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The second victim – supporting JMOs through medical errors

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The term ‘second victim’, coined by Wu in 2000*, encapsulates the impact of medical error on clinicians. Despite public perception, and what hard-line medical culture would have us believe, medical error is inevitable as we are all fallibly human.

We accept the patient as the first victim, and our critical incident review process naturally focuses on their outcomes. But the health professionals who feel responsible can often be neglected.

Although linear root cause analyses attempt to appreciate the multifactorial reasons behind incidents where the individual is only one ‘hole’ in the ‘Swiss Cheese’ model, the emotional impact of errors may be overlooked. Doctors who are second victims may experience psychological distress, burnout, post-traumatic stress, risk-averse practice, and maladaptive behaviours including drug and alcohol abuse, leaving the profession and, in the worst case, suicide.

Junior doctors and trainees (JMOs) are particularly vulnerable to the effects of medical errors. Inexperience, high workload, increased fatigue, burnout, and feelings of inadequate clinical supervision are important contributors. Senior guidance is critical in shifting the perceptive from trauma to growth. Medical errors can be re-envisioned as formative learning experiences – for the second victim, their colleagues and the organisation.

Medical leadership sets the culture for how medical errors are viewed, processed and managed.

The work environment must be a safe place to discuss mistakes. Water-cooler gossip is harmful and should be actively discouraged. JMOs require support when providing open disclosure to the patient, and it helps to understand that admission and apology do not imply legal liability. They should be encouraged to have early discussions with their medical defence organisation to address medico-legal consequences.

1. Assess any acute needs.
Second victims are often distressed immediately after the incident comes to light, needing a safe space for a sounding board and psychological first aid, while steering away from clinical scrutiny. The JMO may need a break or to go home. Disclosing one’s own inevitable experience of medical errors normalises and validates their experience.

2. Facilitate reflection to enable clinical growth.
Have regular check-ins with the JMO and make sure they know the existing hospital and external support available for further assistance. In times of crisis and distress, the Australasian Doctors’ Health Network directs viewers or callers to the state-based health organisations which provide 24/7 advice lines manned by medical practitioners experienced in doctors’ health. These organisations can support doctors acutely, anonymously, and with confidentiality.

Systemic change is needed when approaching medical error. When the second victim is blamed, the healthcare organisation and patients suffer. Consider what simple interventions in everyday practices could prevent future second victims, and integrate early and ongoing support for second victims into the workplace.

Open discussion of medical errors during college education, peer groups and supervision will help dispel the stigma of mistakes. At the end of the day, we are all only human. A human response of empathetic support helps JMOs become better clinicians, moving forward from ‘victim’ to formulating meaning and experience after medical error.

*Reference available on request: marketing@mdanational.com.au

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Locally performed postoperative circulating tumour DNA testing performed during routine clinical care to predict recurrence of colorectal cancer

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Key words
circulating tumour DNA, colorectal neoplasms, recurrence.

Abstract

Background: Identifying patients at high risk for colorectal cancer recurrence is essential for improving prognosis. In the postoperative period, circulating tumour DNA (ctDNA) has been demonstrated as a significant prognostic indicator of recurrence. These results have been obtained under the strict rigours of clinical trials, but not validated in a real-world setting using in-house testing. We report the outcomes of locally performed postoperative ctDNA testing conducted during routine clinical care and the association with the recurrence of colorectal cancer.

Methods: We recruited 36 consecutive patients with newly diagnosed colorectal cancer between 2018 and 2020. Postoperative plasma samples were collected at the first outpatient review following resection. Tumour-informed ctDNA analysis was performed using droplet digital polymerase chain reaction or targeted next-generation sequencing.

Results: At the time of surgery, there were 24 patients (66.7%) with localized cancer, nine (25%) with nodal spread, and three (8.3%) with metastatic disease. The median time from surgery to plasma sample donation was 22 days (IQR 20–28 days). At least one somatic mutation was identified in primary tumour tissue for 28 (77.8%) patients. Postoperative ctDNA was detected in five patients (13.9%). The median duration of follow-up was 32.0 months (IQR 27.2–38.1 months). Two patients (5.56%) developed metastatic recurrence. However, neither had detectable postoperative ctDNA. There were no instances of loco-regional recurrence.

Conclusion: Analysis of postoperative ctDNA testing can be performed locally, however this study did not reproduce the adverse association between detectable postoperative ctDNA and the development of colorectal cancer recurrence seen in clinical trials.

