001
Resection at urgent operation	19				
No Lymphatic vessel invasion	195	0.61 (0.45-0.82)	0.001	0.70 (0.51-0.96)	0.03
Lymphatic vessel invasion	107				
Adenocarcinoma	270	0.73 (0.46-1.14)	0.16		
Other histology	32				
No Positive Margin	294	0.33 (0.15-0.70)	0.004	0.62 (0.26-1.48)	0.29
Positive Margin	8				
Adjuvant chemotherapy	172	0.33 (0.24-0.45)	<0.001	0.52 (0.35-0.77)	0.001
No Adjuvant chemotherapy	130				
Maximum surface dimension ≤ 5cm	206	0.70 (0.51-0.95)	0.02	0.87 (0.63-1.20)	0.39
Maximum surface dimension > 5cm	96				
Number of nodes examined < 12	79	0.87 (0.62-1.22)	0.41		
Number of nodes examined ≥ 12	223				
Number of Comorbidities ≤ 1	226	0.54 (0.39-0.74)	<0.001	0.87 (0.62-1.23)	0.44
Number of Comorbidities > 1	76				
Right Sided	161	1.57 (1.16-2.13)	0.003	1.32 (0.96-1.81)	0.09
Left sided	141				
No Venous invasion	253	0.67 (0.45-0.10)	0.04	0.65 (0.43-0.99)	0.04
Venous invasion	49				

Abbreviation: CRC, Colorectal cancer

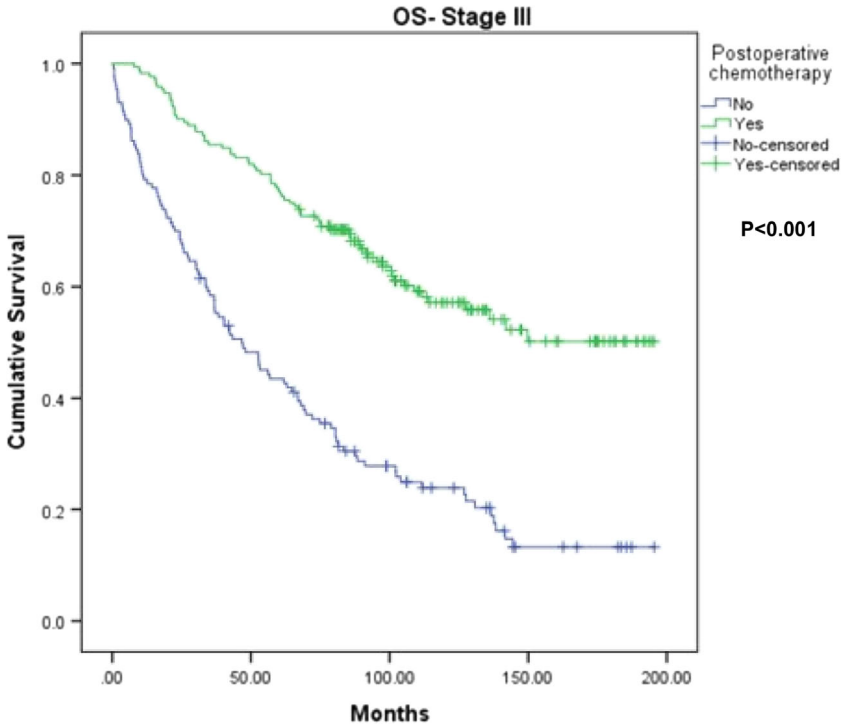

Fig. 5 OS curve by adjuvant chemotherapy in stage III

Widdison et al. (n = 459) showed increasing age was a predictor of worse OS but not CSS [19, 25], indicating that older patients are more likely to die from comorbid illnesses than cancer.

Increasing age was associated with worse OS but not CSS in our total study population indicating that older adults died of intercurrent causes rather than of colon cancer. For stage III colon cancer, however, increasing age was associated with worse CSS, possibly due to the underutilisation of adjuvant chemotherapy in older adults. Worse OS may be due to the differences in tumour and patient characteristic and treatment disparity, but it seems unlikely that they account for all the differences in OS across the three age groups. Better understanding of the reasons behind these differences has important

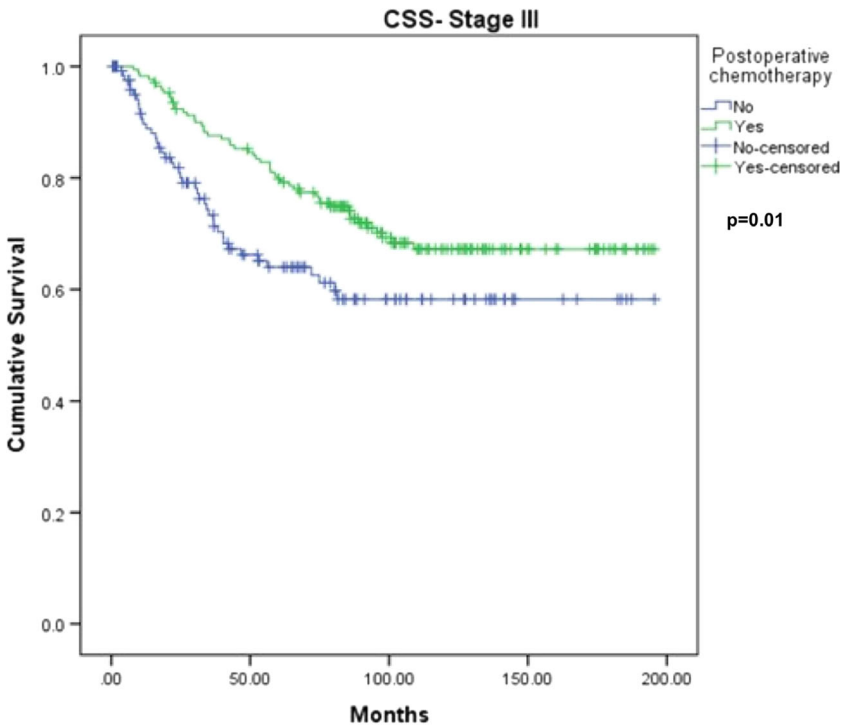

Fig. 6 CSS curve by adjuvant chemotherapy in stage III

implications to prevent older adults with colon cancer being discriminated and denied standard of care treatment due to their chronological age alone or being treated unnecessarily. This is especially important in older adults with stage III colon cancer, where CSS of older adults was worse than in younger adults and where a careful selection approach should be adopted to consider surgery and adjuvant chemotherapy to improve their OS and CSS.

Right-sided tumour location is an established negative prognostic factor in patients with relapsed or stage IV colon cancer, but its impact on outcomes in patients with stage I–III colon cancer is unclear [26, 27]. In our study, right-sided tumour location did not independently predict OS in patients with stage III colon cancer. Kennecke et al. investigated the prognostic impact of tumour sidedness in patients with colon cancer ($n = 5378$) and showed that right-sided tumour location was a favourable prognostic factor in patients with stage II colon cancer and a negative prognostic factor in stage IV colon cancer, but not a prognostic factor in stage III colon cancer [26]. The latter finding may be ameliorated, in part, by the presence of high microsatellite instability (MSI) in 20% of right-sided tumours given the favourable prognostic effect of MSI-high cancers [28]. In a meta-analysis of 66 studies with more than 1.4 million patients with colon cancer, Petrelli et al. found that tumour left-sidedness was associated with a significantly decreased risk of death (hazard ratio, 0.82, 95% CI, 0.79–0.84; $p < 0.001$) independent of stage [27]. Therefore, the actual impact of tumour sidedness on the outcomes of older patients with colon cancer, particularly those with early stage disease, remains unclear.

Comorbidity is very relevant to the management of older adults with colon cancer as it weighs strongly in decisions about cancer treatments. Whilst higher comorbidity has previously been associated with worse OS in patients with colon cancer [29–31], we did not find this in our study. This is possibly due to the selection bias of patients needing to be fit enough to have had a resection of colon cancer to be included in the database and hence the smaller proportion of patients with > 1 comorbid conditions (76/226, 33%). The impact of more comorbidity on CSS, as opposed to OS, is unclear because often OS has been the survival endpoint in the available studies not CSS, but it is likely less significant [29, 32–34].

Older adults in our study, compared with younger patients, received less adjuvant chemotherapy. Older adults (aged > 80 years) had also a lower adjuvant chemotherapy utilisation rate (4%) than older patients in large database studies (8% and15 %) [4, 8]. Reasons why patients did not receive adjuvant chemotherapy would have been useful to review, but these were not captured in the database and are a limitation of the study. The receipt of adjuvant chemotherapy predicted better OS in stage III colon cancer. The demonstrated OS benefit of adjuvant chemotherapy in this study is from non-randomised data, and hence minimal emphasis is placed on this result. The underutilisation of adjuvant chemotherapy may be due to the lack of robust data supporting the use of adjuvant chemotherapy for colon cancer in older adults (aged ≥ 70 years) [35]. Other reasons include clinician nihilism and unwillingness to refer or treat older adults with adjuvant chemotherapy, inadequate skills in assessing older adults' suitability for chemotherapy, and concerns about excess toxicity even with standard doses [36]. Fit older patients with colon cancer benefit equally from adjuvant chemotherapy without a significant increase in toxicity [37, 38]. Ways to increase utilisation of adjuvant chemotherapy in older adults include conducting trials specifically in older adults, the use of geriatric assessments and risk predicting tools to assist oncologists in assessing older adults' suitability for chemotherapy [39–41], and studies determining the optimal adjuvant dosing of chemotherapy agents in older adults [40, 42].

The main strength of our study lies in it being performed on a large prospectively maintained surgical database over one decade with minimal missing data. Limitations include the database only involving a single institution meaning that the surgical and oncological management, patient selection, surgical techniques, post-operative care, and selection for adjuvant chemotherapy may differ from other institutions or healthcare settings. Generalisability of the study may also be limited by the sample bias of only including patients who had had a resection of a primary colon cancer and hence excludes patients who were not suitable or fit for surgery or chose not to have surgery. Details of chemotherapy regimen, completion, or toxicities were also not readily available and required retrieval of individual patient records for which the study was not resourced.

Conclusion

Older adults who had a resection of a stage I–IV colon cancer had higher comorbidity and more frequent right-sided cancers and received less adjuvant chemotherapy. Older adults had worse OS across all stages and worse CSS in stage III disease. These results highlight the need to optimise the treatment of older adults with colon cancer and ways to increase the utilisation of adjuvant chemotherapy.

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Author Contributions Mohsen Shafiei contributed in data collection, statistical analysis, and writing of the manuscript. He is the corresponding author. Philip Beale and Prunella Blinman (lead author) contributed in study design, data validation, final writing, and editing of the manuscript.

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Compliance with Ethical Standards

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492.
2. Australian Institute of Health and Welfare. Colon Cancer ACIM. https://www.aihw.gov.au/reports/cancer/cancer-data-in-australia/acim-books. 2018 Accessed 16 May 2019
3. Audisio RA, Veronesi P, Ferrario L, Cipolla C, Andreoni B, Aapro M. Elective surgery for gastrointestinal tumours in the elderly. Ann Oncol. 1997;8(4):317–26.
4. Patel SS, Nelson R, Sanchez J, Lee W, Uyeno L, Garcia-Aguilar J, et al. Elderly patients with colon cancer have unique tumor characteristics and poor survival. Cancer. 2013;119(4):739–47. https://doi.org/10.1002/cncr.27753.
5. Kamran SC, Clark JW, Zheng H, Borger DR, Blaszkowsky LS, Allen JN, et al. Primary tumor sidedness is an independent prognostic marker for survival in metastatic colorectal cancer: results from a large retrospective cohort with mutational analysis. Cancer Med. 2018;7(7):2934–42. https://doi.org/10.1002/cam4.1558.
6. Loupakis F, Yang D, Yau L, Feng S, Cremolini C, Zhang W, et al. Primary tumor location as a prognostic factor in metastatic colorectal cancer. J Natl Cancer Inst. 2015;107(3). https://doi.org/10.1093/jnci/dju427.
7. Marusch F, Koch A, Schmidt U, Zippel R, Gastmeier J, Ludwig K, et al. Impact of age on the short-term postoperative outcome of patients undergoing surgery for colorectal carcinoma. Int J Color Dis. 2002;17(3):177–84.
8. Kotake K, Asano M, Ozawa H, Kobayashi H, Sugihara K. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. Color Dis. 2015;17(3):205–15. https://doi.org/10.1111/codi.12826.
9. Baquero GA, Rich MW. Perioperative care in older adults. J Geriatr Cardiol. 2015;12(5):465–9. https://doi.org/10.11909/j.issn.1671-5411.2015.05.018.
10. McNicol L, Story DA, Leslie K, Myles PS, Fink M, Shelton AC, et al. Postoperative complications and mortality in older patients having non-cardiac surgery at three Melbourne teaching hospitals. Med J Aust. 2007;186(9):447–52.
11. Sieber FE, Barnett SR. Preventing postoperative complications in the elderly. Anesthesiol Clin. 2011;29(1):83–97. https://doi.org/10.1016/j.anclin.2010.11.011.
12. Crombag MR, Joerger M, Thurlimann B, Schellens JH, Beijnen JH, Huitema AD. Pharmacokinetics of selected anticancer drugs in elderly cancer patients: focus on breast cancer. Cancers (Basel). 2016;8(1). https://doi.org/10.3390/cancers8010006.
13. Hurria A, Dale W, Mooney M, Rowland JH, Ballman KV, Cohen HJ, et al. Designing therapeutic clinical trials for older and frail adults with cancer: U13 conference recommendations. J Clin Oncol. 2014;32(24):2587–94. https://doi.org/10.1200/jco.2013.55.0418.
14. Moth EB, Vardy J, Blinman P. Decision-making in geriatric oncology: systemic treatment considerations for older adults with colon cancer. Expert Rev Gastroenterol Hepatol. 2016;10(12):1321–40. https://doi.org/10.1080/17474124.2016.1244003.
17. Kune GA, Kune S, Field B, White R, Brough W, Schellenberger R, et al. Survival in patients with large-bowel cancer. A population-based investigation from the Melbourne Colorectal Cancer Study. Dis Colon Rectum. 1990;33(11):938–46.
18. McMillan DC, McArdle CS. The impact of young age on cancer-specific and non-cancer-related survival after surgery for colorectal cancer: 10-year follow-up. Br J Cancer. 2009;101:557–60. https://doi.org/10.1038/sj.bjc.6605222.
19. Devon KM, Vergara-Fernandez O, Victor JC, McLeod RS. Colorectal cancer surgery in elderly patients: presentation, treatment, and outcomes. Dis Colon Rectum. 2009;52(7):1272–7. https://doi.org/10.1007/DCR.0b013e3181a74d2e.
20. Chandrasinghe PC, Ediriweera DS, Nazar T, Kumarage S, Hewavisenthi J, Deen KI. Overall survival of elderly patients having surgery for colorectal cancer is comparable to younger patients: results from a South Asian population. Gastroenterol Res Pract. 2017;2017:9670512. https://doi.org/10.1155/2017/9670512.
21. Dekker JW, van den Broek CB, Bastiaannet E, van de Geest LG, Tollenaar RA, Liefers GJ. Importance of the first postoperative year in the prognosis of elderly colorectal cancer patients. Ann Surg Oncol. 2011;18(6):1533–9. https://doi.org/10.1245/s10434-011-1671-x.
22. Jessup JM, McGinnis LS, Steele GD Jr, Menck HR, Winchester DP. The National Cancer Data Base. Report on colon cancer. Cancer. 1996;78(4):918–26. https://doi.org/10.1002/(sici)1097-0142(19960815)78:4<918::aid-cncr32>3.0.co;2-w.
23. Holt PR, Kozuch P, Mewar S. Colon cancer and the elderly: from screening to treatment in management of GI disease in the elderly. Best Pract Res Clin Gastroenterol. 2009;23(6):889–907. https://doi.org/10.1016/j.bpg.2009.10.010.
24. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med. 2005;352(21):2211–21. https://doi.org/10.1056/NEJMra032424.
25. Widdison AL, Barnett SW, Betambeau N. The impact of age on outcome after surgery for colorectal adenocarcinoma. Ann R Coll Surg Engl. 2011;93(6):445–50. https://doi.org/10.1308/003588411x587154.
26. Kennecke HF, Yin Y, Davies JM, Speers CH, Cheung WY, Lee-Ying R. Prognostic effect of sidedness in early stage versus advanced colon cancer. Health Science Reports. 2018;1(8):e54. https://doi.org/10.1002/hsr2.54.
27. Petrelli F, Tomasello G, Borgonovo K, et al. Prognostic survival associated with left-sided vs right-sided colon cancer: a systematic review and meta-analysis. JAMA Oncology. 2017;3(2):211–9. https://doi.org/10.1001/jamaoncol.2016.4227.
28. Iacopetta B, Grieu F, Amanuel B. Microsatellite instability in colorectal cancer. Asia Pac J Clin Oncol. 2010;6(4):260–9. https://doi.org/10.1111/j.1743-7563.2010.01335.x.
29. Sarfati D, Hill S, Blakely T, Robson B, Purdie G, Dennett E, et al. The effect of comorbidity on the use of adjuvant chemotherapy and survival from colon cancer: a retrospective cohort study. BMC Cancer. 2009;9:116. https://doi.org/10.1186/1471-2407-9-116.
30. Sarfati D, Tan L, Blakely T, Pearce N. Comorbidity among patients with colon cancer in New Zealand. N Z Med J. 2011;124(1338):76–88.
31. Tooth L, Hockey R, Byles J, Dobson A. Weighted multimorbidity indexes predicted mortality, health service use, and health-related quality of life in older women. J Clin Epidemiol. 2008;61(2):151–9. https://doi.org/10.1016/j.jclinepi.2007.05.015.

32. He X, Clarke SJ, McLachlan AJ. Clinical pharmacology of chemotherapy agents in older people with cancer. Curr Gerontol Geriatr Res. 2011;2011:628670. https://doi.org/10.1155/2011/628670.
33. Rieker RJ, Hammer E, Eisele R, Schmid E, Hogel J. The impact of comorbidity on the overall survival and the cause of death in patients after colorectal cancer resection. Langenbeck's Arch Surg. 2002;387(2):72–6. https://doi.org/10.1007/s00423-002-0291-0.
34. Søgaard M, Thomsen RW, Bossen KS, Sørensen HT, Nørgaard M. The impact of comorbidity on cancer survival: a review. Clin Epidemiol. 2013;5(Suppl 1):3–29. https://doi.org/10.2147/clep.s47150.
35. Nitsche U, Stöss C, Friess H. Effect of adjuvant chemotherapy on elderly colorectal cancer patients: lack of evidence. Gastrointest Tumors. 2017;4(1-2):11–9. https://doi.org/10.1159/000479318.
36. Burdette-Radoux S, Muss HB. Adjuvant chemotherapy in the elderly: whom to treat, what regimen? Oncologist. 2006;11(3):234–42. https://doi.org/10.1634/theoncologist.11-3-234.
37. Iwashyna TJ, Lamont EB. Effectiveness of adjuvant fluorouracil in clinical practice: a population-based cohort study of elderly patients with stage III colon cancer. J Clin Oncol. 2002;20(19):3992–8. https://doi.org/10.1200/jco.2002.03.083.
38. Sargent DJ, Goldberg RM, Jacobson SD, Macdonald JS, Labianca R, Haller DG, et al. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. N Engl J Med. 2001;345(15):1091–7. https://doi.org/10.1056/NEJMoa010957.
39. Hurria A, Siccion EP. Assessing the 'fit' older patient for chemotherapy. Oncology (Williston Park). 2014;28(7):594, 596, 598–9.
40. Lichtman SM, Villani G. Chemotherapy in the elderly: pharmacologic considerations. Cancer Control. 2000;7(6):548–56.
41. Millan M, Merino S, Caro A, Feliu F, Escuder J, Francesch T. Treatment of colorectal cancer in the elderly. World J Gastrointest Oncol. 2015;7(10):204–20. https://doi.org/10.4251/wjgo.v7.i10.204.
42. Field KM, Kosmider S, Jefford M, Michael M, Jennens R, Green M, et al. Chemotherapy dosing strategies in the obese, elderly, and thin patient: results of a nationwide survey. Journal of Oncology Practice. 2008;4(3):108–13. https://doi.org/10.1200/jop.0832001.

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Five Year Survival Outcomes of Prospectively Recorded Cohort Data for Older Adults versus Younger Adults with Resected Primary Rectal Cancer

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Abstract

Background: Rectal cancer predominantly occurs in older adults. We aimed to compare the long-term outcomes of older adults (≥70 years) versus younger adults (<70 years) who had had a primary resection for stage I-IV rectal cancer. **Methods:** Consecutive patients who had resection of a primary rectal cancer between January 1, 2000 and December 31, 2010 were identified from a prospective database at the Concord Repatriation General Hospital and stratified into two age groups: <70 years and ≥70 years. Age-related differences in patients, cancer, and treatment characteristics were determined by Chi-square tests. 5-year Overall Survival (OS) and Cancer-Specific Survival (CSS) were determined by the Kaplan-Meier method and by multivariable Cox regression analysis. **Results:** Of 714 included patients, the mean age was 65.8 years (range, 21 - 92 years). 407 (57%) patients were aged < 70 years and 307 (43%) were aged ≥ 70 years. Older age (>70 years) predicted more comorbidity ($p < 0.001$) and earlier stage ($p = 0.01$). Of the patients with stage III rectal cancer, older adults (>70 years), compared with younger adults (<70 years), received less neoadjuvant chemotherapy [7/86 (8.1%) vs 25/147 (17.0%), $p = 0.058$], less neoadjuvant radiotherapy [8/86 (9.3%) vs 42/147 (28.6%), $p = 0.001$] and less adjuvant chemotherapy [30/86 (34.9%) vs 117/147 (79.6%), $p < 0.001$]. Older age was associated with worse OS and CSS in stage III ($p < 0.001$ and $p = 0.02$ respectively). Adjuvant chemotherapy independently predicted improved OS ($p < 0.001$) and CSS ($p = 0.008$) regardless of age. **Conclusion:** Older adults who had had a resection of stage I-IV primary rectal cancer received less neoadjuvant and adjuvant therapy and had worse OS and CSS than their younger counterparts.

Keywords

Rectal Cancer, Chemotherapy, Radiotherapy, Overall Survival, Cancer Specific Survival

1. Introduction

Rectal cancer predominantly occurs in older adults with an increasing incidence with increasing age [1]. Worldwide, there were an estimated 704,000 new cases of rectal cancer in 2018 [2] with the highest risk in developed countries. In Australia, there were an estimated 5238 new cases of rectal cancer in 2019 with over half of these patients (58%) aged over 65 years [3]. With increasing life expectancy and the general aging of the population [4], the number of older adults diagnosed with rectal cancer is expected to increase, making optimisation of the management of rectal cancer in older adults an important priority for clinicians involved in their care.