Introduction

Globally, 1.3 million new cases of colorectal cancer are diagnosed annually, making colorectal cancer the third most common cancer.1 While those with nonmetastatic disease may be cured by surgical resection with or without adjunct therapy, recurrence is anticipated in 30%–50% of these patients.2 The gold standard for predicting recurrence focuses on histopathological examination of resected tumour tissue. The shortcomings of this system are well documented.3,4 At best, histopathological markers are surrogate tumour biology measures, not evidence of residual disease following resection. More personalized and innovative strategies are needed to identify those with minimal residual disease, defined as the persistence of micrometastatic disease following curative resection, which cannot be detected using conventional clinical, biochemical, endoscopic methods, or imaging techniques.

Tumour DNA detected in peripheral blood, termed circulating tumour DNA (ctDNA), provides individualized, minimally
invasive, and real-time molecular tumour characterization. Amongst several other clinical applications, ctDNA can identify minimal residual disease. Several clinical trials have demonstrated that detection of postoperative ctDNA following curative resection of colorectal cancer is associated with a significantly worse prognosis.5–12

With a rapidly growing global interest, the body of knowledge regarding the use of ctDNA in colorectal cancer care has been and continues to be, developed under the optimized constructs of clinical trials. Furthermore, this work has primarily been performed under the auspices of medical oncologists without a significant surgical contribution to the field. While it is encouraging to see that the results from these clinical trials are congruent, the results need to be reproduced under real-world conditions. To date, no real-world study has examined the prognostic role of detectable postoperative ctDNA in colorectal cancer using locally performed sample analysis. Unfortunately, the ctDNA trials conducted in Australia have required shipping patient samples to an overseas laboratory. While this was initially necessary due to the lack of local infrastructure, it is not a long-term solution that is practical outside of a clinical trial setting. We endeavoured to perform in-house ctDNA analysis to demonstrate the feasibility of future ctDNA research within local laboratories and capacity for implementation into routine clinical care in Australia. Herein we report the outcomes of locally performed postoperative ctDNA testing conducted during routine clinical care and the association with colorectal cancer recurrence.

Methods

Study design and participants

A visual summary of the study design is provided in Figure 1. This prospective single-site pragmatic study recruited consecutive patients with colorectal cancer. Patients were included in the study if they underwent resection of primary adenocarcinoma of the colon or rectum between May 2018 and January 2020 and provided a blood sample for ctDNA analysis. The sample size was limited by the recruitment end date to provide sufficient follow-up data at the intended time of publication. The study was approved by the St John of God Health Care and University of Western Australia Human Research Ethics Committees (references 1683 and RA/4/20/6297). All patients provided written informed consent.

Decisions regarding the administration of adjuvant chemotherapy were discussed at a multi-disciplinary meeting. All patients underwent routine surveillance, including 3–6 monthly clinical reviews, carcinoembryonic antigen testing, and annual colonoscopy and/or computed tomography of the chest, abdomen, and pelvis.

Tumour tissue mutation profile

Tumour specimens were fixed in 10% buffered formalin at the time of surgical resection and embedded in paraffin wax. DNA was extracted from macro-dissected full-face formalin-fixed, paraffin-embedded (FFPE) tumour samples using QIAamp DNA FFPE Tissue purification kits (QIAGEN, Hilden, Germany). Validation of yield of DNA extracted from tumour tissue was assessed using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA). Tumour tissue was analysed for somatic mutations in 14 genes commonly mutated in colorectal cancer, using the Oncomine Colon cfDNA Assay (Thermo Fisher Scientific). Sequencing was conducted as previously described.13

Tumour-informed mutation model development

For each patient, a tumour-informed mutation model was developed to determine the presence of ctDNA. Low-frequency variants (those with less than 1% of reads covering that allele) were excluded from selection. Candidate variants were cross-referenced with digital droplet polymerase chain reaction (ddPCR) assays in our laboratory, and a single mutation was selected as the tumour marker for each patient (that with the greatest variant frequency with a ddPCR assay on hand). For cases where a ddPCR assay was not available for the mutation detected in the tumour, targeted next-generation sequencing was performed.