The treatment of locally advanced rectal cancer (stage II, ≥ T3-N0 or stage III, any T ≥ N1) has evolved over the last two decades. Surgery is the mainstay of curative treatment with the addition of neoadjuvant and/or adjuvant therapy for resectable locally advanced disease. For fit patients, one standard approach is tri-modality treatment with neoadjuvant radiotherapy ± chemotherapy followed by a Total Mesorectal Excision (TME) and adjuvant chemotherapy. This approach is based on several randomized clinical trials that showed neoadjuvant radiotherapy ± chemotherapy improved local control ranged from 7% (4.4% - 11%, $p = 0.004$) to 16% (11% - 27%, $p < 0.001$) without consistent improvement in Overall Survival (OS) [5] [6]. The addition of adjuvant chemotherapy improved Disease-Free Survival (DFS) (HR 0.59, 95% CI 0.40 - 0.85) and distant recurrence (HR 0.61, 95% CI 0.40 - 0.94) particularly in patients with a tumour 10 - 15 cm from the anal verge [7]. The NCCN and ESMO guidelines recommend adjuvant chemotherapy as standard treatment for all patients with locally advanced rectal cancer after neoadjuvant radiotherapy or Chemoradiotherapy (CRT) and surgery [8] [9].

Older adults with rectal cancer, compared with younger adults with rectal cancer, may be challenging to treat with triple modality therapy due to the intensity and toxicity of the treatment. Older adults have more comorbidities and geriatric syndromes such as falls, polypharmacy, cognitive impairment and malnutrition that reduce their fitness for standard cancer therapy [10] [11]. Older adults are also more likely to discontinue therapy earlier than younger adults due to the higher rates of treatment toxicity [12]. Older adults are less likely to be referred for neoadjuvant and adjuvant therapy for rectal cancer [13] and, when referred, they may not be offered similar treatment as their younger counterparts [13] [14] [15]. Another key factor affecting the management of older adults with rectal cancer is their underrepresentation in pertinent clinical

trials. The abovementioned trials of neoadjuvant CRT and adjuvant chemotherapy in rectal cancer included mostly younger (median age of 60 - 61) and fitter adults (ECOG performance status of 0 or 1) rather than the frail, older adults typical of routine clinical practice [16]. This means little specific randomized evidence in older adults with rectal cancer to help clinicians guide their care.

Observational studies have a role in determining the impact of age on outcomes of rectal cancer when older adults are underrepresented in randomized clinical trials. The results of observational studies determining Overall Survival (OS) and Cancer-Specific Survival (CSS) for rectal cancer generally show worse OS with increasing age, but inconsistent results for CSS [17] [18] [19].

We conducted an observational study to determine the long-term outcomes of older adults who had had a resection of primary rectal cancer and their utilisation of neoadjuvant CRT and adjuvant chemotherapy, compared with their younger counterparts in our local institution. We hypothesized that older adults, compared with younger adults, had worse long-term outcomes and lower rates of utilisation of neoadjuvant and adjuvant therapy.

2. Methods

2.1. Study Design

Consecutive patients over the age of 18 who had undergone curative or palliative surgery for a diagnosis of rectal cancer at the Concord Repatriation General Hospital, Sydney, Australia between 2000 and 2011 were included. Data were extracted from a prospectively collected Colorectal Cancer (CRC) database maintained since 1971 and received approval of the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206). This database included patient characteristics, comorbidity, presentation, investigations, pathology, neoadjuvant therapy, surgical management, complications, receipt of adjuvant therapy and follow-up data. This project included and explored the following variables: patient gender, previous history of colorectal cancer, number of comorbidities, cardiac comorbidity, resection at urgent operation, histological type, maximum surface dimension, staging, lymphatic vessel invasion, venous invasion, positive margin, neoadjuvant therapy and adjuvant chemotherapy. Patients were stratified to two age groups, <70 years and ≥70 years, at the time of diagnosis.

2.2. Statistical Analysis

Patient demographics, tumour and treatment characteristics between the two age-groups (<70 years and ≥70 years) were compared by the use of the log-rank test. Demographic, tumour and treatment characteristics were compared with use of the chi-squared test for association for categorical factors. Kaplan-Meier method was used to construct overall and rectal cancer specific survival curves in patients with stage III rectal cancer. Results of patients in stage III rectal cancer only were analyzed due to the use of adjuvant chemotherapy in this stage in

routine clinical practice. For 5-year CSS and 5-year OS analysis in patients with stage III rectal cancer, the two age groups (<70 years and ≥70 years) were further stratified by gender, resection at urgent operation, lymphatic vessel invasion, positive margin, venous invasion, number of comorbidities and receipt of neoadjuvant CRT and adjuvant chemotherapy. To determine the association between these factors and patient OS and CSS, multivariate cox regression analysis was performed. SPSS (version 24) was used for all statistical analyses. All p values were 2-sided and values <0.05 were considered statistically significant.

3. Results

714 patients were included in the study. The mean age was 65.9 years (range, 21 - 92 years). 407 (57%) patients were aged <70 years and 307 (43%) were ≥70 years. There were more males than females in both the younger (271/407, 67%) and older (182/307, 60%) age groups. Demographic information, presentation and treatment characteristics are presented in Table 1.

Older age group (≥70 years) predicted more comorbidity ($p < 0.001$), cardiac comorbidity ($p < 0.001$), lymphatic vessel invasion ($p = 0.03$), early stage tumour ($p = 0.01$), less neoadjuvant radiotherapy (p 0.001), less neoadjuvant chemotherapy ($p < 0.001$) and less adjuvant chemotherapy (stage III only; $p < 0.001$).

In patients with stage III rectal cancer, older adults (≥70 years), compared with younger adults (<70 years), received less neoadjuvant chemotherapy [7/86 (8.1%) vs 25/147 (17.0%), $p = 0.058$], less neoadjuvant radiotherapy [8/86 (9.3%) vs 42/147 (28.6%), $p = 0.001$] and less adjuvant chemotherapy [8/86 (9.3%) vs 42/147 (28.6%), $p = 0.001$].

Table 1. Tumour and treatment characteristics stratified by age.

Characteristics	Age group years			
	Total N = 714 Mean	<70 N = 407 Mean	≥70 N = 307 Mean	P difference between <70 and ≥70
Previous CRC resected				
No	702 (98.3%)	399 (98.0%)	303 (98.7%)	P = 0.49
Yes	12 (1.7%)	8 (2%)	4 (1.3%)	
No. of comorbidities				
≤1	545 (76.3%)	341 (83.8%)	204 (66.4%)	P < 0.001
>1	169 (23.7%)	66 (16.2%)	103 (33.6%)	
Cardiac comorbidity*				
No	526 (77.8%)	355 (89%)	171 (61.7%)	P < 0.001
Yes	150 (22.2%)	44 (11%)	106 (38.3%)	
Resection at urgent operation				
No	707 (99%)	403 (99%)	304 (99%)	P = 0.99
Yes	7 (1%)	4 (1%)	3 (1%)	

Continued

Histological type of primary				
Adenocarcinoma	661 (92.6%)	371 (91.2%)	290 (94.5%)	P = 0.09
Mucinous Adenocarcinoma/ Signet ring	53 (7.4%)	36 (8.8%)	17 (5.5%)	
Distant metastasis				
No	621 (87.0%)	347 (85.3%)	274 (89.3%)	P = 0.12
Yes	93 (13.0%)	60 (14.7%)	33 (10.7%)	
Lymphatic vessel permeation				
No	569 (79.7%)	313 (76.9%)	256 (83.4%)	P = 0.03
Yes	145 (20.3%)	94 (23.1%)	51 (16.6%)	
Venous invasion				
None	582 (81.5%)	326 (80.1%)	256 (83.4%)	P = 0.26
Yes	132 (18.5%)	81 (19.9%)	51 (16.6%)	
Positive margin				
No	667 (93.4%)	380 (93.4%)	287 (93.4%)	P = 0.95
Yes	47 (6.6%)	20 (6.5%)	27 (6.6%)	
Preoperative radiotherapy				
No	594 (83.2%)	311 (76.4%)	283 (92.2%)	P < 0.001
Yes	120 (16.8%)	96 (23.6%)	24 (7.8%)	
Preoperative chemotherapy				
No	633 (88.7%)	344 (84.5%)	289 (94.1%)	P < 0.001
Yes	81 (11.3%)	63 (15.5%)	18 (5.9%)	
Postoperative radiotherapy				
No	691 (96.8%)	395 (97.1%)	296 (96.4%)	P = 0.64
Yes	23 (3.2%)	12 (2.9%)	11 (3.6%)	
Postoperative chemotherapy				
No	487 (68.2%)	225 (55.3%)	262 (85.3%)	P < 0.001
Yes	227 (31.8%)	182 (44.7%)	45 (14.7%)	
TNM stage				
Stage I	187 (26.2%)	95 (23.3%)	92 (30.0%)	P = 0.01
Stage II	201 (28.2%)	105 (25.8%)	96 (31.3%)	
Stage III	233 (32.6%)	147 (36.1%)	86 (28.0%)	
Stage IV	93 (13.0%)	60 (14.7%)	33 (10.7%)	

*There were 38 missing cases for New York Heart Association evaluation.

The 5-year OS and 5-year CSS between the two age groups stratified by cancer stage are shown in Table 2. Kaplan-Meier survival curves are presented in Figures 1-4. Five-year OS was significantly lower in the older age group irrespective of cancer stage ($p < 0.001$) (Table 2, Figure 1). In patients with stage III rectal cancer, increasing age group was associated with worse 5-year OS [44.2% (≥70 years) vs 71.9% (<70 years), $p < 0.001$], and worse 5-year CSS [62.3% (≥70 years) vs 76.2% (<70 years), $p = 0.02$] (Figure 3 and Figure 4).

Table 2. 5-year overall and cancer specific survival after surgery by age group and pathological stage.

Stage	Age group	No of cases	5-year OS rate	P value	5-year CSS rate	P value
Stage I	<70	95	94.7%		97.8%	
	≥70	92	72.8%		91.1%	
	All	187				
Stage II	<70	105	81.9%		87.3%	
	≥70	96	60.0%		82.6%	
	All	201		< 0.001		0.001
Stage III	<70	147	71.9%		76.2%	
	≥70	86	44.2%		62.3%	
	All	233				
Stage IV	<70	60	11.7%		11.9%	
	≥70	33	0%		0%	
	All	93				

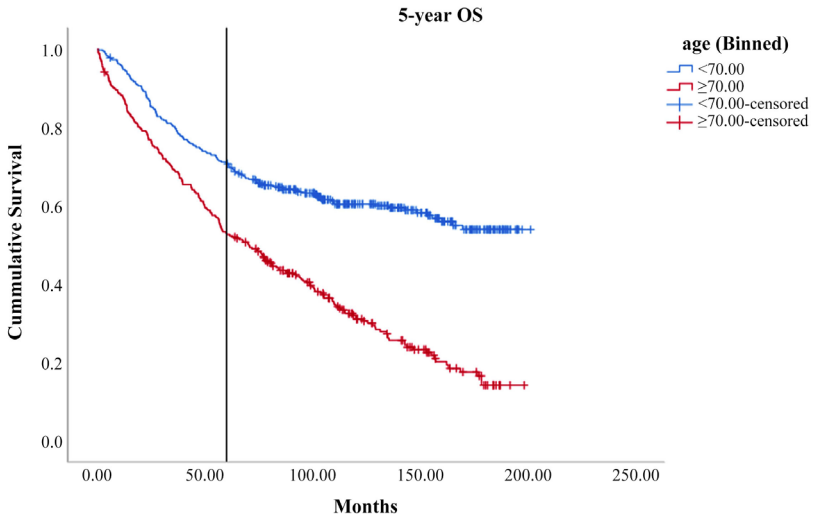

Figure 1. OS curve by age group for all stages. $P < 0.001$.

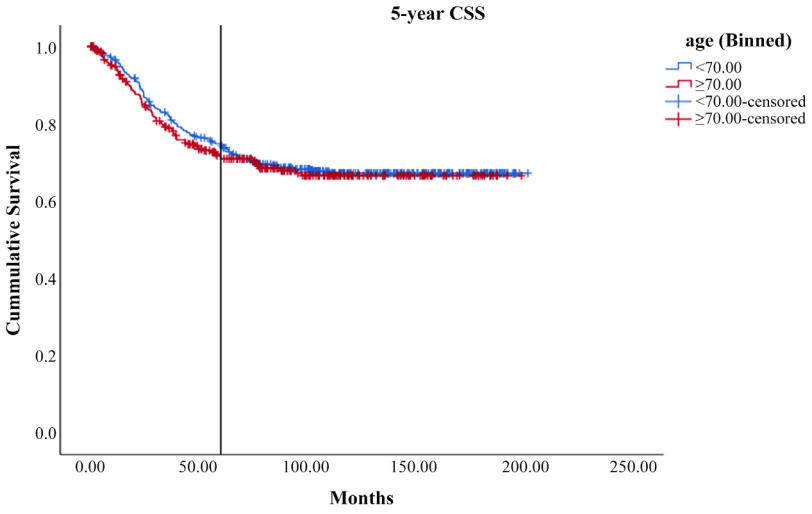

Figure 2. CSS curve by age group for all stages. P = 0.65.

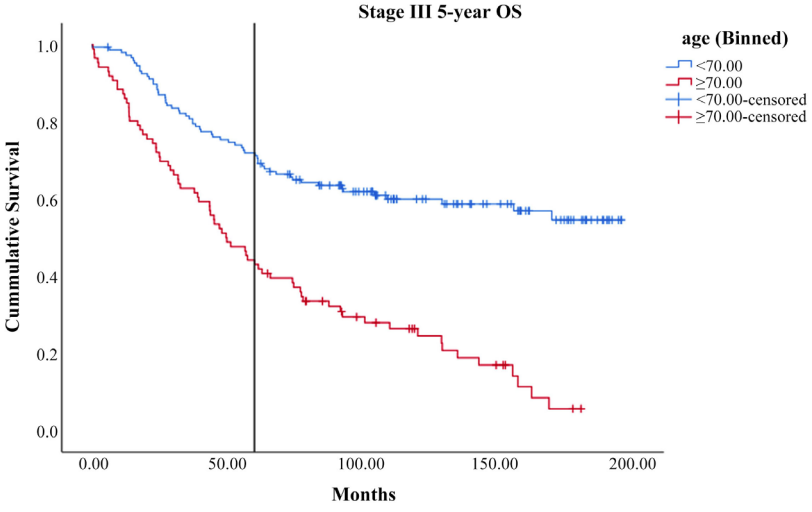

Figure 3. OS curve by age group for stage III. P < 0.001.

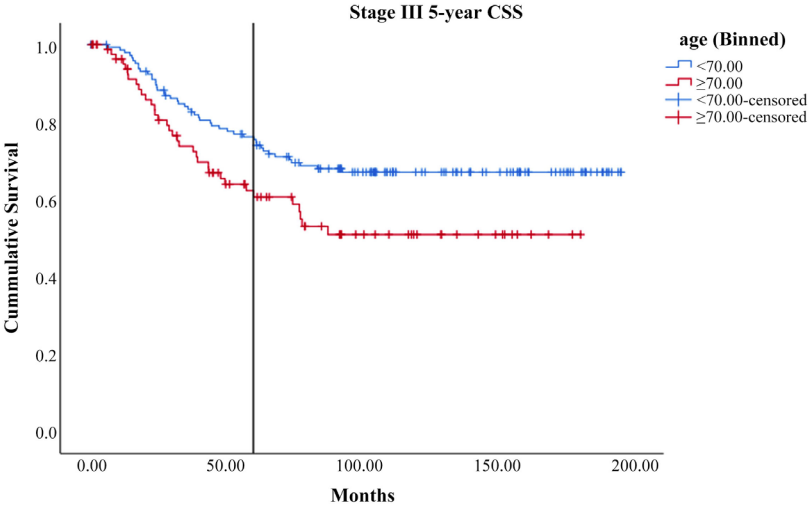

Figure 4. CSS curve by age group for stage III. P = 0.02.

In patients with stage III rectal cancer, bivariate predictors of improved OS were age < 70 years (p < 0.001), no lymphatic vessel invasion (p < 0.001), no positive margin (p < 0.001), receiving adjuvant chemotherapy and less comorbidity (p = 0.002) (Table 3). Neoadjuvant radiotherapy did not improve OS (p = 0.41) but significantly improved CSS (p = 0.038) (Figure 5). On multivariable analysis, improved OS was independently predicted by age < 70 years (hazard ratio, 0.44, p < 0.001), no lymphatic vessel invasion (hazard ratio, 0.47, p < 0.001), no positive margin (hazard ratio, 0.23 p < 0.001) and receiving adjuvant chemotherapy (hazard ratio, 0.50, p = 0.001). Improved CSS was predicted by adjuvant chemotherapy in stage III rectal cancer (p = 0.008) (Figure 6).

Table 3. Bivariate and multivariable survival analysis for only stage III rectal cancer.

Variable	Number	Bivariate hazard Ratio (95% CI)	p	Multivariable hazard Ratio (95% CI)	p
Female Male	86 147	1.13 (0.79 - 1.63)	0.47		
Age < 70 years Age ≥ 70 years	147 86	0.34 (0.24 - 0.48)	<0.001	0.44 (0.30 - 0.65)	<0.001
No Previous CRC Previous CRC	228 5	0.61 (0.19 - 1.93)	0.40		
No Resection at urgent operation Resection at urgent operation	230 3	0.44 (0.11 - 1.77)	0.25		
No Venous invasion Venous invasion	181 52	0.70 (0.48 - 1.04)	0.08		
No lymphatic vessel invasion Lymphatic vessel invasion	156 77	0.49 (0.34 - 0.69)	<0.001	0.47 (0.32 - 0.68)	<0.001
No positive margin Positive margin	212 21	0.16 (0.10 - 0.26)	<0.001	0.23 (0.14 - 0.39)	<0.001
Adenocarcinoma Mucinous adenoCa/ Signet ring	208 25	0.68 (0.41 - 1.13)	0.14		
Neoadjuvant radiotherapy No neoadjuvant radiotherapy	50 183	1.19 (0.78 - 1.80)	0.41		
Neoadjuvant chemotherapy No neoadjuvant chemotherapy	32 201	1.07 (0.64 - 1.78)	0.79		
Adjuvant radiotherapy No adjuvant radiotherapy	14 219	1.40 (0.73 - 2.67)	0.31		
Adjuvant chemotherapy No adjuvant chemotherapy	147 86	0.34 (0.24 - 0.50)	< 0.001	0.50 (0.34 - 0.74)	0.001
Number of nodes examined < 12 Number of nodes examined ≥ 12	60 173	1.30 (0.89 - 1.90)	0.17		
Number of comorbidities ≤ 1 Number of comorbidities > 1	179 54	0.55 (0.38 - 0.81)	0.002	0.76 (0.51 - 1.12)	0.16

CRC, Colorectal cancer.

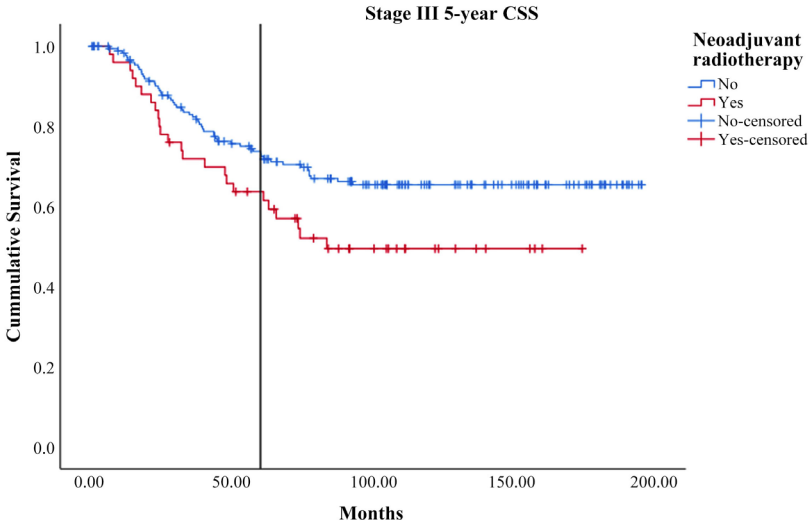

Figure 5. CSS curve by neoadjuvant radiotherapy in stage III rectal cancer. P = 0.038.

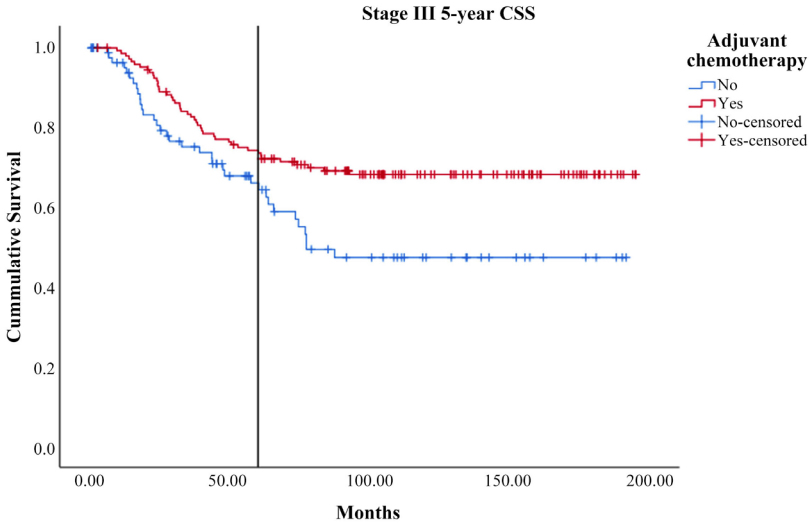

Figure 6. CSS curve by adjuvant chemotherapy in stage III rectal cancer. P = 0.008.

4. Discussion

The key findings of our study were that older adults (≥70 years), compared with younger adults (<70 years), who had had a resection of primary rectal cancer of stage I to IV had higher comorbidity and cardiac comorbidity, more lymphatic vessel invasion and more early stage cancers. Older adults, compared with younger adults, received less neoadjuvant radiotherapy, less neoadjuvant chemotherapy and less adjuvant chemotherapy. 5-year OS declined significantly with the increasing age group. 5-year CSS was significantly worse in older adults with stage III rectal cancer.

The survival outcomes in our study are similar to other published studies. Chang *et al.* conducted an observational study using the Surveillance, Epidemi-

ology, and End Results (SEER) database to examine more than 21,000 patients with locally advanced rectal cancer and found a 31% increase in the relative risk for cancer-specific mortality with each 5-year increase in age ≥ 70 years (RR = 1.31; 95% CI, 1.25 - 1.36; P < 0.0001) [18]. Kotake *et al.* studied included 16,147 patients with rectal cancer in a large study from the Japanese cancer registry and found older age predicted worse 5-year OS (50% in ≥80 years vs 73% in 50 - 64 years, p < 0.001) and worse 5-year CSS (65% in ≥80 years vs 76% in 50 - 64 years, p < 0.001) [17]. Jung *et al.* studied 15,104 patients with rectal cancer from the Swedish Rectal Cancer Registry 1995-2004 of whom more than 11,000 had had curative surgery (stages I-IV). Older adults (≥75 years), compared with younger adults (<75 years), had worse 5-year OS (0.52, 95% CI, 0.50 - 0.54 vs 0.62, 95% CI, 0.61 - 0.63) [19]. Devon *et al.* studied 373 adults undergoing curative surgery for their rectal cancer at the Mount Sinai Hospital, Canada between 1997 and 2006. Older adults (aged > 75 years), compared with younger adults (aged 50 - 75 years), had worse 5-year OS (68.7% vs 57.3%, p = 0.036) but no difference in 5-year CSS (74.0% vs. 74.7%, p = 0.277) [20]. Similarly, Widdison *et al.* studied 218 patients with rectal cancer and showed older age was not a predictor of worse 5-year CSS (72% for younger and older groups) [21].

It was unsurprising that older adults had worse OS in our study, like in the observational studies discussed above, given competing risks for death in older adults. More concerning was that CSS, or the chance of surviving cancer in the absence of other causes of death, was worse for older adults in stage III rectal cancer. Possible reasons for this result highlighted by our study are increased comorbidities and low utilisation rates of neoadjuvant and adjuvant therapy. Other possible reasons include increased toxicity from radiotherapy and chemotherapy and increased post-surgical complications.

The utilisation of neoadjuvant radiotherapy (7.8%) and neoadjuvant chemotherapy (5.9%) in older adults in our study was low, however, similar to other studies [17] [19]. The role of neoadjuvant radiotherapy and CRT in rectal cancer, however, is now well established. Multiple randomized trials and population based studies have shown that neoadjuvant radiotherapy and CRT improve local control in patients aged > 70 years [6] [22] [23] [24] [25]. The large Swedish Rectal Cancer Study Group trial (n = 1168) showed neoadjuvant radiotherapy (25 Gy in 5 fractions), compared with surgery alone, reduced local recurrence by 16% (from 27% to 11%, p < 0.001) and improved both five-year OS by 10% (48% to 58%, p = 0.004) and CSS by 9% (65% to 74%, p = 0.002) (ref Swedish rectal trial). One possible explanation for the low utilisation rates in our study was the dates of data extraction being 2000-2011 (to allow for 5 years of follow-up for survival outcomes) when neoadjuvant radiotherapy ± chemotherapy for older adults was likely a less accepted standard of care. Utilisation rates of neoadjuvant radiotherapy for rectal cancer for older adults have likely increased over time as clinicians have become familiar with the treatment and are generally more confident treating older adults with cancer. The older observational studies such as

Kotake *et al.* (1995 to 2004) showed rates of 0.3% in patients aged ≥ 80 years and 34% in patients aged ≥ 75 years by Jung *et al.* (1995 to 2004) [7] [26]. Later studies such as Zhao *et al.* that analyzed rectal cancer data from the SEER database between 2004 and 2016, showed a utilisation rate of neoadjuvant radiotherapy of 53% for patients aged > 60 years, lower than the 67% rate of patients aged ≤ 60 years [27]. Other reasons for the low utilisation rates include patient preferences for no neoadjuvant and/or adjuvant therapy, and patient and clinician concerns about excess toxicity such as faecal incontinence and sexual dysfunction, which are more pronounced in older patients [28] [29] [30].

In our study, older adults with rectal cancer received less adjuvant chemotherapy (9.3%) than younger adults (28.6%) with rectal cancer as in previous studies [31]. Irrespective of age, there is no clear OS benefit of adjuvant chemotherapy for rectal cancer, and the treatment is largely a translation from the DFS and OS benefit of adjuvant chemotherapy in colon cancer [7] [31] [32] [33] [34] [35]. A meta-analysis of four pivotal randomized control trials examining the benefit of adjuvant chemotherapy for patients with locally advanced rectal cancer demonstrated that adjuvant 5-fluorouracil/capecitabine improves DFS (HR 0.59, 95% CI: 0.40 - 0.85, p = 0.005) and rate of distant recurrence (HR 0.61, 95% CI: 0.40 - 0.94, p = 0.025) in those patients with a tumour 10 to 15 cm above the anal verge but no improvement in OS (HR 0.97, 95% CI: 0.81 - 1.17, p = 0.775) [7]. Common clinical practice, supported by guidelines, is four months of adjuvant chemotherapy for patients who had long course CRT and six months of adjuvant chemotherapy for patients who have not had neoadjuvant therapy [8].

Possible reasons for the low utilisation rates in our study include the paucity of robust evidence supporting the benefit of such therapy in patients of all ages and in older adults (>70 years), referrer bias against the treatment resulting in reduced referrals for adjuvant chemotherapy, and concerns about the increased toxicity of chemotherapy in older adults [36]. Fit older adults with rectal cancer, however, benefit equally from adjuvant chemotherapy without a significant increase in toxicity [37].

Increasing treatment utilisation in older adults with rectal cancer involves optimal assessment of their fitness for treatment to minimise their exclusion from treatment based on their chronological age. This is particularly important in older adults with stage III rectal cancer where the worse CSS in our study highlights the need to improve outcomes and where tri-modality treatment, requiring careful patient selection, is a standard of care. Optimal assessment of older adults can be achieved by the use of formal geriatric assessments and risk predicting tools, as recommended by ASCO guidelines [38] [39]. Integrated geriatric assessment in the care of older adults with cancer has recently been shown to improve quality of life, reduce hospital admissions and reduce early discontinuation of anti-cancer therapy [40] [41] [42]. The key ways to improve treatment utilisation in older adults with rectal cancer include conducting trials and studies specific to older adults, for example, the optimal dosing of adjuvant chemotherapy.

The main strength of our study is the prospective, large surgical database with minimal missing data. Limitations of our study include the database involving a single institution meaning that the surgical and oncological management, patient selection, surgical techniques, pre-operative and post-operative care, and selection for neoadjuvant radiotherapy or chemo-radiotherapy and adjuvant chemotherapy may differ from other institutions or health care settings. Details of radiotherapy (dose, fractionation, completion) and chemotherapy (regimen, dose, toxicities, completion) were not readily available and required manual searching through medical records for which the study was not adequately resourced. The generalisability of the study is limited due to the inclusion of patients who had had a resection of primary rectal cancer and hence excludes patients who were not suitable or fit for surgery or who chose not to have surgery.

In conclusion, older adults who had a resection of a stage I-IV rectal cancer had higher comorbidity, cardiac comorbidity, more lymphatic vessel invasion, early stage tumour, and received less neoadjuvant radiotherapy, less neoadjuvant chemotherapy and less adjuvant chemotherapy. Older adults had worse OS and worse CSS in stage III disease. These results highlight the need to optimise the treatment of older adults with rectal cancer and ways to increase the utilisation of adjuvant chemotherapy.

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Conflicts of Interest

The authors of this manuscript have no relevant affiliations or financial involvement with any organization or entity with a financial interest with the subject matter or materials discussed.

References

[1] Matanoski, G., Tao, X., Almon, L., Adade, A.A. and Davies-Cole, J.O. (2006) Demographics and Tumor Characteristics of Colorectal Cancers in the United States, 1998-2001. *Cancer*, **107**, 1112-1120. https://doi.org/10.1002/cncr.22008

[2] Society, A.C. (2018) Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.

[3] Australia C (2019) Cancer Incidence. https://ncci.canceraustralia.gov.au/diagnosis/cancer-incidence/cancer-incidence

[4] Crimmins, E.M. (2015) Lifespan and Healthspan: Past, Present, and Promise. *The Gerontologist*, **55**, 901-911. https://doi.org/10.1093/geront/gnv130

[5] Quirke, P., Steele, R., Monson, J., Grieve, R., Khanna, S., Couture, J., O'Callaghan, C., *et al.* (2009) Effect of the Plane of Surgery Achieved on Local Recurrence in Patients with Operable Rectal Cancer: A Prospective Study Using Data from the MRC CR07 and NCIC-CTG CO16 Randomised Clinical Trial. *Lancet* (*London*, *England*), **373**, 821-828. https://doi.org/10.1016/S0140-6736(09)60485-2

[6] Cedermark, B., Dahlberg, M., Glimelius, B., Påhlman, L., Rutqvist, L.E. and Wilking, N. (1997) Improved Survival with Preoperative Radiotherapy in Resectable Rectal Cancer. *The New England Journal of Medicine*, **336**, 980-987. https://doi.org/10.1056/NEJM199704033361402

[7] Breugom, A.J., Swets, M., Bosset, J.F., Collette, L., Sainato, A., Cionini, L., Glynne-Jones, R., *et al.* (2015) Adjuvant Chemotherapy after Preoperative (Chemo) radiotherapy and Surgery for Patients with Rectal Cancer: A Systematic Review and Meta-Analysis of Individual Patient Data. *The Lancet Oncology*, **16**, 200-207. https://doi.org/10.1016/S1470-2045(14)71199-4

[8] Network NCC (2020) NCCN Clinical Practice Guidelines in Oncology. Rectal Cancer. https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf

[9] Oncology ESfM (2020) Rectal Cancer: ESMO Clinical Practice Guidelines. https://www.esmo.org/guidelines/gastrointestinal-cancers/rectal-cancer

[10] Hurria, A., Browner, I.S., Cohen, H.J., Denlinger, C.S., deShazo, M., Extermann, M., Ganti, A.K., *et al.* (2012) Senior Adult Oncology. *Journal of the National Comprehensive Cancer Network*: *JNCCN*, **10**, 162-209. https://doi.org/10.6004/jnccn.2012.0019

[11] Farrington, N., Richardson, A. and Bridges, J. (2020) Interventions for Older People Having Cancer Treatment: A Scoping Review. *Journal of Geriatric Oncology*, **11**, 769-783. https://doi.org/10.1016/j.jgo.2019.09.015

[12] Kahn, K.L., Adams, J.L., Weeks, J.C., Chrischilles, E.A., Schrag, D., Ayanian, J.Z., Kiefe, C.I., *et al.* (2010) Adjuvant Chemotherapy Use and Adverse Events among Older Patients with Stage III Colon cancer. *JAMA*, **303**, 1037-1045. https://doi.org/10.1001/jama.2010.272

[13] Luo, R., Giordano, S.H., Freeman, J.L., Zhang, D. and Goodwin, J.S. (2006) Referral to Medical Oncology: A Crucial Step in the Treatment of Older Patients with Stage III Colon Cancer. *The Oncologist*, **11**, 1025-1033. https://doi.org/10.1634/theoncologist.11-9-1025

[14] Samet, J., Hunt, W.C., Key, C., Humble, C.G. and Goodwin, J.S. (1986) Choice of Cancer Therapy Varies with Age of Patient. *JAMA*, **255**, 3385-3390. https://doi.org/10.1001/jama.1986.03370240055036

[15] Goodwin, J.S., Hunt, W.C. and Samet, J.M. (1993) Determinants of Cancer Therapy in Elderly Patients. *Cancer*, **72**, 594-601. https://doi.org/10.1002/1097-0142(19930715)72:2<594::AID-CNCR2820720243>3.0.CO;2-#

[16] Moth, E.B., Kiely, B.E., Martin, A., Naganathan, V., Della-Fiorentina, S., Honeyball, F., Zielinski, R., *et al.* (2019) Older Adults' Preferred and Perceived Roles in Decision-Making about Palliative Chemotherapy, Decision Priorities and Information Preferences. *Journal of Geriatric Oncology*, **11**, 626-632. https://doi.org/10.1200/JCO.2019.37.15_suppl.11546

[17] Kotake, K., Asano, M., Ozawa, H., Kobayashi, H. and Sugihara, K. (2015) Tumour Characteristics, Treatment Patterns and Survival of Patients Aged 80 Years or Older with Colorectal Cancer. *Colorectal Disease*, **17**, 205-215. https://doi.org/10.1111/codi.12826

[18] Chang, G.J., Skibber, J.M., Feig, B.W. and Rodriguez-Bigas, M. (2007) Are We Undertreating Rectal Cancer in the Elderly? An Epidemiologic Study. *Annals of Surgery*, **246**, 215-221. https://doi.org/10.1097/SLA.0b013e318070838f

[19] Jung, B., Pahlman, L., Johansson, R. and Nilsson, E. (2009) Rectal Cancer Treatment and Outcome in the Elderly: An Audit Based on the Swedish Rectal Cancer Registry 1995-2004. *BMC Cancer*, **9**, 68. https://doi.org/10.1186/1471-2407-9-68

[20] Devon, K.M., Vergara-Fernandez, O., Victor, J.C. and McLeod, R.S. (2009) Colorectal Cancer Surgery in Elderly Patients: Presentation, Treatment, and Outcomes. *Diseases of the Colon and Rectum*, **52**, 1272-1277. https://doi.org/10.1007/DCR.0b013e3181a74d2e

[21] Widdison, A.L., Barnett, S.W. and Betambeau, N. (2011) The Impact of Age on Outcome after Surgery for Colorectal Adenocarcinoma. *Annals of the Royal College of Surgeons of England*, **93**, 445-450. https://doi.org/10.1308/003588411X587154

[22] Gérard, J.P., Conroy, T., Bonnetain, F., Bouché, O., Chapet, O., Closon-Dejardin, M.T., Untereiner, M., *et al.* (2006) Preoperative Radiotherapy with or without Concurrent Fluorouracil and Leucovorin in T3-4 Rectal Cancers: Results of FFCD 9203. *Journal of Clinical Oncology*: *Official Journal of the American Society of Clinical Oncology*, **24**, 4620-4625. https://doi.org/10.1200/JCO.2006.06.7629

[23] Bosset, J.F., Calais, G., Mineur, L., Maingon, P., Radosevic-Jelic, L., Daban, A., Bardet, E., *et al.* (2005) Enhanced Tumorocidal Effect of Chemotherapy with Preoperative Radiotherapy for Rectal Cancer: Preliminary Results—EORTC 22921. *Journal of Clinical Oncology*: *Official Journal of the American Society of Clinical Oncology*, **23**, 5620-5627. https://doi.org/10.1200/JCO.2005.02.113

[24] Boulis-Wassif, S., Gerard, A., Loygue, J., Camelot, D., Buyse, M. and Duez, N. (1984) Final Results of a Randomized Trial on the Treatment of Rectal Cancer with Preoperative Radiotherapy Alone or in Combination with 5-Fluorouracil, Followed by Radical Surgery. Trial of the European Organization on Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. *Cancer*, **53**, 1811-1818. https://doi.org/10.1002/1097-0142(19840501)53:9<1811::AID-CNCR2820530902>3.0.CO;2-H

[25] McCarthy, K., Pearson, K., Fulton, R. and Hewitt, J. (2012) Pre-Operative Chemoradiation for Non-Metastatic Locally Advanced Rectal Cancer. *The Cochrane Database of Systematic Reviews*, **12**, Cd008368. https://doi.org/10.1002/14651858.CD008368.pub2

[26] Holm, T., Rutqvist, L.E., Johansson, H. and Cedermark, B. (1996) Postoperative Mortality in Rectal Cancer Treated with or without Preoperative Radiotherapy: Causes and Risk Factors. *The British Journal of Surgery*, **83**, 964-968. https://doi.org/10.1002/bjs.1800830725

[27] Zhao, F., Wang, J., Yu, H., Cheng, X., Li, X., Zhu, X., Xu, X., *et al.* (2020) Neoadjuvant Radiotherapy Improves Overall Survival for T3/4N+M0 Rectal Cancer Patients: A Population-Based Study of 20300 Patients. *Radiation Oncology* (*London*, *England*), **15**, 49. https://doi.org/10.1186/s13014-020-01497-4

[28] Dahlberg, M., Glimelius, B., Graf, W. and Pahlman, L. (1998) Preoperative Irradiation Affects Functional Results after Surgery for Rectal Cancer: Results from a Randomized Study. *Diseases of the Colon and Rectum*, **41**, 543-549. https://doi.org/10.1007/BF02235256

[29] Lange, M.M., den Dulk, M., Bossema, E.R., Maas, C.P., Peeters, K.C., Rutten, H.J., Klein Kranenbarg, E., *et al.* (2007) Risk Factors for Faecal Incontinence after Rectal Cancer Treatment. *The British Journal of Surgery*, **94**, 1278-1284. https://doi.org/10.1002/bjs.5819

[30] Birgisson, H., Pahlman, L., Gunnarsson, U. and Glimelius, B. (2007) Late Adverse Effects of Radiation Therapy for Rectal Cancer—A Systematic Overview. *Acta Oncologica* (*Stockholm*, *Sweden*), **46**, 504-516. https://doi.org/10.1080/02841860701348670

[31] Liu, S.L., O'Brien, P., Zhao, Y., Hopman, W.M., Lamond, N. and Ramjeesingh, R. (2018) Adjuvant Treatment in Older Patients with Rectal Cancer: A Population-Based Review. *Current Oncology*, **25**, e499-e506. https://doi.org/10.3747/co.25.4102

[32] Bosset, J.F., Collette, L., Calais, G., Mineur, L., Maingon, P., Radosevic-Jelic, L., Daban, A., *et al.* (2006) Chemotherapy with Preoperative Radiotherapy in Rectal Cancer. *The New England Journal of Medicine*, **355**, 1114-1123. https://doi.org/10.1056/NEJMoa060829

[33] Sainato, A., Cernusco Luna Nunzia, V., Valentini, V., De Paoli, A., Maurizi, E.R., Lupattelli, M., Aristei, C., *et al.* (2014) No Benefit of Adjuvant Fluorouracil Leucovorin Chemotherapy after Neoadjuvant Chemoradiotherapy in Locally Advanced Cancer of the Rectum (LARC): Long Term Results of a Randomized Trial (I-CNR-RT). *Radiotherapy and Oncology*: *Journal of the European Society for Therapeutic Radiology and Oncology*, **113**, 223-229. https://doi.org/10.1016/j.radonc.2014.10.006

[34] Breugom, A.J., van Gijn, W., Muller, E.W., Berglund, Å., van den Broek, C.B., Fokstuen, T., Gelderblom, H., *et al.* (2015) Adjuvant Chemotherapy for Rectal Cancer Patients Treated with Preoperative (Chemo)radiotherapy and Total Mesorectal Excision: A Dutch Colorectal Cancer Group (DCCG) Randomized Phase III Trial. *Annals of Oncology*: *Official Journal of the European Society for Medical Oncology*, **26**, 696-701. https://doi.org/10.1093/annonc/mdu560

[35] Glynne-Jones, R., Counsell, N., Quirke, P., Mortensen, N., Maraveyas, A., Meadows, H.M., Ledermann, J., *et al.* (2014) Chronicle: Results of a Randomised Phase III Trial in Locally Advanced Rectal Cancer after Neoadjuvant Chemoradiation Randomising Postoperative Adjuvant Capecitabine plus Oxaliplatin (XELOX) versus Control. *Annals of Oncology*: *Official Journal of the European Society for Medical Oncology*, **25**, 1356-1362. https://doi.org/10.1093/annonc/mdu147

[36] Nitsche, U., Stöss, C. and Friess, H. (2017) Effect of Adjuvant Chemotherapy on Elderly Colorectal Cancer Patients: Lack of Evidence. *Gastrointestinal Tumors*, **4**, 11-19. https://doi.org/10.1159/000479318

[37] Millan, M., Merino, S., Caro, A., Feliu, F., Escuder, J. and Francesch, T. (2015) Treatment of Colorectal Cancer in the Elderly. *World Journal of Gastrointestinal Oncology*, **7**, 204-220. https://doi.org/10.4251/wjgo.v7.i10.204

[38] Hurria, A. and Siccion, E.P. (2014) Assessing the "Fit" Older Patient for Chemotherapy. *Oncology*, **28**, 594, 596, 598-599.

[39] Lichtman, S.M. and Villani, G. (2000) Chemotherapy in the Elderly: Pharmacologic Considerations. *Cancer Control*, **7**, 548-556. https://doi.org/10.1177/107327480000700606

[40] Mohile, S.G., Mohamed, M.R., Culakova, E., Xu, H., Loh, K.P., Magnuson, A., Flannery, M.A., *et al.* (2020) A Geriatric Assessment (GA) Intervention to Reduce Treatment Toxicity in Older Patients with Advanced Cancer: A University of Rochester Cancer Center NCI Community Oncology Research Program Cluster

Randomized Clinical Trial (CRCT). *Journal of Clinical Oncology*, **38**. https://doi.org/10.1200/JCO.2020.38.15_suppl.12009

[41] Li, D., Sun, C.-L., Kim, H., Chung, V., Koczywas, M., Fakih, M., Chao, J., *et al.* (2020) Geriatric Assessment-Driven Intervention (GAIN) on Chemotherapy Toxicity in Older Adults with Cancer: A Randomized Controlled Trial. *Journal of Clinical Oncology*, **38**. https://doi.org/10.1200/JCO.2020.38.15_suppl.12010

[42] Soo, W.-K., King, M., Pope, A., Parente, P., Darzins, P. and Davis, I.D. (2020) Integrated Geriatric Assessment and Treatment (INTEGERATE) in Older People with Cancer Planned for Systemic Anticancer Therapy. *Journal of Clinical Oncology*, **38**. https://doi.org/10.1200/JCO.2020.38.15_suppl.12011

Pharmacokinetics of Anticancer Drugs Used in Treatment of Older Adults With Colorectal Cancer: A Systematic Review

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Purpose: Older adults with cancer experience more toxicity from anticancer therapy, possibly because of age-related changes in the pharmacokinetic (PK) profile of anticancer drugs. We aimed to evaluate studies investigating the effect of aging on the PK of anticancer therapies used in the treatment of colorectal cancer (CRC).

Methods: A systematic literature search of EMBASE and PubMed was performed to find eligible studies that assessed the effect of age on the PK of anticancer therapies used in the treatment of CRC.

Results: The 21 eligible studies included 17 prospective studies and 4 pooled analyses of prospective studies. Of these, PK of 5-fluorouracil (5-FU) was determined in 7 studies, oxaliplatin in 2 studies, capecitabine in 3 studies, irinotecan in 4 studies, bevacizumab in 1 study, cetuximab in 3 studies, and panitumumab in 1 study. Studies included a median of 44 patients and had varying definitions for older adults: 65 years or older (3 studies), older than 70 years (3 studies), or older than 75 years (1 study). Increasing age significantly affected the PK parameters of irinotecan with a 7%–8% reduction in CL ($P < 0.001$) for every 10 years in patients older than 60 years and an increase in area under the curve (r = 0.44, $P = 0.007$) and Cmax (r = 0.42, $P = 0.009$).

Conclusions: Older age mainly influences PK of irinotecan and, to some extent, that of capecitabine, 5-FU, and panitumumab, but there is limited evidence for age-related changes in PK of other anticancer therapies used in the management of older adults with CRC. Factors other than PK may be responsible for the greater toxicity of these agents experienced by older adults.

Key Words: anticancer agents, CRC, older adults, PK

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INTRODUCTION

Colorectal cancer (CRC) is a common cancer in older adults and a common cause of cancer death. In 2012, there were an estimated 1.4 million cases of CRC and 693,900 deaths from CRC worldwide.[1] More than 60% of patients diagnosed with CRC are aged 70 years or older, and the absolute numbers of older adults are increasing because of the aging of the population. The management of older adults with CRC is thus an increasing issue for clinicians providing care for older adults.