Circulating tumour DNA analysis

At their first postoperative outpatient review, patients donated 10–20 mL of whole blood using Cell-Free DNA blood collection tubes (Streck, La Vista, USA). Plasma was isolated from whole blood by two-step centrifugation (10 min at 1600 × g followed by 10 min at 4000 × g) and stored at −80°C until use. DNA was extracted from 4 to 5 mL of plasma using the QIAamp Circulating Nucleic Acid purification kit (QIAGEN). Validation of yield and quality of cell-free DNA extracted from plasma were assessed using the D1000 ScreenTape Assay (Agilent Technologies, Santa Clara, USA). Postoperative cell-free plasma DNA samples interrogated by ddPCR were analysed using PrimePCR ddPCR assays (Bio-Rad, Hercules, USA). A positive control, a healthy control and a no-template control were included in each assay. Only tests providing >10 000 droplets were used for analysis. Postoperative cell-free plasma DNA samples interrogated by targeted Next-Generation Sequencing were analysed using the Oncomine Colon cfDNA Assay as previously described.13

Statistical analyses

Statistical analyses were performed using Stata version 17.0 (StataCorp, College Station, USA). Data distribution was assessed using the Shapiro–Wilk test. Survival data was analysed using the Kaplan–Meier estimator with subsequent Log-Rank test for equality of survival functions and Cox proportional hazards regression analysis. Survival analysis was truncated to the point when the number at risk was one-third of the starting figure. Follow-up duration was calculated using the reverse Kaplan–Meier method. Statistical significance was defined as *P < 0.05. Confidence intervals (CI) were set at the 95% level.
Patient characteristics
Thirty-six patients with colorectal cancer were included in this study. Their clinical and histopathological characteristics are summarized in Table 1 and displayed in Figure 2. The median time from surgery to blood donation was 22 days (interquartile range 20–28 days).

All patients underwent resection of their primary tumour. One patient had synchronous ascending (T3N0M0) and descending colon (T1N0M0) cancers. The more advanced ascending tumour was used for staging and analysis. Only one patient had involved resection margins - a 62-year-old male with T3N2bM1a rectal cancer with tumour adherence to the iliac vessels.

Of those administered adjuvant chemotherapy, most patients received first-line FOLFOX regime (60.0%) or capecitabine (33.3%).

The median follow-up time was 32.0 months (interquartile range 27.2–38.1 months). Amongst those with nonmetastatic disease at resection, recurrence occurred in 5.56% (n = 2). There were no instances of local recurrence. One of the two patients with metastatic recurrence underwent metastasectomy and is now disease free. There were three deaths during the follow-up period. No patients were lost to follow-up.

Primary tumour somatic mutations and ctDNA assay selection
Figure 2 summarizes the results from primary tumour targeted next-generation sequencing. The most frequently mutated genes in primary tumour samples were KRAS, followed by APC, PIK3CA, and TP53. Two patients had no mutations detected on sequencing, resulting in a tumour sequencing failure rate of 5.56%. Six patients had only low-frequency variants (i.e. less than the background threshold of 1% for FFPE tissues). For the remaining 28 patients, ctDNA assay selection is summarized in Supplementary Table 1. Eleven ddPCR assays were sufficient to cover 75.0% (n = 21) of the ctDNA testing cohort, with the remaining patients (n = 7) requiring ctDNA analysis by targeted next-generation sequencing.

Relationship between ctDNA status and clinical outcomes
Five patients were positive for postoperative ctDNA, however, this includes one patient with metastatic disease at the time of sampling.

Of those with nonmetastatic disease at the time of plasma sampling, the ctDNA detection rate was 14.8% (n = 4/27). None of the patients with detectable ctDNA developed recurrence, while the recurrence rate for those without detectable ctDNA was 8.7% (Fig. 3). There were no significant differences in disease-free
Our reported recurrence rate amongst stage I–III colorectal cancer patients of 5.56% is significantly lower than all other studies exploring the role of postoperative ctDNA in colorectal cancer. The mean recurrence rate in studies of postoperative ctDNA analysis in stage I–III colorectal cancer is 18.31% (standard deviation 5.00%).\textsuperscript{5,8,11,12,14} The lowest reported recurrence rate was 13.0%, found amongst 38 patients with locally advanced rectal cancer.\textsuperscript{11} While our sample size is smaller than most, our recurrence rate is dramatically lower. This has been achieved with a similar median duration of follow-up compared to of published studies (32.0 months versus 27 months).\textsuperscript{5,12,14,18} Given that this could be due to sampling error, we reviewed our institutional patient registry. Of the 1347 patients with stage I–III colorectal adenocarcinoma that underwent surgery between 2010 and 2018 (inclusive), the recurrence rate was 6.68% ($n = 90$). This included 74 patients with metastatic recurrence, four with local recurrence, and 12 with both local and metastatic recurrence. The median follow-up duration for this cohort was 7.33 years (interquartile range 5.19–10.1 years). This data suggests that the low recurrence rate for this study is not that dissimilar to our institutional standard and is markedly lower than the rates published by others.

We reported a primary sequencing failure rate of 5.56% ($n = 2$). The sequencing failure rate in the literature varies widely. Tarazona \textit{et al.}\textsuperscript{12} and Khakoo \textit{et al.}\textsuperscript{11} obtained a 12.0%\textsuperscript{12} and 9.62%\textsuperscript{11} sequencing failure rate, respectively. Across several of their studies, using the
Safe-SeqS assay in collaboration with Johns Hopkins University, Tie et al. achieved a sequencing failure rate of 0.0%–1.84%. While a watered-down version of Safe-SeqS is commercially available for colorectal liquid biopsy research, access to the full sequencing panel used in these studies is only possible through shipping patient samples to a Baltimore-based laboratory.

We performed in-house tumour-informed ctDNA analysis using the Oncomine Colon cfDNA Assay. A tumour-informed approach uses knowledge of the genetic alterations of the primary tumour to identify ctDNA in the blood, while a tumour-agnostic approach aims to identify ctDNA without prior primary tumour genetic profiling. Under this paradigm, ctDNA is sequenced using sequencing panels to detect mutations frequently involved in carcinogenesis. While tumour-informed ctDNA identification is more resource demanding and requires access to the primary tumour, the personalized nature of this approach is more sensitive and specific compared to tumour-agnostic testing.

In order to limit the costs of our analysis, we preferentially used ddPCR analysis for ctDNA samples. Compared to targeted next-generation sequencing, ddPCR is both dramatically cheaper per sample tested, less time consuming, and less technically challenging to perform ($AU4.00/sample vs. $AU225/sample; 1 versus 7 days). We selected the Oncomine Colon cfDNA Assay due to our local experience with the product and the published performance of the assay. Our ctDNA detection rate of 14.8% was similar to the rates reported in the literature. Those using ddPCR for ctDNA analysis reported a ctDNA detection rate, amongst nonmetastatic colorectal...
cancer patients, of 13.0%–28.6%. Those using targeted next-generation sequencing reported ctDNA detection rate varied by stage and disease location. Tie et al. found a ctDNA detection rate of 7.87%, 20.8%, and 11.9% for stage I, and stage II colon cancer, and locally advanced rectal cancer, respectively. In other studies, using targeted next-generation sequencing, amongst patients with nonmetastatic colorectal cancer, the ctDNA detection rate is reported at 8.3%–14.3%. The rate of ctDNA detection tends to increase with an increasing duration between surgery and sample collection. Henriksen et al. found that all postoperative ctDNA samples taken less than 2 months following curative resection were negative, but when sampled after 2 months, 80% of patients developed detectable ctDNA. This relationship likely reflects the surge in cell-free DNA following surgery, lasting up to 4 weeks. This process increases the ratio of wild-type-to-mutant cell-free DNA present in plasma, thereby diluting ctDNA below the test limit of detection.

Our study was unable to validate the association between detectable postoperative ctDNA and recurrence of colorectal cancer, partly due to a combination of the modest sample size ($n = 36$) and our low recurrence rate (5.56%). This study served as a proof of concept for in-house ctDNA analysis within the routine clinical setting. By design, consecutive sampling was utilized with limiting of the sample size by the study end date to permit adequate follow-up of participants at the time of intended publication. An a posteriori power calculation, at a recurrence rate of 6.0%, with the alpha set at 0.05 and power at 0.8, using the Cox proportional hazards model, a sample size of 104 patients would be required to reproduce the mean effect size (HR = 9.5) reported by trials of recurrence-free survival estimates using postoperative ctDNA analysis in stage I–III colorectal cancer. As a result, our study was underpowered to reproduce these findings. However, our study was adequately sized to demonstrate the feasibility of in-house ctDNA analysis in the routine clinical setting. Our a priori power calculation used the mean recurrence rate (18.31%) and mean effect size (HR = 9.5) reported by studies of postoperative ctDNA analysis in stage I–III colorectal cancer. Based on these figures, a sample size of 34 was required at an alpha of 0.05 and a power of 0.8. Smaller trials than ours have been published regarding postoperative ctDNA analysis. Most studies assessing colorectal cancer recurrence risk based on postoperative ctDNA are larger than ours, with a median trial sample size of 95 and an interquartile range of 53–160 patients. Acknowledging the limitations of our low recurrence rate amongst a modest sample size, continued patient recruitment is underway, with an expanded set of centres, to facilitate a more extensive future study.