Older adults with CRC, compared with younger adults with CRC, experience more toxicity from anticancer therapy.[2] Data from randomized trials and large pooled analyses in the adjuvant setting showed that older adults experienced more chemotherapy-related toxicity with 5-fluorouracil (5-FU),[3] capecitabine,[4] FOLFOX [5-FU/leucovorin (LV) and oxaliplatin],[5,6] and XELOX (capecitabine and oxaliplatin).[5] In the metastatic setting, older adults experienced more chemotherapy toxicity with capecitabine,[4,7] irinotecan,[8–10] and regimens such as FOLFOX,[8,11] XELOX,[12] and FOLFIRI (5-FU/LV and irinotecan).[8–10,12] Of the targeted therapies in the metastatic setting, older adults experienced more toxicity with bevacizumab.[13]

Aging is a heterogeneous process with variable decline in physiological reserve and functional status, and physiological age often has little relationship with chronological age. Prescribing anticancer therapies to older adults can be challenging with wide variation in response, more treatment toxicity as described previously, and worse survival regardless of the cancer stage.[14–17] Older adults are also underrepresented in clinical trials,[18,19] meaning dosing and efficacy data are predominantly derived from clinical trials of younger, fitter patients, physiologically distinct from the majority of older adults seen in routine clinical practice.

Aging is associated with changes in the clinical pharmacology of anticancer therapies, namely pharmacokinetics (PK) and pharmacodynamics (PD).[20] PK is the study of "what the body does to a drug," that is, the uptake of a drug by the body and its time course of absorption, distribution, metabolism, and excretion. PD, however, is the study of what a drug does to the body, meaning the relationship between the concentration of a drug at the site of action in the body and its biochemical and physiological effects.[20,21] Age-related changes in the PK of anticancer therapies occur due to physiological changes affecting the absorption, distribution, metabolism, and excretion of drugs.[21,22] Renal clearance, for example, typically declines with increasing age and impairs the excretion of renally excreted drugs, which results in increased drug exposure and toxicity.[23] Changes in PK associated with aging are important for medical oncologists to

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understand because they are potentially ameliorated, at least in part, by dose modifications or use of a less-toxic alternative.[20]

To better understand the role of age-associated changes in PK and its impact on the toxicity of anticancer therapies used in older adults with CRC, we conducted a systematic literature review aiming to investigate and evaluate trials studying the effect of aging on the PK of anticancer therapies commonly used in the treatment of CRC.

METHODS

Two independent reviewers (M.S. and R.Y.) conducted a systematic literature search of the electronic databases EMBASE and PubMed. Studies were included if they assessed the effect of age on the PK of the following chemotherapy or biologic anticancer therapies used in the treatment of CRC. The key words "elderly," "aging," "geriatrics," "old," AND "metabolism," "pharmaco*," "AUC" (area under the curve), "Cmax" (maximum concentration), "drug kinetics," and "drug clearance" were used, and the results were combined with each of the following anticancer agents with at least level II evidence for use in the treatment of patients with CRC: "irinotecan," "5-fluorouracil," "capecitabine," "oxaliplatin," "panitumumab," "cetuximab," "bevacizumab," "regorafenib," "ramucirumab," and "trifluridine/tipiracil" (also searched as "TAS-102"). We did not include immunotherapy agents because of their application in the small subset of patients with dMMR (mismatch repair deficient) CRC and the lack of level II evidence for its efficacy. All solid cancer types were included (Table 1), not just CRC. Results were limited to studies in humans and publication dates through June 2018 (Fig. 1).

The independent reviewers extracted and tabulated data for preplanned data fields for each study. Results were then reviewed together for consensus on each data field for each study. Disagreements were resolved with discussion and repeat review of the relevant study as needed.

RESULTS

Twenty-one publications met the eligibility criteria and were included in the review. All results are presented in Table 1. Seventeen publications were prospective studies, and 4 studies were pooled PK data analyses of prospective studies. The PK of irinotecan was assessed in 3 studies,[24–26] 5-FU in 7 studies,[27–33] capecitabine in 2 studies,[34,35] oxaliplatin in 2 studies,[36,37] bevacizumab in 1 study,[38] and cetuximab in 2 studies.[39,40] Four studies examined the PK of panitumumab, irinotecan, capecitabine, and cetuximab from pooled analysis of prospective studies.[41–44] Studies included a median of 44 patients (range 19–1200), with the age definition of an older adult varying across studies (≥65 years, >70 years, or >75 years). Six studies determined the PK of the anticancer therapies in CRC, whereas 15 studies analyzed other cancer types. PK parameters significantly affected by age were CL (drug clearance), AUC, Cmax, and Vmax (maximum rate of process) across 8 studies.[24–26,29,30,35,41,42]

Irinotecan

The doses of irinotecan examined in phase I dose-escalating studies ranged from 20 to 340 mg/m^2. Irinotecan is commonly dosed as 180 mg/m^2 every 2 weeks[45] or 350 mg/m^2 every 3 weeks to treat patients with CRC in clinical practice.[46] Three of the 4 studies found a significant association between the PK of irinotecan and increasing age. Klein et al[42] conducted a dose-escalation study of irinotecan (n = 78) in solid tumors and found a 7.2% reduction in irinotecan clearance (CL) for every 10 years in patients older than 60 years ($P < 0.001$). Miya et al[25] investigated factors influencing PK of irinotecan and showed increased AUC (r = 0.44) and Cmax (r = 0.42) of irinotecan with increasing age (age range 29–75 years, $P = 0.007$, $P = 0.009$, respectively). Poujol et al[26] showed a significant 8% decline in the CL of irinotecan with increasing age (median age 62 years, r = 0.42, $P = 0.009$).

5-FU

The doses of 5-FU ranged from 320 to 2400 mg/m^2. 5-FU is commonly dosed from 400 mg/m^2 (bolus) to 2400 mg/m^2 (a 46-hour continuous infusion) every 2 weeks in clinical practice.[47] Denham et al[30] (n = 44) found an increasing AUC of 5-FU with increasing age ($P = 0.02$). Etienne et al[29] (n = 104) assessed the effect of patient factors on the PK of 5-FU and found a statistically significant decrease in the CL of 5-FU with increasing age ($P < 0.001$). The other 5 studies showed no association between the PK of 5-FU and increasing age.

Capecitabine

All studies used the same dose of capecitabine (1000 mg/m^2). Capecitabine is commonly dosed from 1000 to 1250 mg/m^2 twice daily for 14 days every 3 weeks in clinical practice.[48] Louie et al[35] investigated the PK of capecitabine in older adults with CRC (n = 29) and found that older patients (aged >70 years), compared with younger patients (aged <60 years), had a statistically significant 71% decline in CL ($P = 0.03$) and a 150% increase in the AUC ($P = 0.04$) of capecitabine, but no difference in the PK parameters of the metabolites (5′DFCR, 5′DFUR, and 5-FU) of capecitabine. Daher Abdi et al[43] compared the PK data of capecitabine in 20 older patients with breast cancer or CRC (aged >75 years) with 40 younger patients (aged <60 years) from 2 previous clinical trials. Elimination parameters of capecitabine and its metabolites were not affected by age. Significantly higher median exposures of capecitabine and its metabolites occurred in older patients who experienced hand–foot syndrome, compared with older patients who did not experience hand–foot syndrome. Cassidy et al[34] in a small study (n = 25) of adults with solid tumors showed that age, sex, body surface area (BSA), or creatinine clearance did not affect PK parameters of capecitabine and its metabolites.

Oxaliplatin

The doses of oxaliplatin ranged from 50 to 130 mg/m^2. Oxaliplatin is commonly dosed as 85 mg/m^2 every 2 weeks and 130 mg/m^2 every 3 weeks in clinical practice.[47] Bastian et al[36] investigated the effect of age on the PK of oxaliplatin in 56

TABLE 1. Pharmacokinetics of Anticancer Drugs in Older Adults

Anticancer Drug	Participants, Median Age (Range)	Cancer Type	Study Purpose	Findings and Comments	Author, Year
Irinotecan	n = 28 (<65 yrs: n = 16 and ≥65 yrs: n = 12), 63 yrs (29–82 yrs)	CRC, unknown primary, uterus, and renal cell	Phase I dose-escalation PK study of oral irinotecan in patients with solid tumors to characterize the MTD, DLTs, PK profile, and antitumor effects. Doses were 20, 40, 66, and 100 mg/m^2/d, daily for 5 days every 3 weeks.	High interindividual PK variability. Advanced age was associated with reduced drug tolerance; patients aged ≥65 years had DLT at lower dose (66 mg/m^2/d) than patients aged <65 years (80 mg/m^2/d).	Drengler et al,[24] 1999
	n = 36, 60 yrs (29–75 yrs)	Lung, head and neck, colon, and uterus	Observational study examining influence of sex, age, BSA, and SCr on PK of irinotecan and its metabolites. Dose was 100 mg/m^2 weekly.	Irinotecan AUC significantly increased with increasing age ($P = 0.007$), male sex ($P = 0.008$), and poor SCr. Irinotecan Cmax significantly increased with increasing age ($P = 0.009$), male sex ($P = 0.007$), and BSA ($P = 0.023$).	Miya et al,[25] 2001
	n = 78, 61 yrs (31–80 yrs)	Solid tumors and lymphoma	PK analysis of 2 dose-escalation studies to develop a population PK model. Doses were 100–175 mg/m^2 and 240–340 mg/m^2.	Increasing age and poorer performance status significantly correlated with decreased irinotecan CL ($P < 0.01$). Irinotecan CL decreased 2.1 L/h (7.2%) for every 10 years in patients older than 60 years.	Klein et al,[42] 2002
	n = 35, 62 yrs (mean)	Digestive system	Prospective observational PK study investigating the effect of patient factors on irinotecan CL. Dose was 180 mg/m^2 2 weekly.	Irinotecan CL significantly declined with increasing age and explained 8% of the interindividual variability in CL.	Poujol et al,[26] 2005
5-FU	n = 26 (≥70 yrs: n = 4 and <70 yrs: n = 22), 53 yrs (43–75 yrs)	CRC, breast, and esophagus	Observational study investigating the effect of sex, age, and BSA on the PK of 5-FU. Doses were 320–960 mg/m^2 weekly to 3 weekly.	Advanced age correlated with reduced 5-FU CL but not statistically significant. ↑ 5-FU CL was associated with ↑ BSA, male sex, and ↑ dose ($P < 0.001$).	Port et al,[27] 1991
	n = 360 (>70 yrs: n = 58, 51–70 yrs: n = 245, and <50 yrs: n = 57), 62 yrs (25–91 yrs)	HNSCC	Prospective observational study examining the effect of sex and age on 5-FU CL. The mean 5-FU dose was 857.5 mg/m^2 (range, 365–1224 mg/m^2) on days 1–5.	5-FU CL was not influenced by age ($P = 0.45$) but was 10% lower in women ($P = 0.0005$).	Milano et al,[28] 1992
	n = 104, 59 yrs (31–84 yrs)	Head and neck and esophagus	Prospective study investigating the effect of patient factors including age on 5-FU CL. 5-FU dose was 1000 mg/m^2/d on days 2–6.	Increasing age correlated with reduced 5-FU CL ($P < 0.001$).	Etienne et al,[29] 1998
	n = 44, 72 yrs (42–91 yrs)	Esophageal	Observational study investigating causes of increased rate of myelosuppression in older patients on chemoradiotherapy including PK of 5-FU (as 5-FU/cisplatin). 5-FU dose was 800 mg/m^2/d by continuous i.v. infusion over 4 ± 5 days.	Advanced age correlated with higher 5-FU AUC ($P = 0.02$).	Denham et al,[30] 1999
	n = 181, 65 yrs (34–87 yrs)	CRC	Observational study examining patient factors including age on 5-FU AUC and association with toxicity in adjuvant setting. Dose used was 425 + 20 mg/m^2 daily for 5 days every 4 weeks.	5-FU AUC or CL not influenced by age. ↑ drug dose ($P < 0.0001$), ↑ body weight ($P < 0.0001$), and female sex ($P < 0.0001$) were correlated with ↑ 5-FU AUC.	Gusella et al,[31] 2006
	n = 103 (<65 yrs: n = 55, 59 yrs (33–64 yrs), ≥65 yrs: n = 48), 70 yrs (65–80 yrs)	mCRC	Prospective study of PK-guided dosing of 5-FU in patients with mCRC assessing the impact of age on PK of 5-FU. Dose used was 425 + 20 mg/m^2 daily for 5 days every 4 weeks.	5-FU CL, Vd, $t_{1/2}$, and AUC not influenced by age ($P = 0.1$). Patients aged ≥65 years tolerated dose intensification similar to the patients aged <50 years ($P = 0.9$).	Duffour et al,[32] 2010
	n = 31 (≥65 yrs: n = 14 and <65 yrs: n = 17), 63 yrs (31 –81 yrs)	Gastrointestinal	Prospective single-arm study investigating the effect of sex, age, BSA, SCr, liver dysfunction, and DPYD genotype on PK (AUC, CL and Vd) of 5-FU. 5-FU bolus dose was 400 mg/m^2 followed by a 46-hour continuous infusion at a dose of 2400 mg/m^2.	5-FU CL was ↑ in male sex ($P < 0.01$) and not affected by age.	Mueller et al,[33] 2012

(*continued on next page*)

TABLE 1. (*Continued*) Pharmacokinetics of Anticancer Drugs in Older Adults

Anticancer Drug	Participants, Median Age (Range)	Cancer Type	Study Purpose	Findings and Comments	Author, Year
Capecitabine	n = 25, 62 yrs (41–80 yrs)	CRC and breast	Randomized cross-over bioequivalence study of 2 capecitabine tablet formulations, examining the effect of age, sex, BSA, and creatinine CL on PK of capecitabine PK. Dose was 1250 mg/m^2, twice daily for 14 days.	PK of capecitabine and its metabolites not influenced by age ($P > 0.15$), BSA ($P = 0.03$), or creatinine CL ($P = 0.29$) but were only ↑ in female sex ($P = 0.001$).	Cassidy et al,[34] 1999
	n = 29 (A: ≥70 yrs: n = 24, 76 yrs (mean), B: <60 yrs: n = 5), 55 yrs (mean)	Unresectable CRC	Prospective study investigating the influence of age on PK of capecitabine and its metabolites. Dose used was 1000 mg/m^2 twice daily for 14 days.	Advanced age was associated with 71% decrease in capecitabine CL ($P = 0.03$) and 150% increase in capecitabine AUC ($P = 0.04$).	Louie et al,[35] 2013
	n = 60 (<75 yrs: n = 40, 54 yrs (30–73 yrs), ≥75 yrs: n = 20), 80 yrs (75–92 yrs)	Breast and CRC	Prospective observational study examining effect of age on PK of capecitabine and its metabolites and investigating the exposure–effect relationship in the older age group (>75 yrs). Dose used was 1250 mg/m^2 twice daily for 14 days.	PK of capecitabine not influenced by age ($P = 0.59$). Higher exposure of capecitabine and its metabolites was observed in patients who developed hand and foot syndrome in cycle 2 of treatment ($P = 0.01$).	Daher Abdi et al,[43] 2014
Oxaliplatin	n = 56, 59 yrs (41–79 yrs)	Solid tumors (majority CRC)	Prospective phase I and phase I/II studies to develop a population PK model and to investigate the influence of covariates (including age) on PK of oxaliplatin. Doses used were 50, 65, 75, 85, 100, or 130 mg/m^2 in 2- or 4-hour i.v. infusions.	Oxaliplatin CL not influenced by age, but was positively correlated with body weight ($P < 0.001$), negatively correlated with SCr ($P < 0.001$), and was greater in male patients ($P < 0.01$).	Bastian et al,[36] 2003
	n = 40, 59 yrs (29–82 yrs)	CRC	Prospective observational phase I study to explore association between patient factors and PK parameters of oxaliplatin. Doses ranged from 80 to 130 mg/m^2 2 weekly to 3 weekly.	PK parameters of oxaliplatin not influenced by age, but ↑ CL was significantly correlated with ↑ SCr, ↑ BSA, and ↓ Hb.	Delord et al,[37] 2003
Panitumumab	n = 1200, male 62 yrs (mean), female 59 yrs (mean)	CRC, lung, and kidney	Pooled data analysis to determine population PK modeling of panitumumab from 14 prospective trials and to explore the impact of baseline covariates on PK parameters of panitumumab. Doses ranged from 0.01 to 9 mg/kg but mostly 2.5 mg/kg weekly, 6 mg/kg 2 weekly, and 9 mg/kg 3 weekly.	Advanced age was correlated with reduced panitumumab Vmax ($P < 0.001$), but effect size was small (0.7% of variance in AUC versus weight-based dose regimen effect of 69.2%).	Ma et al,[41] 2009
Bevacizumab	n = 19, 60 yrs (37–73 yrs)	CRC	Prospective study to develop a population PK model for bevacizumab. Doses used were 5 mg/kg 2 weekly and 7.5 mg/kg 3 weekly.	PK of bevacizumab not influenced by age ($P > 0.01$).	Panoilia et al,[38] 2015
Cetuximab	n = 40, 60 yrs (22–85 yrs)	CRC	Prospective study to evaluate the PK of cetuximab given as to different doses. Effects of patient factors on cetuximab CL were assessed. Loading doses of 50, 100, 250, 400, or 500 mg/m^2 followed by weekly fixed dose of 250 mg/m^2.	Cetuximab CL not influenced by age but increased with BSA ($P = 0.002$), weight ($P = 0.002$), and dose ($P < 0.0001$).	Tan et al,[39] 2006
	n = 156, 56 yrs (23–77 yrs)	HNSCC	Pooled data analysis of PK of cetuximab from early-phase I/II & II studies to evaluate the PK of cetuximab and to identify the effects of covariates on its PK. Loading dose of 400 mg/m^2 followed by a weekly fixed dose of 250 mg/m^2.	Cetuximab PK parameters not influenced by age. Cetuximab CL predicted by ideal body weight ($P < 0.001$) and white blood cell count ($P < 0.001$).	Dirks et al,[44] 2008
	n = 96, 63 yrs (38–80 yrs)	mCRC	Prospective phase II study, investigating influence of interindividual variability in cetuximab PK on progression-free survival of patients with CRC. Loading dose of 400 mg/m^2 followed by a weekly fixed dose of 250 mg/m^2.	Cetuximab PK parameters not influenced by age. ↑ BSA correlated with ↑cetuximab Vd ($P = 0.01$) and ↑ pretreatment serum albumin correlated with↓ cetuximab CL ($P = 0.006$).	Azzopardi et al,[40] 2011

Cmax, maximum concentration; DLT, dose-limiting toxicities; Hb, hemoglobin; HNSCC, head and neck squamous cell carcinoma; i.v., intravenous; mCRC, metastatic colorectal cancer; MTD, maximum-tolerated dose; SCr, serum creatinine; $t_{1/2}$, half-life; Vd, volume of distribution.

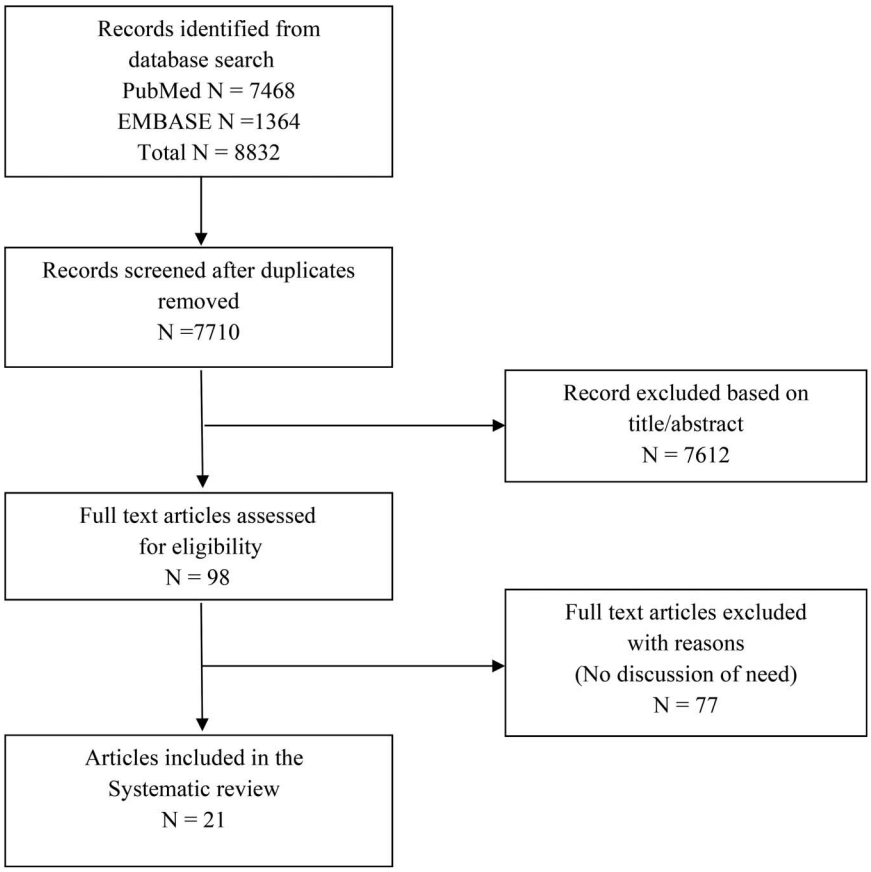

FIGURE 1. PRISMA flow diagram.

patients (41–79 years) with solid tumors (majority CRC) from phase I and phase I/II studies. CL of oxaliplatin was not affected by age, but decreased CL was correlated with lower body weight and higher serum creatinine level. Delord et al[37] conducted an observational phase I study in 40 patients aged 29–82 years with CRC, exploring the impact of multiple covariates including age, sex, anemia, BSA, and renal function on the PK of oxaliplatin. PK of oxaliplatin was not affected by age, but increased CL was significantly correlated with increased serum creatinine level, higher BSA, and hemoglobinemia.

Panitumumab

The doses of panitumumab examined in phase I dose-escalating studies ranged from 0.01 to 9 mg/kg. Panitumumab is dosed as 6 mg/kg every 2 weeks in clinical practice.[49] Ma et al[41] investigated the PK of panitumumab in a pooled data analysis of 14 prospective clinical trials including 1200 patients with solid tumors. The population PK of panitumumab was explained by both linear (dose-proportional manner) and nonlinear (saturable binding to epidermal growth factor receptor) elimination pathways. Age was negatively correlated with Vmax of panitumumab (nonlinear clearance) with an increase in age from 50 years to 70 years yielding a 15.3% decrease in Vmax. However, the contribution of age to the variance of AUC at steady state (AUC_{SS}) was small at only 0.7% compared with that of the weight-based dose regimen around 69.2%.

Bevacizumab

In the only published relevant study, the dose of bevacizumab was 5 mg/kg. Bevacizumab is dosed as 5 mg/kg every 2 weeks to 7.5 mg/kg every 3 weeks in clinical practice.[50] Panoilia et al[38] conducted a small study (n = 19) primarily designed to characterize bevacizumab's population PK. In this study, age had no significant effect on bevacizumab PK.

Cetuximab

All 3 studies of cetuximab used the same dose of 250 mg/m^2. Cetuximab is dosed as 500 mg/m^2 every 2 weeks in clinical practice.[51] In each case, the PK of cetuximab was not influenced by age.[39,40,44] Azzopardi et al[40] examined patient factors that influenced the PK of cetuximab in a PK-guided dose intensification study of cetuximab in 96 patients with metastatic CRC aged 38–80 years. Only BSA and initial serum albumin concentration were significantly correlated with CL of cetuximab, but not other covariates including age. Tan et al[39] (n = 40) and Dirks et al[44] (n = 156) investigated the effect of patient factors on the PK of cetuximab and showed that patients' BSA and weight affected PK parameters, but not age.

DISCUSSION

Older age was associated with PK parameters in all studies concerning irinotecan,[24–26,42] the one study

concerning panitumumab,[41] and some, but not all, of the studies concerning 5-FU[29,30] and capecitabine.[35] No association between increasing age and PK parameters was found in the included studies concerning oxaliplatin, bevacizumab, or cetuximab.[36–40,44] There were overall few studies that determined the effect of age as a primary outcome on the PK of anticancer therapies used in the management of CRC.

We conducted this review to help determine whether changes in PK are responsible for the increased toxicity symptoms such as fatigue, diarrhea, myelosuppression, dehydration, and consequent hospitalizations[8] experienced by older adults with CRC on these drugs. The most consistent findings for an effect of older age on PK were in the studies concerning irinotecan. Where age-related PK changes were found, however, the reported effect sizes were small, all less than 10% and so unlikely to be of clinical significance. There are no guidelines for the interpretation and clinical significance of PK parameters, but Joerger[52] has suggested a minimum change of at least 20% in major PK parameters, mainly drug elimination, to be considered as clinically significant.

The most consistent finding of changes in the PK of anticancer therapies with older age is the decline in CL. The study by Louie et al included in this review showed a 71% decline in CL of capecitabine in older adults. Studies in other cancer types have also shown a decline in CL in older adults such as a 31% decline in the CL of carboplatin in lung cancer[53] and a 30% decline in the CL of doxorubicin in breast cancer in 2 studies, which both defined older adults as aged >70 years.[54] Such knowledge of the PK of anticancer therapies in older adults provides an opportunity to overcome the heterogeneity of the aging process and to refine prescribing, by better understanding treatment-related toxicity and optimize dosing for maximum efficacy.

Factors other than age-related changes in the PK of anticancer therapies are likely responsible for the excess treatment toxicity seen in older adults with CRC. Age-related changes in PD can explain, for example, the greater hematological toxicity from chemotherapy due to reduced hematopoiesis with increasing age. Geriatric syndromes are another likely cause. The presence of multiple comorbidities in older adults can lead to frailty, vulnerability, and limited physiological reserve to tolerate serious treatment toxicities.[55] Polypharmacy, for example, carries the potential risk of serious drug–drug interactions with anticancer therapies (eg, QT-prolonging drugs). Cognitive impairment can cause confusion and impede compliance with usual medications and oral chemotherapy, such as capecitabine, usually taken independently at home. Limited social support and social isolation can cause late presentations to medical care, leading to more severe and prolonged toxicity.

Optimal selection of older adults for anticancer therapy and tailored prescribing are imperative for providing quality care to older adults with cancer. Patient selection can be aided by the use of Complex Geriatric Assessment or an abbreviated version of such, and/or the use of risk prediction tools that estimate the risk of severe chemotherapy toxicity.[56,57] Complex Geriatric Assessments are recommended for all older adults with cancer[58] and have been shown to identify impairments and frailty, predict survival and treatment toxicity, and help develop appropriate supportive care interventions. There are no studies determining the relationship, if any, between the results of geriatric assessments and PK.

Prescribing anticancer therapies involves careful consideration and application of relevant cancer treatment guidelines. International cancer treatment guidelines do not recommend dose modifications for older age per se for the anticancer therapies in our review.[48,59,60] The Australian EVIQ guidelines recommend a lower starting dose of capecitabine when used as monotherapy in the metastatic setting in "elderly patients and other patients considered at risk of toxicity" (from 1250 to 1000 mg/m^2 bid).[61] Dose modifications across guidelines are recommended in people with renal and hepatic impairment, commonly seen in older adults, for 5-FU, capecitabine, irinotecan, and oxaliplatin. Importantly, older age should never be seen as a reason to not actively treat an older patient with CRC, especially where there is genuine consideration for a positive outcome.[62] What is clear from these PK data is the need to consider dose individualization and careful monitoring to guide dosing in older people.[20]

An important limitation of the available PK studies is their tendency to be conducted in clinical trials enrolling predominantly younger, fitter patients. Even where older adults are eligible and included in clinical trials, they typically comprise only a small proportion of the total trial population, and the included older adults are also a very fit subset of the entire population of older adults with cancer.[63] These limit the generalizability of the PK results and consequent dosing recommendations to the typical older adults in clinical practice. This limitation applies to several of the studies included in our review.

Other limitations of our review include the methodological heterogeneity across studies, the small number of studies for each drug included in the review, the small number of patients in the included studies, and the variable definitions of aging and older adults. Some studies, for example, used 65 years as a dichotomous cutoff for older versus younger patients, whereas other studies used 75 years. These limitations make it difficult to draw firm conclusions and reduce the applicability of the results to typical older adults having anticancer therapy for CRC in clinical practice. The small sample sizes of the included studies and the fact that age is typically explored as a potential predictor in subgroup analyses rather than as a primary outcome reduce the power to detect age as a significant covariate.

CONCLUSION

Older age is significantly associated with the PK parameters of some anticancer therapies used in older adults with CRC, but the effects are small and not easily translated into recommended dose modifications of these drugs for older adults. PK and PD studies including older adults typical of routine clinical practice to optimize dosing of anticancer therapy are warranted.

REFERENCES

1. Arnold M, Sierra MS, Laversanne M, et al. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017;66:683–691.
2. Millan M, Merino S, Caro A, et al. Treatment of colorectal cancer in the elderly. *World J Gastrointest Oncol.* 2015;7:204–220.
3. Stein BN, Petrelli NJ, Douglass HO, et al. Age and sex are independent predictors of 5-fluorouracil toxicity. Analysis of a large scale phase III trial. *Cancer*. 1995;75:11–17.
4. Hall M, Krell D, Glynne-Jones R. Single-agent capecitabine use in patients age 70 or older with colorectal cancer. *J Clin Oncol.* 2015;33:764.
5. Cen P, Liu C, Du XL. Comparison of toxicity profiles of fluorouracil versus oxaliplatin regimens in a large population-based cohort of elderly patients with colorectal cancer. *Ann Oncol.* 2012;23:1503–1511.
6. Kim JY, Kim YJ, Lee KW, et al. Practical outcome of adjuvant FOLFOX4 chemotherapy in elderly patients with stage III colon cancer: single-center study in Korea. *Jpn J Clin Oncol.* 2013;43:132–138.
7. Cassidy J, Twelves C, Van Cutsem E, et al. First-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. *Ann Oncol.* 2002;13:566–575.
8. McKibbin T, Frei CR, Greene RE, et al. Disparities in the use of chemotherapy and monoclonal antibody therapy for elderly advanced colorectal cancer patients in the community oncology setting. *Oncologist.* 2008;13:876–885.
9. Folprecht G, Rougier P, Saltz L, et al. Irinotecan in first line therapy of elderly and non-elderly patients with metastatic colorectal cancer: meta-analysis of four trials investigating 5-FU and irinotecan. *J Clin Oncol.* 2006;24:3578.
10. Tam VC, Rask S, Koru-Sengul T, et al. Generalizability of toxicity data from oncology clinical trials to clinical practice: toxicity of irinotecan-based regimens in patients with metastatic colorectal cancer. *Curr Oncol.* 2009;16:13–20.
11. Figer A, Perez-Staub N, Carola E, et al. FOLFOX in patients aged between 76 and 80 years with metastatic colorectal cancer: an exploratory cohort of the OPTIMOX1 study. *Cancer*. 2007;110:2666–2671.
12. Lichtman SM, Villani G. Chemotherapy in the elderly: pharmacologic considerations. *Cancer Control*. 2000;7:548–556.
13. Cunningham D, Lang I, Marcuello E, et al. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2013;14:1077–1085.
14. Korc-Grodzicki B, Boparai MK, Lichtman SM. Prescribing for older patients with cancer. *Clin Adv Hematol Oncol.* 2014;12:309–318.
15. Korc-Grodzicki B, Holmes HM, Shahrokni A. Geriatric assessment for oncologists. *Cancer Biol Med.* 2015;12:261–274.
16. Kotake K, Asano M, Ozawa H, et al. Tumour characteristics, treatment patterns and survival of patients aged 80 years or older with colorectal cancer. *Colorectal Dis.* 2015;17:205–215.
17. Patel SS, Nelson R, Sanchez J, et al. Elderly patients with colon cancer have unique tumor characteristics and poor survival. *Cancer*. 2013;119:739–747.
18. Moth EB, Vardy J, Blinman P. Decision-making in geriatric oncology: systemic treatment considerations for older adults with colon cancer. *Expert Rev Gastroenterol Hepatol.* 2016;10:1321–1340.
19. Zulman DM, Sussman JB, Chen X, et al. Examining the evidence: a systematic review of the inclusion and analysis of older adults in randomized controlled trials. *J Gen Intern Med.* 2011;26:783–790.
20. He X, Clarke SJ, McLachlan AJ. Clinical pharmacology of chemotherapy agents in older people with cancer. *Curr Gerontol Geriatr Res.* 2011; 2011:628670.
21. Hilmer SN, McLachlan AJ, Le Couteur DG. Clinical pharmacology in the geriatric patient. *Fundam Clin Pharmacol.* 2007;21:217–230.
22. McLachlan AJ, Hilmer SN, Le Couteur DG. Variability in response to medicines in older people: phenotypic and genotypic factors. *Clin Pharmacol Ther.* 2009;85:431–433.
23. Wasil T, Lichtman SM. Clinical pharmacology issues relevant to the dosing and toxicity of chemotherapy drugs in the elderly. *Oncologist*. 2005;10:602–612.
24. Drengler RL, Kuhn JG, Schaaf LJ, et al. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. *J Clin Oncol.* 1999;17:685–696.
25. Miya T, Goya T, Fujii H, et al. Factors affecting the pharmacokinetics of CPT-11: the body mass index, age and sex are independent predictors of pharmacokinetic parameters of CPT-11. *Invest New Drugs*. 2001;19:61–67.
26. Poujol S, Bressolle F, Duffour J, et al. Pharmacokinetics and pharmacodynamics of irinotecan and its metabolites from plasma and saliva data in patients with metastatic digestive cancer receiving Folfiri regimen. *Cancer Chemother Pharmacol*. 2006;58:292–305.
27. Port RE, Daniel B, Ding RW, et al. Relative importance of dose, body surface area, sex, and age for 5-fluorouracil clearance. *Oncology*. 1991;48:277–281.
28. Milano G, Etienne MC, Cassuto-Viguier E, et al. Influence of sex and age on fluorouracil clearance. *J Clin Oncol.* 1992;10:1171–1175.
29. Etienne MC, Chatelut E, Pivot X, et al. Co-variables influencing 5-fluorouracil clearance during continuous venous infusion. A NONMEM analysis. *Eur J Cancer*. 1998;34:92–97.
30. Denham JW, Ackland SP, Burmeister B, et al. Causes for increased myelosuppression with increasing age in patients with oesophageal cancer treated by chemoradiotherapy. *Eur J Cancer*. 1999;35:921–927.
31. Gusella M, Crepaldi G, Barile C, et al. Pharmacokinetic and demographic markers of 5-fluorouracil toxicity in 181 patients on adjuvant therapy for colorectal cancer. *Ann Oncol.* 2006;17:1656–1660.
32. Duffour J, Roca L, Bressolle F, et al. Clinical impact of intensified 5-Fluorouracil-based chemotherapy using a prospective pharmacokinetically-guided dosing approach: comparative study in elderly and non-elderly patients with metastatic colorectal cancer. *J Chemother.* 2010;22:179–185.
33. Mueller F, Büchel B, Köberle D, et al. Gender-specific elimination of continuous-infusional 5-fluorouracil in patients with gastrointestinal malignancies: results from a prospective population pharmacokinetic study. *Cancer Chemother Pharmacol.* 2013;71:361–370.
34. Cassidy J, Twelves C, Cameron D, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. *Cancer Chemother Pharmacol.* 1999;44:453–460.
35. Louie SG, Ely B, Lenz HJ, et al. Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). *Br J Cancer*. 2013;109:1744–1749.
36. Bastian G, Barrail A, Urien S. Population pharmacokinetics of oxaliplatin in patients with metastatic cancer. *Anticancer Drugs*. 2003;14:817–824.
37. Delord JP, Umlil A, Guimbaud R, et al. Population pharmacokinetics of oxaliplatin. *Cancer Chemother Pharmacol.* 2003;51:127–131.
38. Panoilia E, Schindler E, Samantas E, et al. A pharmacokinetic binding model for bevacizumab and VEGF165 in colorectal cancer patients. *Cancer Chemother Pharmacol.* 2015;75:791–803.
39. Tan AR, Moore DF, Hidalgo M, et al. Pharmacokinetics of cetuximab after administration of escalating single dosing and weekly fixed dosing in patients with solid tumors. *Clin Cancer Res.* 2006;12:6517–6522.
40. Azzopardi N, Lecomte T, Ternant D, et al. Cetuximab pharmacokinetics influences progression-free survival of metastatic colorectal cancer patients. *Clin Cancer Res.* 2011;17:6329–6337.
41. Ma P, Yang BB, Wang YM, et al. Population pharmacokinetic analysis of panitumumab in patients with advanced solid tumors. *J Clin Pharmacol.* 2009;49:1142–1156.
42. Klein CE, Gupta E, Reid JM, et al. Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. *Clin Pharmacol Ther.* 2002;72:638–647.
43. Daher Abdi Z, Lavau-Denes S, Prémaud A, et al. Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. *Cancer Chemother Pharmacol.* 2014;73: 1285–1293.
44. Dirks NL, Nolting A, Kovar A, et al. Population pharmacokinetics of cetuximab in patients with squamous cell carcinoma of the head and neck. *J Clin Pharmacol.* 2008;48:267–278.
45. Colucci G, Gebbia V, Paoletti G, et al. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol.* 2005;23:4866–4875.
46. Fuchs CS, Moore MR, Harker G, et al. Phase III comparison of two irinotecan dosing regimens in second-line therapy of metastatic colorectal cancer. *J Clin Oncol.* 2003;21:807–814.
47. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol.* 2000;18:2938–2947.
48. Van Cutsem E, Nordlinger B, Cervantes A, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2014;25:iii1–iii9.

49. Price TJ, Peeters M, Kim TW, et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol.* 2014;15:569–579.
50. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004;350:2335–2342.
51. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med.* 2007;357:2040–2048.
52. Joerger M. Covariate pharmacokinetic model building in oncology and its potential clinical relevance. *AAPS J.* 2012;14:119–132.
53. Merino-Sanjuan M, Monteiro JF, Porta-Oltra B, et al. Effect of age on systemic exposure and haematological toxicity of carboplatin in advanced non-small cell lung cancer patients. *Basic Clin Pharmacol Toxicol.* 2011;109:457–464.
54. Robert J, Hoerni B. Age dependence of the early-phase pharmacokinetics of doxorubicin. *Cancer Res.* 1983;43:4467–4469.
55. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* 2001;56:M146–M156.
56. Hurria A, Togawa K, Mohile SG, et al. Predicting chemotherapy toxicity in older adults with cancer: a prospective multicenter study. *J Clin Oncol.* 2011;29:3457–3465.
57. Extermann M, Boler I, Reich RR, et al. Predicting the risk of chemotherapy toxicity in older patients: the chemotherapy risk assessment scale for high-age patients (CRASH) score. *Cancer*. 2012;118:3377–3386.
58. Wildiers H, Heeren P, Puts M, et al. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. *J Clin Oncol.* 2014;32:2595–2603.
59. Network NCC. NCCN guidelines, version 2.2018. Colon cancer. 2018. Available at: https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf. Accessed May 8, 2018.
60. Labianca R, Nordlinger B, Beretta GD, et al. Early colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2013;24(suppl 6):vi64–vi72.
61. EVIQ. Colorectal metastatic capecitabine. 2018. Available at: https://www.eviq.org.au/medical-oncology/colorectal/metastatic/94-colorectal-metastatic-capecitabine. Accessed May 8, 2018.
62. Mitry E, Rougier P. Review article: benefits and risks of chemotherapy in elderly patients with metastatic colorectal cancer. *Aliment Pharmacol Ther.* 2009;29:161–171.
63. Hamaker ME, Stauder R, van Munster BC. Exclusion of older patients from ongoing clinical trials for hematological malignancies: an evaluation of the National Institutes of Health Clinical Trial Registry. *Oncologist*. 2014;19:1069–1075.

Dried Blood Spot Sampling in the Monitoring of Anticancer Therapy for Solid Tumors: A Systematic Review

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Background: Dried blood spot (DBS) sampling is a convenient alternative to whole-blood sampling for therapeutic drug monitoring (TDM) in clinical practice. The aim of this study was to systematically review studies that have examined and used DBS sampling for the TDM of chemotherapy and targeted therapy agents for the treatment of patients with solid cancers.

Methods: Using the PRISMA guidelines, a systematic literature search of EMBASE and PUBMED was performed to identify eligible clinical studies that used DBS sampling to monitor chemotherapy or targeted therapy for the treatment of solid cancers.

Results: Of the 23 eligible studies, 3 measured concordance between drug concentrations determined by DBS and whole-blood, 7 developed analytical methods of DBS, and 13 performed both. DBS was employed for the TDM of everolimus (3 studies), vemurafenib (2 studies), pazopanib (2 studies), abiraterone (2 studies), mitotane, imatinib, adavosertib, capecitabine, 5-fluorouracil, gemcitabine, cyclophosphamide, ifosfamide, etoposide, irinotecan, docetaxel, gefitinib, palbociclib/ribociclib, and paclitaxel (one study each). The studies included a median of 14 participants (range: 6–34), with 10–50 μL of blood dispensed on DBS cards (20) and Mitra devices (3). Seventeen of the 20 studies that used DBS found no significant impact of the hematocrit on the accuracy and precision of the developed method in the normal hematocrit ranges (eg, 29.0%–59.0%). DBS and plasma or venous concentrations were highly correlated (correlation coefficient, 0.872–0.999) for all drugs, except mitotane, which did not meet a predefined level of significance ($r > 0.872$; correlation coefficient, $r = 0.87$, $P < 0.0001$).

Conclusions: DBS provides an alternative sampling strategy for the TDM of many anticancer drugs. Further research is required to establish a standardized approach for sampling and processing DBS samples to allow future implementation.

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Key Words: dried blood spot, therapeutic drug monitoring, solid cancers, chemotherapy and targeted therapy

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INTRODUCTION

Patients treated with chemotherapy or targeted therapy (eg, tyrosine kinase inhibitors) have a risk of significant toxicities from overdosing or compromised treatment due to inadvertent underdosing. Therapeutic drug monitoring (TDM) is a valuable tool used to avoid treatment failure or treatment-related harms by guiding individualized dosing of anticancer therapy, especially for drugs with a narrow therapeutic index and wide interindividual variation in pharmacokinetics (PKs).[1] TDM uses PK-guided dosing instead of body surface area–guided dosing or flat dosing, both of which do not account for interindividual variability in the PKs of agents.[2]

TDM-based dosing strategies rely on an established relationship between the PKs of anticancer therapies and clinical outcomes (efficacy and toxicity).[3–5] In clinical practice, TDM-based dosing has been partially implemented for a small number of anticancer drugs according to observational, retrospective, and randomized control trials, including carboplatin,[6] methotrexate,[7] busulfan,[8] and mitotane.[9] TDM for other agents, such as imatinib, 5-fluorouracil, and pazopanib, has not been implemented despite evidence of benefit and feasibility in well-designed randomized control trials.[1,3,10,11] Challenges of routine implementation of TDM for cancer include (i) measured plasma concentrations requiring clinical and pharmacological interpretation to guide decision making; (ii) the need for venipuncture collection of 1- to 5-mL sample volume over multiple time points and the infrastructure for such collections; and (iii) limited availability of TDM assays.[1,3,12] To overcome these challenges, new methods and analytical assays for small volumes using robust and convenient sampling techniques are required.

Novel techniques for microsampling and precise analytical investigations for TDM indicate that PK-guided individualized dosing is now more feasible in clinical practice.[13] There are several microsampling methods for the TDM of drugs. A commonly used method is dried blood spot (DBS) sampling that uses capillary blood from a finger prick with an automatic lancet. Briefly, a drop of blood is collected to fill a premarked circle on the absorbent paper. Thereafter, the blood drop is dried at room temperature, and the filter paper is packed for transportation to the laboratory. A disc is punched from the DBS paper on which the analyte is measured using an analytical technique. The advantages of DBS sampling over venipuncture include the use of a very

small volume of blood, convenience, simplification of logistics for remote sampling with reduced workforce requirements, increased sample stability, and easier storage and shipping.[12] The Food and Drug Administration (FDA) guidelines define the necessary parameters for the validation of quantitative DBS-based methods and on the application of validated methods in routine clinical practice.[14] Accordingly, validation should include assessing the effects of storage and handling temperatures, homogeneity of sample spotting, hematocrit, stability, carryover, and reproducibility, including incurred sample reanalysis.

Another microsampling method is volumetric absorptive microsampling (VAMS) using the Mitra device. This device has a relatively simple collection process and can be used by patients at home. It absorbs a small (10–30 μL) volume of blood from a finger prick into a tip, which is then used to extract the analytes, eliminating the need for a subpunch from a DBS card and problems of homogeneity of the sample. DBS sampling and VAMS have been used to assist in the diagnosis, investigation, and measurement of a wide variety of pathogens, including HIV, HBV, HCV, and inherited metabolic disorders drugs.[15–17]

Given the potential application of DBS and VAMS in improving barriers for TDM implementation and the interest in individualized dosing of anticancer therapy, the aim of this systematic review was to identify and describe published studies that used DBS sampling and VAMS for TDM of chemotherapy and targeted therapy agents in the treatment of patients with solid cancers. This review also investigated the agreement between conventional venous blood samples and DBS sampling approaches for the TDM of anticancer drugs. We hypothesized that VAMS and DBS sampling methods can be used in the TDM of anticancer therapy and are feasible, less invasive, and are as effective as venous sampling methods.