Four patients had detectable ctDNA following curative resection and, to date, have no evidence of recurrence. Their median ctDNA concentration was 1.35 copies per millilitre of plasma (interquartile range 1.05–20.8 copies per millilitre of plasma). These patients harboured mutations in KRAS and TP53. Theoretically, these false positives could result from clonal haematopoiesis of indeterminate potential (CHIP), whereby somatic mutations have arisen in haematopoietic stem cells in an individual without a detectable haematologic malignancy. Typically, CHIP occurs in 10% of people aged greater than 70 years old, amongst patients with solid organ malignancy, and commonly involves mutations in KRAS and TP53. Concomitant interrogation of the buffy coat and plasma ctDNA samples would help identify CHIP. Although this doubles the laboratory and reagent cost per patient, in future, studies should consider performing concurrent ctDNA and buffy coat analysis in order to minimize false positives due to CHIP.

This is the first series to consider the practical implications of implementing postoperative ctDNA testing into routine clinical practice. We have utilized convenience-based ctDNA sampling, whereby patients provide samples at their first postoperative visit with in-house laboratory analysis. As a real-world application, convenience-based sampling allows researchers to understand how inherent patient-and clinician-based factors impact results. Not only does this allow variation in sample timing and volume, but it also incorporates the impact that prolonged or complicated admissions post-resection and rurality may have on ctDNA detection. These key factors are poorly represented in clinical trials, which typically are strictly regimented, and often have a selection bias against patients from rural and remote backgrounds. Importantly our study has shown that in-house ctDNA analysis is feasible in routine clinical care settings. Within Australia, the colorectal cancer ctDNA field has primarily been developed in collaboration with major international research institutions. We hope that this study will encourage others to explore ways in which we can continue to develop local researchers and fund local research facilities. Furthermore, in this study we have demonstrated that the practical application of postoperative ctDNA analysis requires significant further consideration before widespread implementation. In addition to the factors mentioned above, there needs to be further studies cross-validating ctDNA analysis platforms, economic viability studies and consideration of how to manage patients that, despite having active disease, have undetectable ctDNA. For the latter, the rate of undetectable preoperative ctDNA in patients with known colorectal cancer varies from 11.5% to 32%. At the time of commencing this study 5 years ago, there was only one pathology company in Australia and New Zealand that offered colorectal cancer ctDNA testing. They offer tumour-agnostic testing using the Aspect Liquid Biopsy panel (Australian Clinical Labs, Victoria, Australia), which tests for 107 somatic mutations in KRAS, NRAS, BRAF, PIK3CA, and EGFR. This test has a 2% limit of detection and has an out-of-pocket cost of AU$550 per sample. Interestingly at the time of writing, no additional companies are offering colorectal ctDNA analysis. As such, groups wanting to establish their own in-house ctDNA analysis pipeline will need to either use this service or collaborate with a local genomic research group.

In this study, we have been unable to reproduce the adverse association between the detection of postoperative ctDNA and the development of colorectal cancer recurrence, however we have demonstrated the feasibility of in-house ctDNA analysis in the routine clinical setting. This result is a powerful reminder of the need for further standardization and validation prior to the widespread implementation of the paradigm shift promised by trials that advocate for postoperative ctDNA testing to become the gold standard in recurrence risk prediction.

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Author contributions

Ryan Cohen: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; validation; visualization; writing – original draft.

Aaron Beasley: Investigation; validation; writing – review and editing. Melanie McCoy: Funding acquisition; supervision; writing – review and editing. Cameron Platell: Funding acquisition; supervision; writing – review and editing. Katie Meehan: Supervision; writing – review and editing. Elin Gray: Conceptualization; resources; supervision; writing – review and editing. Kathy Fuller: Supervision; writing – review and editing.

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Conflict of interest statement

None declared.

Data availability statement

De-identified data generated from this study can be made available by contacting the corresponding author, however requests will be subject to St John of God Healthcare Human Research Ethics Committee approval.

References


Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Supplementary Table 1. De-identified clinicopathologic data, primary tumour sequencing data summary, and ctDNA assay selection.