METHODS

A systematic literature search of the electronic databases, Web of Science, EMBASE, and PUBMED was conducted by 2 independent reviewers (MS, AM) using PRISMA guidelines.[18] A combination of MeSH terminology associated with the Medline database and relevant keywords was used to capture more studies. Studies were included if they assessed the use of DBS sampling or VAMS in the TDM of chemotherapy or biological anticancer therapies used for the treatment of solid cancers. Only original articles with available full text were eligible for inclusion in the review: The key words, "DBS," "dried blood spot," "microsampling," "volumetric absorptive microsampling," "finger prick*," AND "metabolism," "pharmaco*", "TDM," "therapeutic drug monitoring," "drug kinetics," and "drug clearance," were used. The results were then combined (AND) with the search results of "cancer," "chemotherapy," "targeted therapy," "tyrosine kinase inhibitor," "solid tumo*," and "cytotoxic." The results were limited to studies performed with humans, written in English, and had publication dates up to July 2022 (see Table 1 and Fig. 1). Studies that investigated the anticancer therapy used in the treatment of hematological malignancies and hormonal therapies were excluded. The extracted and tabulated data were reviewed together for consensus in each data field for each study. Disagreements were resolved through discussion, which involved the authors repeating the review of the relevant study to reach a consensus.

RESULTS

Twenty-three studies met the eligibility criteria and were included in this review. Of these studies, 20 described analytical method development, and 14 of the 20 studies investigated the agreement between the concentrations determined in DBS and whole-blood samples (Table 1). Three studies (involving vemurafenib, pazopanib, and everolimus) only examined the agreement between the concentrations determined in DBS and whole-blood samples. DBS sampling was used in the TDM of 10 chemotherapy agents, including 5-fluorouracil, capecitabine, gemcitabine, cyclophosphamide, ifosfamide, etoposide, irinotecan, docetaxel, paclitaxel, and mitotane (each 1 study). TDM was also explored for the measurement and comparison of 9 targeted therapy agents in 13 studies: everolimus (3 studies), vemurafenib (2 studies), pazopanib (2 studies), gefitinib, abiraterone (2 studies), palbociclib and ribociclib combined (one study), adavosertib, and imatinib. The studies had a median of 14 participants (range: 6–34) and involved the dispensing of 10–50 μL of blood on DBS cards (18 studies) or the Mitra VAMS device (3 studies).

According to most studies (20 of 23), the assay validation process was conducted and reported according to the US FDA guidelines.[14] Ranges of 85%–115% were considered acceptable limits of accuracy and precision. The lowest limit of quantification (LLOQ) was defined as the lowest concentration that could be measured with a precision within 20% and an accuracy between 80% and 120% in all studies. Using Passing–Bablok, Demming regression, or Bland–Altman analysis, DBS and plasma or venous concentrations were found to display strong agreement (correlation coefficient, ranging from 0.872 to 0.999; see Table 1) for all drugs, except mitotane by Friedl et al (2019). In this study, an HPLC-UV assay was developed and validated to measure mitotane concentrations using a Mitra VAMS 20-μL microsampler.[28] The DBS samples were stable at room temperature and 2–8°C for 1 week but unstable at 37°C when a significant amount of analytes were potentially lost through evaporation. Mitotane concentration, as measured by plasma sampling, and DBS by VAMS was not significantly correlated ($r = 0.87$, $P < 0.0001$, where a positive correlation was predefined as $r > 0.872$). The authors concluded that VAMS was neither feasible nor reliable for the measurement of mitotane in TDM.

Utilization of Methods In Actual TDM To Guide Dosing of Anticancer Agents

The aim of most of the included studies (20 of 23) was to develop an analytical method to measure drug concentrations using DBS; however, the concentration ranges detected by DBS were only compared with the accepted target concentration ranges (therapeutic ranges) in studies involving paclitaxel and etoposide.[24,27]

TABLE 1. TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Drug	Approach	Device/Material	Volume	Analysis	Evaluation Study Design/Sample Collection Time Point
Singhal et al 2015[19]	Capecitabine	Venous blood (plasma kept at −20°C) dried for 2 h at RT	Absorbent paper	10 μL of spiked concentration	LC–MS/MS	Extracted blank plasma > ULOQ sample > extracted blank plasma > LLOQ sample
Radovanovic et al 2021[20]	Capecitabine/5-FU	Venous capillary blood dried for 2 h at RT	MITRA (VAMS)	One drop	LC–MS/MS	Aliquots were removed at 4 time points (0, 1, 2, 4 h)
Kumar et al 2015[21]	Gemcitabine	Venous blood, plasma kept at −20°C (without THU), dried for 2 h at RT	DBS cards	50 μL of plasma and venous spiked concentration	LC–MS/MS	Spiked known concentration (HQC level) into whole human blood in the presence and absence of THU Aliquots removed at multiple time points
Harahap et al 2020[22]	Cyclophosphamide	Venous blood containing analytes dried at RT for 3 h	DBS paper	30 μL	UPLC–MS/MS	Blood samples collected at 2 h and 4 h
Torres et al 2015[23]	Ifosfamide	Venous blood and capillary blood dried 6 h then stored at −80°C.	Absorbent paper	40 μL	UPLC-MS/MS	Capillary blood at 12 h and 24 h Median concentration values compared
Kukec et al 2016[24]	Etoposide	Venous blood dried for 1 h at RT.	DBS paper	20 μL	HPLC-FL	Samples collected during 4 chemotherapy cycles on days 1, 2, and 3 of each cycle at 3 h, 6 h, and 24 h
Hahn et al 2018[25]	Irinotecan	Capillary blood and venous blood dried at RT for 3 h.	Absorbent paper	50 μL	HPLC-FL	Blood samples collected at 1 h and 24 h
Raymundo et al 2018[26]	Docetaxel	Spiked venous blood dried at RT within 3–24 h.	Absorbent paper	(25 μL) and 1 drop of capillary blood	LC-MS/MS	Venous and capillary samples collected. Approach not reported.
Andriguetti et al 2018[27]	Paclitaxel	Nonspiked venous blood, dried for 3 h	Absorbent paper	(50 μL) and 1 drop of capillary blood.	LC-MS/MS	Venous and capillary samples collected at 18 h and 30 h
Bettina Friedl et al 2019[28]	Mitotane	Whole-blood samples kept at 2–8°C.	MITRA (VAMS)	Spiked (20 μL) whole blood	HPLC-UV	Paired plasma and venous blood sample collected, approach not reported
Yang Xu et al 2012[29]	Adavosertib	Spiked venous blood and plasma dried overnight	Absorbent paper	40 μL	HPLC-MS/MS	Samples collected on day 1 predose, day 3 predose, and 3 h and 8 h postdose
Nijenhuis et al 2014[30]	Vemurafenib	4 drops of capillary blood dried for 3 h at RT.	Absorbent paper	10 μL of spiked venous blood	HPLC-MS/MS	4 drops of capillary samples at an unknown time
Nijenhuis et al 2016[31]	Vemurafenib	Whole-blood samples dried at RT for 3 h.	Absorbent paper	4 drops of capillary bloods	HPLC-MS/MS	4 drops of capillary samples and venous samples at an unknown time
Verheijen et al 2016[32]	Pazopanib	Capillary samples dried at RT for 3 h	Absorbent paper	15 μL of spiked venous blood	LC–MS/MS	Paired DBS and (venous) plasma samples
de Wit et al 2015[33]	Pazopanib	Whole-blood samples dried for 2 h at RT	Absorbent paper	15 μL of venous blood and capillary blood	HPLC-MS/MS	Day 14 of treatment: venipuncture samples at pre-dose and 1, 2, 3, 4, 6, 8, 10, and 24 h and capillary samples at pre-dose, and 3 and 8 h
Lotte M. Knapen et al 2018[34]	Everolimus	Venous blood dried overnight at RT	Absorbent paper	30 μL of venous blood	UPLC-MS/MS	Venous blood samples collected, collection time not reported

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TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Drug	Approach	Device/Material	Volume	Analysis	Evaluation Study Design/Sample Collection Time Point
Willemsen et al 2018[35]	Everolimus	Capillary blood	Absorbent paper	Two drops	UPLC MS/MS	Whole-blood (plasma, DBS) and finger prick samples on day 7 or after
Verheijen et al 2019[36]	Everolimus	Whole-blood dried at RT	MITRA (VAMS)	20 μL	LC-MS/MS	Whole-blood and VAMS samples - collection time not reported
Kei Irie et al 2018[37]	Gefitinib	Capillary blood and venous blood dried at RT for 2 h.	Absorbent paper	10 μL	LC-MS/MS	Predose capillary and venous samples
Valentina Iacuzzi et al 2019[38]	Imatinib	Venous blood and capillary blood dried for 3 h at RT.	Absorbent paper	20 μL and 2 drops of capillary blood	LC-MS/MS	Pre-dose capillary and venous trough samples
Atul Bhatnagar et al 2019[39]	Abiraterone	Plasma samples, dried for 2 h at RT.	Absorbent paper	15 μL	UPLC-MS/MS	Predose venous blood samples
Dillenburg Weiss et al 2021[40]	Abiraterone	Whole-blood and capillary samples dried for 3 h at RT	Absorbent paper	18 μL	UPLC-MS/MS	Predose capillary, venous and plasma samples
Poetto AS et al 2021[41]	Palbociclib, ribociclib	Venous and finger prick samples	Absorbent paper	20 μL of spiked blood and capillary samples	LC-MS/MS	Predose capillary, venous and plasma samples

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Singhal et al 2015[19]	1 ex vivo sample	10-10,000 ng/mL	Assessed interassay and intraassay precision (within 5%), accuracy (within 6%) and linearity $r^2 = 0.9995$	Not done	Hematocrit 24% and 45%; no impact on accuracy and precision	60	**Advantages**: Long-term stability Low resource (absorbent paper, not a device) **Disadvantages:** Venous blood not capillary blood No comparison of the venous and DBS methods Could be used in TDM
Radovanovic et al 2021[20]	Ex vivo, 10 patients on capecitabine and 20 patients on 5-FU	5-Fu: 4.24–47.9 μg/mL capecitabine: 11–7712 μg/mL	Intraday and interday precision within 8.1% and 13.3%, respectively. Accuracy (within 14%) for all analytes and linearity $r^2 > 0.990$	Venous and capillary samples compared using passing–Bablok analysis and Bland–Altman comparison High correlation between capillary and venous blood samples	N/A	270	**Advantages**: Capillary samples **Disadvantages:** Higher resource (device) Could be used in TDM

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TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Kumar et al 2015[21]	Ex vivo, 6 healthy volunteers	5–5000 ng/mL	Demonstrated interassay and intraassay precision (within 6%), accuracy (within 15%) and linearity r^2 = >0.99	Not done	Hematocrit value of 43% (examined 25%–62%) showed a negligible effect on accuracy and precision	90	**Advantages**: Long-term stability Low resource (absorbent paper, not device) **Disadvantages:** Venous blood not capillary blood No comparison of the venous and DBS methods Potential to be used in TDM
Harahap et al 2020[22]	Blood samples of 17 patients	50–30,000 ng/mL	Assessed interassay and intraassay precision (within 12%) and accuracy (within 20%) and linearity r^2 = >0.99	Not done	Hematocrit and plasma effect not assessed	1	**Advantages:** Short extraction time Low resource (absorbent paper) **Disadvantages:** Venous blood not capillary blood No comparison of the venous and DBS methods Short stability Potential for use in TDM
Torres et al 2015[23]	Capillary blood samples (28) from 14 patients	100–10,000 ng/mL	Intrad and interday assay at 30% hematocrit, accuracy: Within 5%. Precision (% CV): within 11%. Linearity: r^2 = 0.97	Not done	Hematocrit between 30% and 45% (examined 20%–50%) had no impact on accuracy and precision	28	**Advantages:** Capillary blood. Long-term stability Low resource (absorbent paper) **Disadvantages:** No comparison of the venous and DBS methods Requires storage at −80°C before analysis Potential for use in TDM
Kukec et al 2016[24]	216 samples from 6 patients	500–20,000 ng/mL	Intraday and interday precision (% CV): within 10.1% Accuracy: within 3.9% Linearity: r^2 = 0.9753. Plasma concentration = DBS concentration/ 1- hematocrit	Not done	Hematocrit effect assessed at 30%, 40% and 60%: no impact on accuracy and precision (deviation <15%)	28	**Advantages**: Short extraction time Long-term stability Low resource (absorbent paper) **Disadvantages:** Venous blood not capillary blood No comparison of the venous and DBS methods Could be used in TDM

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TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Hahn et al 2018[25]	19 patients	10 to 3000 ng/mL	Accuracy: within ≤5.74% Intraassay and interassay precision (%CV): within ≤4.72%	Correlation between DBS and plasma samples at 1h postinfusion: r = 0.949	Hematocrit effect assessed at 25%, 35%, and 50%: no impact (deviation <7.5%)	14	**Advantages**: Capillary (DBS) and venous the methods compared Long-term stability Low resource (absorbent paper) **Disadvantages:** Nil identified Potential for use in TDM
Raymundo et al 2018[26]	31 patients	50 to 3000 ng/mL	Precision (% CV): <9.8%. Accuracy: within 3%	Venous and DBS methods compared using passing–Bablok regression analysis. r = 93% (high correlation between DBS-derived estimated plasma concentrations and plasma samples, $P < 0.01$)	Hematocrit effect assessed at 30%, 45% and 60%: No impact (deviation <12.1% for 60% and <10.1% for 30%)	18	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** Nil identified Potential for use in TDM
Andriguetti et al 2018[27]	34 patients	2.5–400 ng/mL	Intraassay and interassay precision (% CV): within 6.89% and 8.74% Accuracy within 9.92%	Venous and DBS methods compared using Passing–Bablok analysis and Bland–Altman comparison. r = 0.986 (high correlation between DBS and venous blood)	Spotted volume influence: Accuracy within 12.7% Hematocrit effect between 25% and 46% assessed: Accuracy within 14.8%	21	**Advantages**: Long-term stability Low resource (absorbent paper) Assessed capillary bloods on DBS **Disadvantages:** Nil identified Potential for use in TDM
Bettina Friedl et al 2019[28]	51 samples from 6 patients	1–50 mg/mL	A nonlinear model may be necessary to relate Mitra and plasma concentrations Poor correlation between mitotane concentration in DBS and venous plasma ($P <$ 0.0001)	Venous and VAMS methods compared using Passing–Bablok analysis and Bland–Altman comparison Poor agreement defined: r < 0.90. r = 0.87 (concordance correlation coefficient: 0.60)	Hematocrit effect of adjusted levels 30%–55% assessed: accuracy within 13%	0 (at 2–8°C: 7)	**Disadvantages:** Venous blood not capillary blood Higher resource (device) Unstable at RT Should not be used in TDM
Yang Xu et al 2012[29]	12 patients	2 to 1000 ng/mL	Intraday and interday precision (% CV): within 7.2% Accuracy within 14%	Mean DBS to plasma ratio of 1.29, indicating good agreement	Spot size and punch location effect on accuracy: within 5.8% Hematocrit effect between 16% and 85% assessed: accuracy within 15%	420	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** Venous blood not capillary blood Not a commonly used anticancer therapy Could be used in TDM

TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Nijenhuis et al 2014[30]	8 patients	1000 to 100,000 ng/mL	Assessed intraassay and interassay accuracy: within 13.6% and precision (%CV) within 6.5%, linearity: $r^2 = 0.997$	Not done	Blood spreadability impact: bias within 9.4% and precision within 4.6% DBS volume impact: finger prick volume within 15% Hematocrit 24% and 45% - impact on accuracy (<11.4%) and precision (<4.1%)	163	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** No comparison of the venous and DBS methods Could be used in TDM
Nijenhuis et al 2016[31]	43 capillary samples and plasma samples from 8 patients	1000 to 100,000 ng/mL	Not done	Bland–Altman and weighted Deming regression analysis Highly correlated (r = 0.964) but consistently lower than the corresponding plasma concentration with a slope of 0.64 (95%CI, 0.60 to 0.68), (vemurafenib in plasma = vemurafenib in DBS/0.64)	Hematocrit effect between 27% and 49% assessed: accuracy within 11.4%	827	**Advantages**: Long-term stability Low resource (absorbent paper) Could be used in TDM
Verheijen et al 2016[32]	329 samples from 30 patients	1000–50,000 ng/mL	Interrun and intrarun precision (CV) ≤8.6%	Venous (plasma) and DBS methods compared using weighted Deming fit and Bland–Altman comparison. $r^2 = 0.872$ (good correlation between DBS and plasma concentrations) (slope: 0.709, intercept: −0.182)	Blood spot homogeneity: bias within 3.5% Effect of blood spot volume: accuracy within 9.5%. Effect of blood hematocrit (35%–50%): accuracy within 14.2%	398	**Advantages**: Assessed capillary DBS method and compared with plasma method Long-term stability Low resource (absorbent paper) Could be used in TDM
de Wit et al 2015[33]	12 patients	100–50,000 ng/mL	Within-run and between-run precision: within 14.7% Accuracy within 5.5% Mean ratio of calculated to measured concentrations 0.94 (95% CI, 0.65–1.23) 92.6% (88/95) of the data points within clinical acceptance limits	Bland–Altman and Passing–Bablok analysis Constant bias between plasma and DBS (intercept estimate, 4.68; 95% CI, 6.48 to 2.47), (slope estimate, 0.63; 95% CI, 0.57 to 0.68)	Blood hematocrit effect (20%–65%): Bias within 12.6%	75	**Advantages**: Compared capillary DBS method and plasma method Long-term stability Low resource (absorbent paper) Could be used in TDM

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TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Lotte M. Knapen et al 2018[34]	6 healthy volunteers	3–75 ng/mL	Intraassay and interassay precision (%CV): Within 10.7% Accuracy within 4.4% (for hematocrit values of ≥25%)	Not done	Blood hematocrit effect (20%–50%): precision within 14.8% but hematocrit <25% not accurate-bias >15%. Spot volume effect: precision within 3.5%	17	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** No comparison of the venous and DBS methods Venous blood not capillary blood Potential for use in TDM
Willemsen et al 2018[35]	20 patients	3.7–33.3 ng/mL	Mean ratio of everolimus in WB to DBS concentrations 0.90 (95% LoA 0.71–1.08). r = 0.97 and r^2 = 0.95	Bland–Altman analysis and Passing–Bablok analysis No constant bias (intercept 0.02; 95% CI 0.93–1.35) and a small proportional bias (slope 0.89; 95% CI 0.76–0.99)	Blood hematocrit effect (25%–45%): assumed no impact for >25%	Not done	**Advantages**: Compared the venous and DBS (capillary) methods Low resource (absorbent paper) **Disadvantages:** Unreported stability period Potential for use in TDM
Verheijen et al 2019[36]	10 patients	2.50–100 ng/mL	Intrarun precision (%CV): within 14.6% Intrarun accuracy: within 11.1% r > 0.99	Compared concentrations obtained by VAMS and DBS Advantage of VAMS over DBS not shown	Hematocrit range (30%–50%) effect: considerable biases from 20% to 31%	362	**Advantages:** Nil identified **Disadvantages:** Venous blood not capillary blood Higher resource (device) Significant impact of low hematocrit Should not be used in TDM
Kei Irie et al 2018[37]	10 patients	37.5 to 2400 ng/mL	Intraday and interday precision and accuracy of all samples were within 15% Linearity: r^2 = 0.99	Venous and DBS methods compared using Bland–Altman analysis and Passing–Bablok analysis. r^2 = 0.99	Hematocrit range (31%–43%) Impact not assessed	150	**Advantages**: Compared the venous and DBS methods Patients self-performed sampling. Low resource (absorbent paper) **Disadvantages:** Not assessed hematocrit impact Potential for use in TDM
Valentina Iacuzzi et al 2019[38]	26 patients	50–7500 ng/mL	Intraday and interday precision (%CV): Within 5.6% Intraday and interday accuracy within 11.1%. Linearity: r^2 > 0.996	Plasma, venous DBS, and finger prick DBS methods compared using Bland–Altman analysis and Passing–Bablok analysis. r^2 = 0.9967	Hematocrit (29%–59%) effect: accuracy and precision within 4.8%. Blood spot volume effect: accuracy and precision within 10.1%	480	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** Nil identified Potential for use in TDM

TABLE 1. (*Continued*) TDM of Anticancer Agents in Patients With Solid Tumors Using Microsampling (DBS or VAMS)

Author, Year, Citation	Samples	Working Concentration Range	Accuracy and Precision	DBS Concentrations Compared with Venous Concentrations	Influential Factors (Hematocrit, Serum/Plasma Etc.)	RM Stability (d)	Comments
Atul Bhatnagar et al 2019[39]	22 patients	0.132–196.0 ng/mL	Intraday and interday accuracy and precision (% CV): within 11.3%, linearity: r = 0.99	Venous DBS and plasma methods compared using Bland–Altman analysis, Passing–Bablok analysis, Pearson correlation coefficient and *t* test (nonparametric) $r^2 = 0.9921$	Effect of hematocrit or spot volume not assessed	30	**Advantages**: Long-term stability Low resource (absorbent paper) **Disadvantages:** Venous blood not capillary blood Hematocrit effect not assessed Potential for use in TDM
Dillenburg Weiss et al 2021[40]	10 patients	1−400 ng/mL	Between-run and within-run precision (%CV): Within 9.72% Accuracy: within 7% Linearity $r^2 = 1.0$	Plasma and finger prick DBS methods compared using Bland–Altman analysis and Passing–Bablok analysis Concentrations overestimated using the DBS approach (15%)	Hematocrit (28%–44%) effect: not significant Spot volume effect: precision within 12.1%	7	**Advantages**: Assessed capillary DBS method and compared with plasma Low resource (absorbent paper) **Disadvantages:** Short-term stability Could be used in TDM
Poetto AS et al 2021[41]	38 samples from 18 patients	1–250 ng/mL for palbociclib, 40–10,000 ng/mL for ribociclib	Intraday and interday precision (CV (%)) within 11.4% and accuracy within10%. Linearity: $r^2 = 0.9979$	Passing–Bablok regression analysis, Bland–Altman plots, and Lin concordance correlation coefficient $r^2 = 0.958$	Hematocrit (25%–49%) effect: precision within 14.8% Spot size sample homogeneity precision within 15%	75	**Advantages:** Assessed capillary DBS method and compared with plasma Low resource (absorbent paper) Could be used in TDM

DBS, dried blood spot; RT, room temperature; VAMS, volumetric absorptive microsampling; 5-FU, 5-fluorouracil; ULOQ, upper limit of quantification; LLOQ, lower limit of quantification; THU, tetra-hydro-uridine; HQC, higher quality control; UPLC–MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry; LoA, limits of agreement; WB, whole blood.

Andriguetti et al[27] developed and validated a liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) assay using DBS sampling of paclitaxel. The plasma concentration above a threshold of 0.05 μmol/L (Tc > 0.05 μmol/L) was considered to be the therapeutic range that would represent the relation between exposure to paclitaxel and clinical response.[42,43] The developed LC-MS/MS assay was validated for the concentration range of 2.5–400 ng/mL, which would cover the known therapeutic range. The precision (CV %) and accuracy at various concentrations were within acceptable ranges (Table 1). The authors concluded that paclitaxel could be accurately measured using DBS, which could thus be used for the TDM of paclitaxel.

Kukec et al (2016) developed and validated a high-performance liquid chromatography-fluorescence assay through DBS sampling to establish TDM for etoposide.[24] The therapeutic range was considered to be 2000–6000 ng/mL and 8000–14,000 ng/mL for the trough and peak serum concentrations, respectively.[44] The developed method covered a concentration range of 500–20,000 ng/mL with a linear relationship ($r^2 = 0.9753$). The accuracy of ≥96.1% and precision (%CV) of ≤10.1% were within the accepted criteria (accuracy: 85%–115%, precision: ≤15%). Notably, etoposide levels significantly correlated between the plasma and DBS samples ($r^2 = 0.97$; $P < 0.05$). The developed method was reported to be a patient friendly and reliable alternative to conventional plasma methods for the TDM of etoposide.

The relationship between concentration range and toxicity or efficacy was not investigated in any of the studies included in this review.

Physicochemical Factors Impacting Concentration Results

Most studies (19 of 23) evaluated the hematocrit effect, except for the studies with 5-fluorouracil,[20] cyclophosphamide,[22] gefitinib,[37] and abiraterone.[39] Of the 19 studies, most (17 of 19) found no significant impact (bias >15%) on the

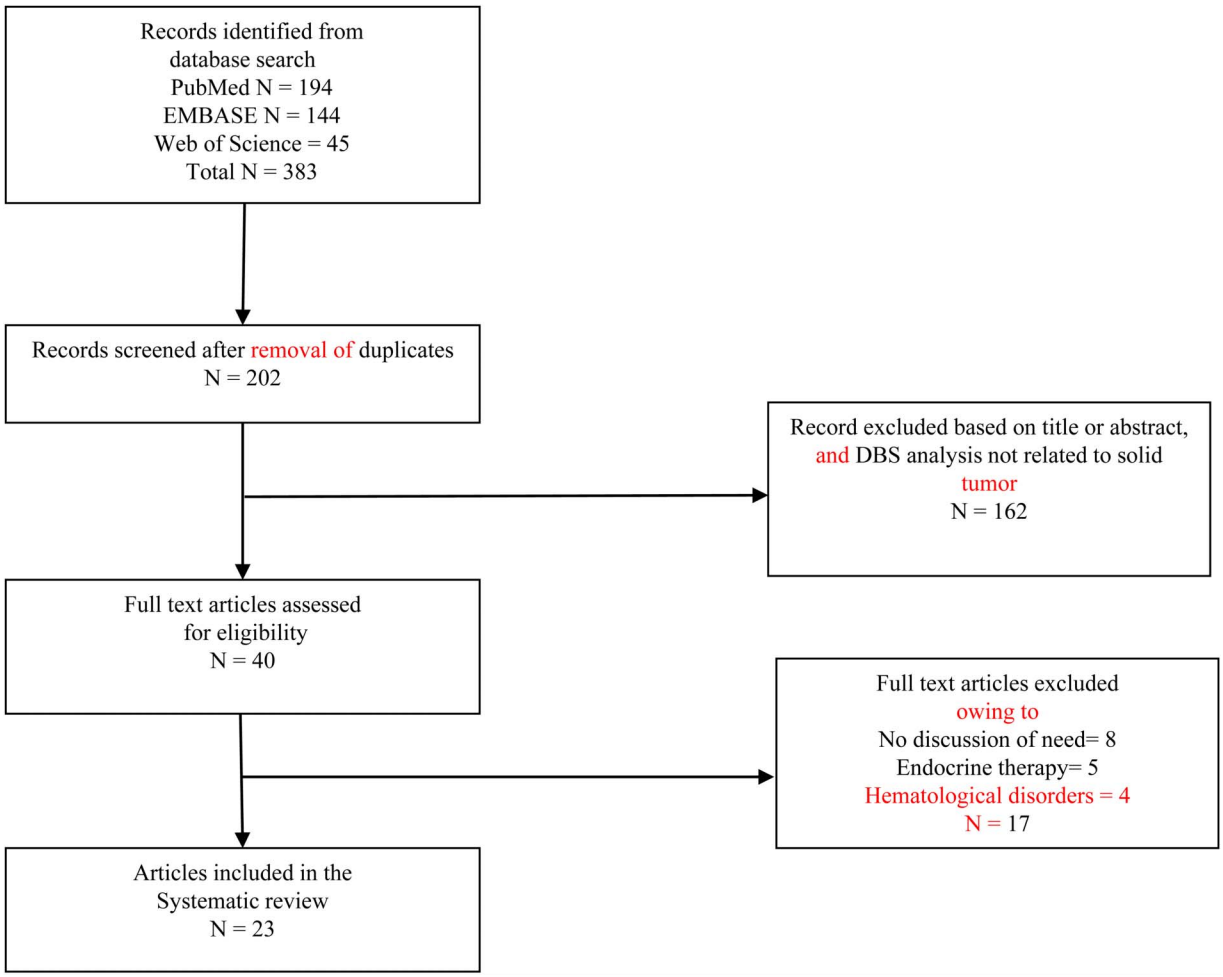

FIGURE 1 . PRISMA flow diagram 1.

determination of drug concentration in the normal hematocrit ranges (eg, 29.0%−59.0%). Some studies (eg, etoposide and pazopanib)[24,32,45] used the following equation: plasma concentration = DBS concentration/(1 − hematocrit) to adjust for hematocrit impact and reported an acceptable bias of <15%.

A lower range of hematocrit (<25% and <31%) was reported to cause unacceptable bias in 2 studies on everolimus. Knapen et al[34] developed and validated an UPLC-MS/MS assay to measure the concentrations of everolimus in DBS samples. The effect of blood hematocrit (20%–50%) on the measured concentration was assessed with an unacceptable precision of >15% for hematocrit values of <25%. Similarly, Verheijen et al[36] developed and validated an LC-MS/MS method to quantify everolimus concentrations through VAMS sampling. Considerable bias (>15%) was observed for hematocrit ranges from 20% to 31% (hematocrit range assessed: 20%–50%).

Approximately half of the studies (11 of 23) examined other factors affecting the concentration results, including spot homogeneity or the spot volume effect[19,21,25,27,29,30,32,34,38,40] (Table 1). For example, Verheijen et al[32] developed and validated a DBS assay using LC–MS/MS to measure the concentration of pazopanib by DBS sampling for TDM. Interindividual variability was observed in the results that could not be explained by the hematocrit effect because the samples were collected from the same patients at the same time. Other factors, such as spot homogeneity and spot volume, could have contributed to this variability. In their study, by avoiding the use of very large, very small, or irregular spots, the researchers demonstrated an acceptable bias of within 3.5% for blood spot homogeneity and an acceptable accuracy of within 9.5% for the effect of blood spot volume.

Application to Patient Samples

Capillary blood sampling and venous DBS sampling were both used in 14 of the 23 studies[20,23,25–27,30–33,35,37,38,40,46] (Table 1). In 13 of the 14 studies, DBS sampling for the participants was performed by a research team (physicians, nurses, etc); the DBS was performed by participants in one study.[37] Kei Irie et al[37] used LC-MS/MS to develop and validate a method to quantify gefitinib in DBS sampling. Self-performed capillary samples from 10 patients with NSCLC receiving gefitinib (daily or every other day) were collected for analysis. Capillary samples were obtained by puncturing the fingertips of the participants with a lancet,

immediately before gefitinib administration (trough concentration). Venous samples were also collected from the participants within 10 minutes of DBS sampling. Good agreement was observed between the gefitinib concentrations measured using the DBS method and the plasma concentrations (r^2 = 0.99). The feasibility of finger-prick testing by patients was not adequately investigated in the included studies.

Sample Preparation Time

Most studies reported an analytical run time (14 of 23)[19–21,25–29,33,34,37–39,41] ranging from 2.3 to 8.5 minutes, and a sample preparation time (20 of 23),[19–30,33,34,36–41] ranging from 80 to 160 minutes. However, the actual hands-on time was not revealed in any of the included studies. For example, Raymundo et al[26] developed and validated an LC–MS/MS method to measure docetaxel in DBS samples. The reported total analytical run time was 7 minutes, the DBS sample dry time was 3 hours, and DBS sample preparation took 75 minutes.

DISCUSSION

The key findings of our systematic review are as follows: all but one included study showed that DBS and VAMS sampling methods were feasible and had good correlation with plasma sampling methods, as per the FDA guidelines.

To our knowledge, another systematic review summarized the use of DBS sampling to measure the concentration of chemotherapy and targeted anticancer therapy for TDM in routine clinical practice.[46] Lacuzzi et al reviewed studies that used DBS to measure anticancer drug concentrations from November 2008 to May 2020 and investigated the physicochemical factors of the drugs and their impact on blood distribution, the influence of hematocrit on DBS concentrations, and the reported approach to normalize DBS concentrations to those measured in plasma. The authors found that DBS sampling could replace standard venous blood or plasma sampling without compromising outcomes when appropriate conversion methods were used. The key differences between our review and that of Lucuzzi et al include the inclusion of anticancer agents used in hematological malignancies (eg, radotinib) and hormonal anticancer therapies (eg, estrogen receptor modulators, tamoxifen) and a methodological focus on conversion and normalization approaches to correlate plasma and DBS concentrations.

The primary objective of most of the included studies (20 of 23) was to develop and validate DBS assays to implement TDM for anticancer therapy. TDM for anticancer therapy has been logistically difficult to implement in clinical practice using venipuncture, as presented in the introduction. By contrast, DBS can be performed by patients; the samples do not have to be processed on site and are dried, stable, and easily transported. This review has highlighted other advantages of DBS for TDM for use in clinical practice, including (i) the very small volumes of blood required for DBS (range, 10–50 μL) compared with plasma sampling (range, 1–5 mL); (ii) stability of samples ranging from 9 days (at −20 to 45°C for docetaxel)[26] to 16 months (at room temperature for imatinib), adequately covering a typical 2–4 weeks stability time needed for its use in guiding dose adjustment in routine clinical care.[38] Ideally, for TDM, the sampling and analytic method covers the drug therapeutic range if known to propose meaningful dose adjustments to avoid underdosing and excess toxicity. Evidence of known or accepted therapeutic ranges only exists for several agents, such as paclitaxel, etoposide, mitotane, imatinib, and pazopanib.[3–5] In our review, 2 studies (eg, those of paclitaxel and etoposide) covered the known accepted therapeutic range; however, other studies either did not cover the known therapeutic range or the therapeutic range was unknown.

A challenge in DBS is the hematocrit effect, which is the predominant source of interindividual variability. Increased hematocrit reflects an increased blood viscosity, which can cause less homogenous spread of the blood sample on the absorbent paper used for DBS[47]; this may affect the measurement of drug concentration by variation in the location of the punch within the heterogeneous spot and subsequent extraction method. International guidelines recommend the evaluation of samples from a central or peripheral punch at low and high concentrations of a given drug at low, medium, and high hematocrit levels. These conditions must then be analyzed in quintuplicate.[48,49] Studies that evaluated the hematocrit effect in this review (19 of 23) adhered to the FDA guidelines, and most of those studies (17 of 19) found that normal hematocrit ranges (eg, 29.0%–59.0%) had no significant impact on the accuracy and precision of the developed method. Low hematocrit (<29%) interfered with the accuracy and precision of the developed methods (imatinib). The validation of a low hematocrit range (<29%) is very important because many patients in oncology may have a hematocrit level of less than 29% (anemia) due to cancer and/or systemic anticancer therapy.

VAMS has been introduced as an alternative DBS sampling technique to overcome the challenges of the hematocrit effect and punch area bias.[50] Previous studies suggested that VAMS could reduce or, for selected analytes, eliminate the influence of hematocrit.[51] In a study on the DBS of everolimus, Verheijen et al[36] revealed that the VAMS sampling method was strongly influenced by hematocrit in a concentration-dependent manner, and VAMS was not superior to the DBS methods. The VAMS method as a solution to the hematocrit impact remains to be determined in future studies of other drugs used in oncology. The articles identified in this review only used DBS and VAMS; other available techniques were not used. Therefore, only these 2 techniques were included in the review.

There were several issues with the studies included in this review. The first issue is the research setting because all studies performed microsampling in the research environment but not in the at-home environment where microsampling occurs for TDM in routine clinical practice. The DBS cards or Mitra devices were prepared by the research nurse or study staff rather than the participants and hence do not reflect at-home sampling where patients perform these tasks themselves. Previous studies on DBS use for antiretroviral and immunosuppressive drugs have shown that 87.5%–98% of the samples obtained by patients were suitable for

analysis,[52,53] suggesting that preparation of the DBS cards by patients is feasible. The included studies did not consistently assess the impact of lower hematocrit levels (<30%) on the determination of drug concentrations. Other issues of the included studies were publication bias because all but one included study had positive results, and selection bias, as only certain chemotherapy and targeted therapy agents were investigated in the published studies.

Overall, the limitations of this systematic review include the heterogeneity in the methods across studies, different DBS methods, such as Mitra and absorbent paper, the small number of studies for each drug included in the review (mostly single study), the small number of patients or samples in the included studies, and the variable methods used to adjust for the hematocrit effect. These limitations, particularly the paucity of studies on individual drugs, make it difficult to draw firm conclusions on the applicability of broad methods to individual drugs.

CONCLUSION

The reviewed articles mainly support the use of developed microsampling methods for the measurement of various chemotherapy and targeted therapy agents using the preselected equivalent concentration range. Given the feasibility and advantages of DBS over venipuncture, further studies are warranted to evaluate the clinical utility of microsampling by patients themselves using quality of life measures and comparing clinical outcomes. Clinical research data showing the comparative benefits of DBS would ultimately improve the uptake of TDM, dosing of anticancer therapy, and patient care.

REFERENCES

1. Kim HY, Martin JH, McLachlan AJ, et al. Precision dosing of targeted anticancer drugs—challenges in the real world. *Transl Cancer Res.* 2017; 6:1500–1511.
2. Gurney H. Dose calculation of anticancer drugs: a review of the current practice and introduction of an alternative. *J Clin Oncol.* 1996;14:2590–2611.
3. Menz BD, Stocker SL, Verougstraete N, et al. Barriers and opportunities for the clinical implementation of therapeutic drug monitoring in oncology. *Br J Clin Pharmacol.* 2021;87:227–236.
4. Mizoguchi K, Nakamura Y, Sano K, et al. Pharmacokinetic parameters of gefitinib predict efficacy and toxicity in patients with advanced non-small cell lung cancer harboring EGFR mutations. *Cancer Chemother Pharmacol.* 2016;78:377–382.
5. Teng JFT, Mabasa VH, Ensom MHH. The role of therapeutic drug monitoring of imatinib in patients with chronic myeloid leukemia and metastatic or unresectable gastrointestinal stromal tumors. *Ther Drug Monit.* 2012;34:85–97.
6. Moeung S, Chevreau C, Broutin S, et al. Therapeutic drug monitoring of carboplatin in high-dose protocol (TI-CE) for advanced germ cell tumors: pharmacokinetic results of a phase II multicenter study. *Clin Cancer Res.* 2017;23:7171–7179.
7. Pignon T, Lacarelle B, Duffaud F, et al. Dosage adjustment of high-dose methotrexate using Bayesian estimation: a comparative study of two different concentrations at the end of 8-h infusions. *Ther Drug Monit.* 1995;17:471–478.
8. Bartelink IH, Bredius RG, Ververs TT, et al. Once-daily intravenous busulfan with therapeutic drug monitoring compared to conventional oral busulfan improves survival and engraftment in children undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2008; 14:88–98.
9. Mauclere-Denost S, Leboulleux S, Borget I, et al. High-dose mitotane strategy in adrenocortical carcinoma: prospective analysis of plasma mitotane measurement during the first 3 months of follow-up. *Eur J Endocrinol.* 2012;166:261–268.
10. Westerdijk K, Desar IM, Steeghs N, et al. Imatinib, sunitinib and pazopanib: from flat-fixed dosing towards a pharmacokinetically guided personalized dose. *Br J Clin Pharmacol.* 2020;86:258–273.
11. Paci A, Veal G, Bardin C, et al. Review of therapeutic drug monitoring of anticancer drugs part 1-cytotoxics. *Eur J Cancer*. 2014;50:2010–2019.
12. Lei BUW, Prow TW. A review of microsampling techniques and their social impact. *Biomed Microdevices*. 2019;21:81.
13. Wilhelm AJ, den Burger JCG, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clin Pharmacokinet.* 2014;53:961–973.
14. Guidance for industry, bioanalytical method validation. Food and drug administration, US department of health and human services web site. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry. Published 2018. Accessed May 8, 2022.
15. Lange B, Roberts T, Cohn J, et al. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples - a systematic review and meta-analysis. *BMC Infect Dis.* 2017;17:693.
16. Smit PW, Sollis KA, Fiscus S, et al. Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. *PLoS One*. 2014;9:e86461.
17. Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. *Int J Neonatal Screen.* 2020;6:26.
18. Moher D, Liberati A, Tetzlaff J, et al., GroupPRISMA Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151:264–269.
19. Singhal P, Shah P, Shah J, et al. Determination of capecitabine-an anticancer drug in dried blood spot by LC-ESI-MS/MS. *Int J Pharm Pharm Sci.* 2015;7:238–245.
20. Radovanovic M, Schneider JJ, Shafiei M, et al. Measurement of 5-fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. *J Chromatogr B*. 2022;1188:123075.
21. Kumar DP, Palavan C, Jvln SR. Development and validation of a dried blood spot LC-MS/MS assay to quantify gemcitabine in human whole blood: a comparison with and without cytidine deaminase inhibitor. *Int J Pharm Pharm Sci.* 2015;7:75–81.
22. Harahap Y, Gustiyanti N, Sunanto AE, et al. Method development on analysis cyclophosphamide and 4-hydroxycyclophophamide in dried blood spot and its application in breast cancer patients. *Int J Appl Pharm.* 2020;12:119–125.
23. Torres LM, Rivera-Espinosa L, Chávez-Pacheco JL, et al. A new method to quantify ifosfamide blood levels using dried blood spots and UPLC-MS/MS in paediatric patients with embryonic solid tumours. *PLoS One*. 2015;10:e0143421.
24. Režonja Kukec R, Grabnar I, Mrhar A, et al. A simple dried blood spot method for clinical pharmacological analyses of etoposide in cancer patients using liquid chromatography and fluorescence detection. *Clinica Chim Acta*. 2016;452:99–105.
25. Hahn RZ, Arnhold PC, Andriguetti NB, et al. Determination of irinotecan and its metabolite SN-38 in dried blood spots using high-performance liquid-chromatography with fluorescence detection. *J Pharm Biomed Anal.* 2018;150:51–58.
26. Raymundo S, Muller VV, Andriguetti NB, et al. Determination of docetaxel in dried blood spots by LC-MS/MS: method development, validation and clinical application. *J Pharm Biomed Anal.* 2018;157:84–91.
27. Andriguetti NB, Hahn RZ, Lizot LF, et al. Analytical and clinical validation of a dried blood spot assay for the determination of paclitaxel using high-performance liquid chromatography-tandem mass spectrometry. *Clin Biochem.* 2018;54:123–130.
28. Friedl B, Kurlbaum M, Kroiss M, et al. A method for the minimally invasive drug monitoring of mitotane by means of volumetric absorptive microsampling for a home-based therapeutic drug monitoring. *Anal Bioanal Chem.* 2019;411:3951–3962.
29. Xu Y, Fang W, Zeng W, et al. Evaluation of dried blood spot (DBS) technology versus plasma analysis for the determination of MK-1775 by

HILIC-MS/MS in support of clinical studies. *Anal Bioanal Chem.* 2012; 404:3037–3048.
30. Nijenhuis CM, Rosing H, Schellens JH, et al. Quantifying vemurafenib in dried blood spots using high-performance LC-MS/MS. *Bioanalysis*. 2014;6:3215–3224.
31. Nijenhuis CM, Huitema AD, Marchetti S, et al. The use of dried blood spots for pharmacokinetic monitoring of vemurafenib treatment in melanoma patients. *J Clin Pharmacol.* 2016;56:1307–1312.
32. Verheijen RB, Bins S, Thijssen B, et al. Development and clinical validation of an LC-MS/MS method for the quantification of pazopanib in DBS. *Bioanalysis*. 2016;8:123–134.
33. de Wit D, den Hartigh J, Gelderblom H, et al. Dried blood spot analysis for therapeutic drug monitoring of pazopanib. *J Clin Pharmacol.* 2015; 55:1344–1350.
34. Knapen LM, Beer Yd, Brüggemann RJM, et al. Development and validation of an analytical method using UPLC-MS/MS to quantify everolimus in dried blood spots in the oncology setting. *J Pharm Biomed Anal.* 2018;149:106–113.
35. Willemsen AECAB, Knapen LM, de Beer YM, et al. Clinical validation study of dried blood spot for determining everolimus concentration in patients with cancer. *Eur J Clin Pharmacol.* 2018;74:465–471.
36. Verheijen RB, Thijssen B, Atrafi F, et al. Validation and clinical application of an LC-MS/MS method for the quantification of everolimus using volumetric absorptive microsampling. *J Chromatogr B*. 2019; 1104:234–239.
37. Irie K, Shobu S, Hiratsuji S, et al. Development and validation of a method for gefitinib quantification in dried blood spots using liquid chromatography-tandem mass spectrometry: application to finger-prick clinical blood samples of patients with non-small cell lung cancer. *J Chromatogr B*. 2018;1087-1088:1–5.
38. Iacuzzi V, Posocco B, Zanchetta M, et al. Development and validation of LC-MS/MS method for imatinib and norimatinib monitoring by finger-prick DBS in gastrointestinal stromal tumor patients. *PLoS One*. 2019; 14:e0225225.
39. Bhatnagar A, McKay MJ, Thaysen-Andersen M, et al. Bioanalytical evaluation of dried plasma spots for monitoring of abiraterone and Δ(4)-abiraterone from cancer patients. *J Chromatogr B*. 2019;1126-1127:121741.
40. Dillenburg Weiss TL, Gössling G, Venzon Antunes M, et al. Evaluation of dried blood spots as an alternative matrix for therapeutic drug monitoring of abiraterone and delta(4)-abiraterone in prostate cancer patients. *J Pharm Biomed Anal.* 2021;195:113861.
41. Poetto AS, Posocco B, Gagno S, et al. A new dried blood spot LC-MS/MS method for therapeutic drug monitoring of palbociclib, ribociclib, and letrozole in patients with cancer. *J Chromatogr B*. 2021;1185: 122985.
42. Krens SD, McLeod HL, Hertz DL. Pharmacogenetics, enzyme probes and therapeutic drug monitoring as potential tools for individualizing taxane therapy. *Pharmacogenomics*. 2013;14:555–574.
43. Joerger M, Kraff S, Huitema ADR, et al. Evaluation of a pharmacology-driven dosing algorithm of 3-weekly paclitaxel using therapeutic drug monitoring: a pharmacokinetic-pharmacodynamic simulation study. *Clin Pharmacokinet.* 2012;51:607–617.
44. Moffat AC, Osselton MD, Widdop B, et al. *Clarke's analysis of drugs and poisons*. Vol 3. London: Pharmaceutical press; 2011. https://www.worldcat.org/title/clarkes-analysis-of-drugs-and-poisons-in-pharmaceuticals-body-fluids-and-postmortem-material/oclc/733491270. Accessed May 8, 2022. Published.
45. Li W, Tse FLS. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. *Biomed Chromatogr.* 2010;24:49–65.
46. Iacuzzi V, Posocco B, Zanchetta M, et al. Dried blood spot technique applied in therapeutic drug monitoring of anticancer drugs: a review on conversion methods to correlate plasma and dried blood spot concentrations. *Pharm Res.* 2021;38:759–778.
47. Denniff P, Spooner N. The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs. *Bioanalysis*. 2010;2:1385–1395.
48. Capiau S, Veenhof H, Koster RA, et al. Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. *Ther Drug Monit.* 2019;41:409–430.
49. Cobb Z, de Vries R, Spooner N, et al. In-depth study of homogeneity in DBS using two different techniques: results from the EBF DBS microsampling consortium. *Bioanalysis*. 2013;5:2161–2169.
50. Denniff P, Spooner N. Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis. *Anal Chem.* 2014;86:8489–8495.
51. De Kesel PMM, Lambert WE, Stove CP. Does volumetric absorptive microsampling eliminate the hematocrit bias for caffeine and paraxanthine in dried blood samples? A comparative study. *Analytica Chim Acta*. 2015;881:65–73.
52. Cheung CY, van der Heijden J, Hoogtanders K, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. *Transpl Int.* 2008;21:140–145.
53. Kromdijk W, Mulder JW, Smit PM, et al. Therapeutic drug monitoring of antiretroviral drugs at home using dried blood spots: a proof-of-concept study. *Antivir Ther.* 2012;18:821–825.

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ORIGINAL ARTICLE

Influence of age on pharmacokinetics of capecitabine and its metabolites in older adults with cancer: a pilot study

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Abstract

Background Capecitabine is an oral chemotherapy prodrug of 5-fluorouracil (5-FU) with unpredictable toxicity, especially in older adults. The aim of this study was to evaluate the pharmacokinetics (PK) of capecitabine and its metabolites in younger adults (< 70 years) and older adults (≥ 70 years) receiving capecitabine for solid cancer.

Methods Eligible participants receiving capecitabine had 2 venous samples collected on day 14 of cycle 1 and cycle 2 of their treatment. Capecitabine and metabolite concentrations were determined using liquid chromatography with tandem mass spectrometry. A Bayesian estimation approach was used to generate individual estimates of PK parameters for 5-FU. A linear mixed-effect analysis of variance (ANOVA) model was used to compare dose-normalised log-transformed PK parameters between age groups. Correlations were determined by linear regression and logistic regression analyses.

Results Of the total 26 participants, 58% were male with a median age of 67 years (range, 37–85) with 54% aged < 70 years and 46% aged ≥ 70 years. Participants aged ≥ 70 years, compared to those aged < 70 years, had a greater 5-FU exposure based on area under the concentration–time curve (AUC) of 17% (90% CI 103–134%; 0.893 vs. 0.762 mg h/L) and 14% increase in maximal concentration, C_{max} (90% CI 82.1–159%; 0.343 vs. 0.300 mg/L). The 5-FU C_{max} was positively associated with time up and go (TUG) (Pearson's correlation 0.77, $p = 0.01$), but not other geriatric assessment domains or severe toxicity.

Conclusion 5-FU exposure was significantly increased in older adults compared to younger adults receiving equivalent doses of capecitabine, and is a possible cause for increased toxicity in older adults.

Keywords Pharmacokinetics · Capecitabine · Older adults · Cancer · Toxicity · Geriatric tools

Background

Cancer is predominantly a disease of older adults with an increasing incidence with increasing age [1]. Worldwide, the absolute number of older adults with cancer is expected to increase due to the ageing of the population [2]. The definition of an older adult varies with many studies using age limit of ≥ 70 years but others using different age limits (e.g., ≥ 65 years and ≥ 75 years) [3].

Older adults with cancer are commonly treated with capecitabine, a convenient oral fluoropyrimidine chemotherapy agent [4]. Capecitabine, a prodrug of 5-fluorouracil (5-FU), has common toxicities including fatigue, hand foot syndrome, and diarrhoea [4]. Compared with younger adults, older adults on capecitabine require more dose modifications (delays, reductions, and omissions), and with, for example, dose reductions required in 51% in those aged ≥ 70 years versus 39% in those aged < 70 years) [5]. Given this, prescribing capecitabine can be challenging in the older, frailer population.

Changes in 5-FU pharmacokinetics (PK) due to physiological changes with ageing may be responsible for the excess toxicity of capecitabine in older adults. Such

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changes can alter decrease gastric acid secretion, reduce gastric emptying, and slow colonic transit times to alter the absorption of orally administered agents. A decline in renal function and changes in fat distribution with ageing can also affect drug disposition [6]. Capecitabine is dosed by body surface area (BSA) dosing, but it is unclear if this is the optimal dosing method in older adults. An alternative is PK-guided dosing where measured 5-FU PK parameters are used to refine the dosing of capecitabine in individual patients.

There are limited data on the PK of capecitabine and its metabolites (5-FU, 5-DFCR, 5-DFUR) in older adults with cancer with conflicting results among the few published studies. Two studies (Abdi et al. $n=60$, Louie et al. $n=24$) [7, 8] investigated the PK of capecitabine in the treatment of a small group of older and younger patients with colorectal cancer. Both studies demonstrated significant differences in capecitabine clearance (CL/F) and volume of distribution (Vd/F) and rate of absorption (=ka) among older adults (aged > 70 years). Abdi et al. [8] also showed a positive correlation between capecitabine PK parameters and its common toxicity, and hand and foot syndrome (HFS) ($p=0.01$). Another study by Cassidy et al. [9] showed no impact of age, sex, BSA, or creatinine clearance on PK parameters of capecitabine and its metabolites in adult patients ($n=25$) with solid tumours. The US FDA does not recommend specific dose adjustments of capecitabine for age [10].

The aim of this study was to investigate the PK of capecitabine and its metabolites (5-DFCR, 5-DFUR and 5-FU) in younger (< 70 years) and older (≥ 70 years) adults receiving treatment for breast or gastrointestinal (gastric, pancreas, colorectal, and biliary) cancer and to explore the correlation between PK of capecitabine and chemotherapy-related toxicity and geriatric assessment domains.

Methods

Study design

This was a pilot pharmacokinetic study in adult participants who had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, breast cancer or gastrointestinal cancer (gastric, pancreas, colorectal, and biliary) and were planned for treatment with capecitabine (adjuvant or palliative) either as monotherapy or in combination with other anti-cancer drugs. Older adult was defined as age ≥ 70 years [3]. The study was conducted at three hospitals from November 2017 to February 2020. Ethics committee approval was obtained from the Sydney Local Health District Ethics Committee (CH62/6/2017-133, HREC/17/CRGH/198) and participants provided written, informed consent.

Sample size

Sample size was determined assuming a normal distribution of capecitabine AUC according to the observation by Louie et al. [7], a sample size of 12 in each group, with a parallel study design, would achieve 80% power to reject the null hypothesis of equal means with a significance level (alpha) of 0.05 using a two-sided two-sample unequal-variance *t* test.

Study procedures

Capecitabine was prescribed and administered as per routine clinical practice protocols. Participants attended clinic on day 14 of cycle one (21 day cycle) of capecitabine (i.e., at steady state) and day 14 of cycle two for study assessments. Venous blood samples were collected pre-treatment, and 1 h, 2 h, and 4 h after dosing for the quantification of plasma concentrations of capecitabine and its primary metabolites (5-DFCR, 5-DFUR, and 5-FU) [11]. Participant's demographic information, inflammatory markers, and renal function data were recorded prior to commencement of the study drug. Toxicity data were recorded during treatment and up to 6 months after completion of treatment for all patients.

Geriatric assessment of the included domains [score/instrument (range of score)] are as follows: cognition [the OMCT-Score (0–28)], functional ability/frailty [Timed Up and Go test (TUG)-score, the Katz index (0–6), OARS-score (0–14), MOS-score (10–30)], comorbidity and polypharmacy [CIRS-G- score (0–4)], psychosocial function [Geriatric Depression Scale (1–5), the modified MOS social support score (4–20)], nutrition [MNA-score (0–14)], and screening instrument [the G8 score (0–17)] [12].

Assay

Using Mitra® microsampling devices for sample collection, an LC–MS/MS method was developed to simultaneously measure capecitabine, 5-DFCR, 5-DFUR, and 5-FU according to Radovanovic et al. [13].

Pharmacokinetic analysis

A Bayesian PK estimation approach using observed metabolite concentration–time data and an existing population PK model was employed to estimate individual estimates of PK parameters [14, 15]. Results were then statistically compared using a standard industry approach to determine any impact of age on PK. The selected population pharmacokinetic model [14] is illustrated in (Online Appendix1).

The selected model was then simulated for a typical patient and compared to data presented by Gieschke et al. to confirm model coding [14]. Individual estimates of PK parameters were determined by empiric Bayesian estimation using the PK of the drug (i.e., model), individual patient factors (i.e., body surface area, estimated creatinine clearance, and serum alkaline phosphatase activity) and the measured drug and metabolite concentration(s). Determined model parameters were then used to calculate the following PK parameters on day 14 for each treatment cycle, and these included area under the plasma concentration–time curve over the 12-h dosing interval (AUCτ), maximum plasma concentration over the 12-h dosing interval (Cmax), and time of maximum plasma concentration (Tmax). Dose-normalised data (to a dose of 1500 mg) were calculated for AUCτ and Cmax parameters, calculated as: Dose Normalised PK Parameter $= \frac{1500\text{mg}}{\text{Dose Administered (mg)}} \times$PK Parameter.

Statistical analysis

A linear mixed-effect analysis of variance (ANOVA) model was used to compare dose-normalised Ln-transformed PK parameters between age groups. The residual error (error mean square) was used to construct the 90% confidence intervals for the ratio of treatment means. To construct the 90% confidence intervals, the younger group (i.e., < 70 years) was used as the reference. Equivalence was concluded if the 90% confidence intervals were within the standard limits of 80–125%. Significance was set at an α-level of 0.05. Linear regression and logistic regression analysis were used to determine the correlation between capecitabine and metabolite PK and domains of geriatric assessment, inflammatory markers, and toxicity. Toxicity was graded according to NCI CTCAE version 3.0 during chemotherapy cycles.

Software

Population PK modelling and simulation was conducted using NONMEM® VIII (ICON Development Solutions, Ellicott City, MD, USA) software with an Intel Fortran compiler (Intel Visual Fortran Composer XE 2013) and Wings for NONMEM 7 interface (http://wfn.sourceforge.net). Data processing was conducted using R® Version 3.3.2 (R Foundation for Statistical Computing). Statistical comparisons were performed using Phoenix® WinNonlin® Version 8.2 (Pharsight®, a Certera™ company). XLSTAT (version 2021.4) software was used for linear regression and logistic regression analysis.

Results

Of a total 26 participants, the median age was 67 years (range, 37–85 years) and 58% were male. 14/26 (54%) were aged < 70 years and 12/26 (46%) were aged ≥ 70 years. All 26 participants were included in the PK analysis of concentration–time data for capecitabine and its metabolites (Online Appendix 2).

Concentration–time data were collected from 1 treatment cycle for all participants and for 2 treatment cycles for 13/26 (50%) participants. The mean capecitabine dose was 1666 mg twice daily (range, 1000–2000 mg) in the older adult group and 1750 mg twice daily (range, 1500–2000 mg) in the younger adults' group. The mean dose-normalised 5-FU concentration–time profiles showed a 17% increase in total exposure (AUCτ 90% CI 103–134%) and 14% increase in maximal concentrations (Cmax5-FU 90% CI 82.1–159%) over the dosing interval in the older age group, compared to the younger group (Online Appendix 3).

Individual empiric Bayesian estimates of PK model parameters are presented in Table 1. The calculated PK parameters for Cycle 1 and Cycle 2 are summarised in Online Appendices 4 and 5, respectively.

Minimal differences between age groups were observed in mean dose-normalised 5-DFUR profiles. The 90% confidence intervals for AUCτ were contained within the limits of 80–125%. 5-DFUR Cmax values exhibited great variability, such that the 90% confidence intervals were 78.7–146%, extending beyond the standard limits; nonetheless, the mean ratio was approximately 100% and no differences found in 5-DFUR PK between older and younger patients. Predicted and observed 5-DFUR and

Table 1 Empiric Bayesian estimates of individual model parameters (capecitabine)

Age group	Younger $n = 14$ mean (CV %)	Older $n = 12$ mean (CV %)
Ka (h-1)	1.65 (74.9)	1.52 (59.7)
V2 (L)	88.4 (14.6)	89.4 (9.83)
CL2 (L/h)	92.5 (17.1)	84.1 (11.7)
V3 (L)	17.8 (0.00)	17.8 (0.00)
CL3 (L/h)	2000 (21.1)	1710 (20.3)
V4 (L)	89.0 (17.5)	66.8 (16.2)
CL4 (L/h)	35.4 (18.5)	24.7 (14.8)

CV % Coefficient of variation, *ka* absorption rate constant, *CL2* apparent 5DFUR clearance, *CL3* apparent 5FU clearance, *CL4* apparent FBAL clearance, *V2* apparent 5DFUR volume, *V3* apparent 5FU volume, *V4* apparent FBAL volume

5-FU concentration–time profiles for each individual are presented in Online Appendices 6 and 7, respectively.

Mean predicted dose-normalised (to a capecitabine dose of 1500 mg) concentration–time profiles on Cycle 1, Day 14 for 5-DFUR and 5-FU, stratified by age group, are presented in Online Appendix 8. The geometric mean ratio of older/younger group PK data and associated 90% confidence intervals are presented in Online Appendix 9.

Logistic regression analysis revealed no significant correlation between PK parameters of capecitabine (AUC5-FU, Cmax5-FU, AUC5-DFUR, Cmax5-DFUR) and capecitabine toxicity [diarrhoea (11/26) ($p=0.43$)], hand and foot syndrome [(11/26) ($p=0.07$)], grade 3 & 4 toxicity [(10/26) ($p=0.11$)], hospitalisation [(4/26) ($p=0.56$)], and any toxicity [(20/26) ($p=0.21$)]. No significant association was found between PK parameters of capecitabine and inflammatory markers C-reactive protein (CRP) ≥ 10 (10/26) ($p=0.33$) and Neutrophil–Lymphocyte Ratio (NLR) ≥ 5 (5/26) ($p=0.19$) (Online Appendix 10). 5-FU Cmax and 5-FU AUC were positively associated with the functional ability based on the Timed Up and Go [TUG- score (median = 9) (Pearson correlation 0.77, $p=0.01$ and 0.79, $p=0.03$, respectively)], but not other domains of geriatric assessment.

Discussion

In the present study, older adults, compared with younger adults, who had standard dose capecitabine for breast or gastrointestinal cancer had a statistically significant higher exposure to 5-FU. The increased exposure to 5-FU among older adults was positively correlated with the TUG-score (a measure of functional ability), but not other geriatric assessment variables, rates of severe chemotherapy-related toxicity or inflammatory markers.

Previous studies determining the effect of age on capecitabine PK have showed differences between older adults and younger adults. Abdi et al. [8] found that the capecitabine absorption rate constant was lower in the older adults (> 75 years; $n=20$, 20/60) compared with younger adults (mean ka value of 0.84 h^{-1} in older adults versus 1.86 h^{-1} in the younger adults). The elimination rate constant of the 5-FU metabolite (k40) decreased significantly over time (after 2 consecutive weeks), but this time effect was not different between the two age groups [8]. From the second cycle of capecitabine, a significant correlation was found between the higher exposures of capecitabine and its metabolites (5-DFCR, 5-DFUR, 5-FU) and grade 2 or 3 hand–foot syndrome ($p=0.01$; $p=0.03$; $p=0.006$; $p=0.008$, respectively). Similarly, in the present study, a higher Cmax for 5-FU was found in older adults, but there was only a trend among patients (older and younger) with high exposure of 5-FU and hand and foot syndrome toxicity ($p=0.07$). Other chemotherapy–related toxicity and PK of capecitabine and its metabolites were not correlated, possibly due to low numbers of older adults providing blood samples in cycle 2 ($n=3$). Louie et al. [7] investigated capecitabine PK in older adults (> 70 years; $n=24$) compared with younger adults (< 70 years; $n=5$). Cmax and AUC of capecitabine were threefold higher among older adults, compared to younger adults, but there was no difference in the PK parameters of 5-DFCR, 5-DFUR, or 5-FU. Correlation between capecitabine exposure and chemotherapy-related toxicity was not examined in their study. This greater variation in PK in older adults with cancer is possibly due to a reduction in renal and hepatic clearance and an increase in volume of distribution of lipid soluble drugs with age [16].

To our knowledge, the association between the PK of capecitabine, or any chemotherapy, and geriatric assessment variables has not been previously investigated. Geriatric assessment variables are correlated with a higher risk of chemotherapy-related toxicity, hospitalisation, and early death [12, 17]. A systematic review investigated the use of geriatric assessment to predict outcomes in older adults with cancer [12]. Geriatric assessment tools were associated with poor health outcomes, such as chemotherapy-related toxicity and mortality [12]. An association between the geriatric assessment variables and PK parameters would enable clinicians, following completion of a geriatric assessment of older adults commencing chemotherapy, to identify older adults at, for example, increased risk of severe chemotherapy toxicity, hospitalisation, and/ or mortality due to change in PK parameters (e.g., exposure and Cmax) of a chemotherapy agent and prescribe appropriate dose modifications to minimise these risks. The American Society of Clinical Oncology (ASCO) guideline (2018) recommends geriatric assessment be performed for all patients with cancer who are older than 65 years [18].

In the present study, functional ability (based on the TUG-score) was the only geriatric assessment variable positively correlated with 5-FU PK. No geriatric assessment variable was associated with chemotherapy-related toxicity. Older adults in our study predominantly had adjuvant capecitabine (9/12, 75%) for colorectal cancer (11/12, 92%), possibly reflecting better overall fitness with a great ability to tolerate chemotherapy.

Strengths of the present study include the inclusion of real-world older and younger patients receiving standard chemotherapy, rather than clinical trial participants, to improve the applicability and generalisability of results to day-to-day clinical practice. Prospective collection of toxicities, geriatric assessment variables, and inflammatory markers at the point of care strengthened the outcome data. Another strength included determining the relationship between geriatric assessment variables and capecitabine PK

and being one of only few reported studies to examine the effect of age on the PK of capecitabine and its metabolites.

In addition to previously mentioned limitations of the study, others include a lower participation rate of older adults in the second cycle of the study (3/12), though comparable to other similar studies and fairly typical of PK studies. We had estimated a sample size of at least 12 participants to have 80% power of detecting an effect (p value < 0.05). The low number of participants in the entire study ($n = 26$) also reduced the power to detect a significant association between the variables. Generalisability of the findings is likely limited by the majority of participants having adjuvant chemotherapy and hence of better fitness for chemotherapy, rather than palliative chemotherapy for advanced cancer, and hence not representative of all patients having capecitabine in routine clinical practice.

Conclusions

Compared to younger adults, older adults having capecitabine chemotherapy at the standard dose have significantly increased exposure to 5-FU but not to the other metabolites of capecitabine. The clinical significance of these findings requires further investigation in a larger cohort to determine whether it contributes to excess toxicity and/or provides a rationale for dose modifications in older adults receiving capecitabine.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors of this manuscript have no relevant affiliations or financial involvement with any organization or entity with a financial interest with the subject matter or materials discussed. Authors of this manuscript have no relevant financial or other relationships to disclose.

References

1. Society. AC. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Available at: https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21492 [Accessed 20 Sep 2022].
2. Australian Institute of Health and Welfare & Australasian Association of Cancer Registries 2012. Cancer in Australia: an overview, 2012. Cancer series no. 74. Cat. no. CAN 70. AIHW.
3. Wildiers H, Heeren P, My P et al (2014) International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. J Clin Oncol 32:2595–2603
4. Stockler MR, Harvey VJ, Francis PA et al (2011) Capecitabine versus classical cyclophosphamide, methotrexate, and fluorouracil as first-line chemotherapy for advanced breast cancer. J Clin Oncol 29:4498–4504
5. Bajetta E, Procopio G, Celio L, Gattinoni L, Della Torre S, Mariani L, Catena L, Ricotta R, Longarini R, Zilembo N, Buzzoni R (2005) Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. J Clin Oncol 23:2155–2161
6. McLachlan AJ, Hilmer SN, Le Couteur DG (2009) Variability in response to medicines in older people: phenotypic and genotypic factors. Clin Pharm Therap 85:431–433
7. Louie SG, Ely B, Lenz HJ et al (2013) Higher capecitabine AUC in elderly patients with advanced colorectal cancer (SWOGS0030). Br J Cancer 109:1744–1749
8. Daher Abdi Z, Lavau-Denes S, Prémaud A et al (2014) Pharmacokinetics and exposure-effect relationships of capecitabine in elderly patients with breast or colorectal cancer. Cancer Chemother Pharmacol 73:1285–1293
9. Cassidy J, Twelves C, Cameron D et al (1999) Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. Cancer Chemother Pharmacol 44:453–460
10. Food and Drug Administration Xeloda (Capecitbine), NDA 020896. 2015. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/020896s037lbl.pdf
11. Gieschke R, Burger HU, Reigner B et al (2003) Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. Br J Clin Pharmacol 55:252–263
12. Puts MT, Hardt J, Monette J et al (2012) Use of geriatric assessment for older adults in the oncology setting: a systematic review. J Natl Cancer Inst 104:1134–1164
13. Radovanovic M, Schneider JJ, Shafiei M et al (2022) Measurement of 5- fluorouracil, capecitabine and its metabolite concentrations in blood using volumetric absorptive microsampling technology and LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 1188:123075
14. Gieschke R, Reigner B, Blesch KS, Steimer JL (2002) Population pharmacokinetic analysis of the major metabolites of capecitabine. J Pharmacokinet Pharmacodyn 29:25–47
15. Gieschke R, Burger HU, Reigner B, Blesch K, Steimer JL (2003) Population pharmacokinetics and concentration-effect relationships of capecitabine metabolites in colorectal cancer patients. Br J Clin Pharmacol 55:252–263
16. Crombag MR, Joerger M, Thürlimann B, Schellens JH, Beijnen JH, Huitema AD (2016) Pharmacokinetics of selected anticancer drugs in elderly cancer patients: focus on breast cancer. Cancers 8:6
17. Feliu J, Espinosa E, Basterretxea L et al (2021) Prediction of chemotoxicity, unplanned hospitalizations and early death in older patients with colorectal cancer treated with chemotherapy. Cancers 14:127
18. Dale W, Williams GR, MacKenzie AR et al (2021) How is geriatric assessment used in clinical practice for older adults with cancer? A survey of cancer providers by the american society of clinical oncology. JCO Oncol Pract 17:336–344