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# Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer

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**IMPORTANCE** For patients with resected, nonmetastatic colorectal cancer (CRC), the optimal surveillance protocol remains unclear.

**OBJECTIVE** To evaluate whether serial circulating tumor DNA (ctDNA) levels detected disease recurrence earlier, compared with conventional postoperative surveillance, in patients with resected CRC.

**DESIGN, SETTING, AND PARTICIPANTS** This study included patients (n = 58) with stage I, II, or III CRC who underwent radical surgical resection at 4 Swedish hospitals from February 2, 2007, to May 8, 2013. Eighteen patients received adjuvant chemotherapy at the discretion of their clinicians, who were blinded to the ctDNA results. Blood samples were collected at 1 month after the surgical procedure and every 3 to 6 months thereafter for ctDNA analysis. Patients were followed up until metachronous metastases were detected, or for a median of 49 months. Data analysis was performed from March 1, 2009, to June 23, 2018.

**MAIN OUTCOMES AND MEASURES** Sensitivity and timing of ctDNA positivity were compared with those of conventional surveillance modalities (computed tomographic scans and serum carcinoembryonic antigen tests) for the detection of disease recurrence.

**RESULTS** This study included 319 blood samples from 58 patients, with a median (range) age of 69 (47-83) years and 34 males (59%). The recurrence rate among patients with positive ctDNA levels was 77% (10 of 13 patients). Positive ctDNA preceded radiologic and clinical evidence of recurrence by a median of 3 months. Of the 45 patients with negative ctDNA throughout follow-up, none (0%; 95% CI, 0%-7.9%) experienced a relapse, with a median follow-up of 49 months. However, 3 (6%; 95% CI, 1.3%-17%) of the 48 patients without relapse had a positive ctDNA result, which subsequently fell to undetectable levels during follow-up.

**CONCLUSION AND RELEVANCE** Although these findings need to be validated in a larger, prospective trial, they suggest that ctDNA analysis could complement conventional surveillance strategies as a triage test to stratify patients with resected CRC on the basis of risk of disease recurrence.

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Colorectal cancer (CRC) is the third most common cause of cancer death in the United States, with more than 135 000 new cases diagnosed each year.[1] Surgical resection is the primary treatment for most nonmetastatic disease (stages I, II, and III). However, even after curative-intent surgical procedure, 30% to 50% of these patients ultimately relapse.[2,3]

For oligometastatic recurrences, particularly within the liver or lung, prompt surgical resection offers curative potential.[4,5] Therefore, early diagnosis of disease recurrence has been a high priority. A meta-analysis of 4055 patients with resected, nonmetastatic CRC found that intensive follow-up strategies, such as serum carcinoembryonic antigen (CEA) tests and computed tomography (CT) scans every 3 to 6 months, were associated with a significantly higher chance of curative resection and overall survival.[6] Accordingly, intensive postoperative surveillance, including measurements of serum CEA level every 3 to 6 months and CT scans every 6 to 12 months, is recommended by the American Society of Clinical Oncology and the National Comprehensive Cancer Network for patients with resected stage II or III CRC. For patients with resected stage I tumors, aggressive surveillance has generally not been recommended because of the high cure rate of surgical intervention; however, a secondary analysis of data from the Clinical Outcomes of Surgical Therapy trial had shown that patients with stage I colon cancer would experience similar advantages from intensive postoperative surveillance.[7] In general, however, the optimal protocol for surveillance of resected colorectal cancer remains uncertain.

The only recommended blood marker for CRC surveillance is serum CEA, an oncofetal protein that is elevated in the serum of patients with a variety of disease conditions, including CRC.[8] Unfortunately, its utility is limited by the lack of sensitivity and specificity.[9] The addition of CT imaging improves surveillance but is associated with nonspecific findings and a small risk of second malignant neoplasms owing to radiation exposure.[10] Magnetic resonance imaging has a high sensitivity for the detection of liver metastases and pelvic recurrence of rectal cancer.[11] However, its higher cost and limited utility in detecting lung metastases preclude its routine use in surveillance.[3]

Genome-wide sequencing studies have identified mutations associated with tumorigenesis in CRC and other tumor types.[12-14] Tumor DNA fragments carrying those mutations, termed *circulating tumor DNA* (ctDNA), are shed into the bloodstream and are sensitive, dynamic markers of disease burden.[15,16] The recent development of extremely sensitive mutation detection methods, such as the Safe-Sequencing System (Safe-SeqS), enables the detection of mutations in the peripheral circulation at low frequency.[17-19] In this current study, we determined whether serial ctDNA levels detected disease recurrence earlier, compared with radiographic imaging alone, in patients with resected, nonmetastatic CRC.

### Key Points

**Question** Can circulating tumor DNA provide a measurement of disease burden to stratify the risk of recurrence in patients with resected colorectal cancer during postoperative surveillance?

**Findings** In this study of 58 patients with resected, nonmetastatic colorectal cancer, 45 patients with negative circulating tumor DNA levels were recurrence-free, whereas 10 of 13 patients with positive circulating tumor DNA levels relapsed during follow-up. Circulating tumor DNA positivity preceded radiologic or clinical evidence of recurrence in all 10 patients by a median of 3 months.

**Meaning** Serial circulating tumor DNA levels during postoperative surveillance can be used as a triage test to stratify patients with resected colorectal cancer on the basis of their risk of recurrence.

## Methods

We enrolled 63 patients with stage I, II, or III CRC who underwent radical resection of their tumors from February 2, 2007, to May 8, 2013, at 4 hospitals in Sweden (Eskilstuna General District Hospital, Örebro University Hospital, Falun General District Hospital, and Gävle General District Hospital). The study was approved by the Human Research Ethical Review Board in Stockholm, Sweden. All participants provided written informed consent. Data analysis was performed from March 1, 2009, to June 23, 2018.

For study inclusion, patients must have had no signs of metastases on preoperative imaging 4 to 6 weeks before surgical resection. In addition, there must be a verified R0 resection of the primary tumor. Blood samples from participants were collected at 1 month after the surgical procedure and then every 3 to 6 months thereafter for ctDNA level analysis. Plasma samples were sent to Johns Hopkins Ludwig Center for ctDNA analysis, which was performed in a blinded fashion. Participants were followed up until metachronous metastases were detected, or for a median of 49 months.

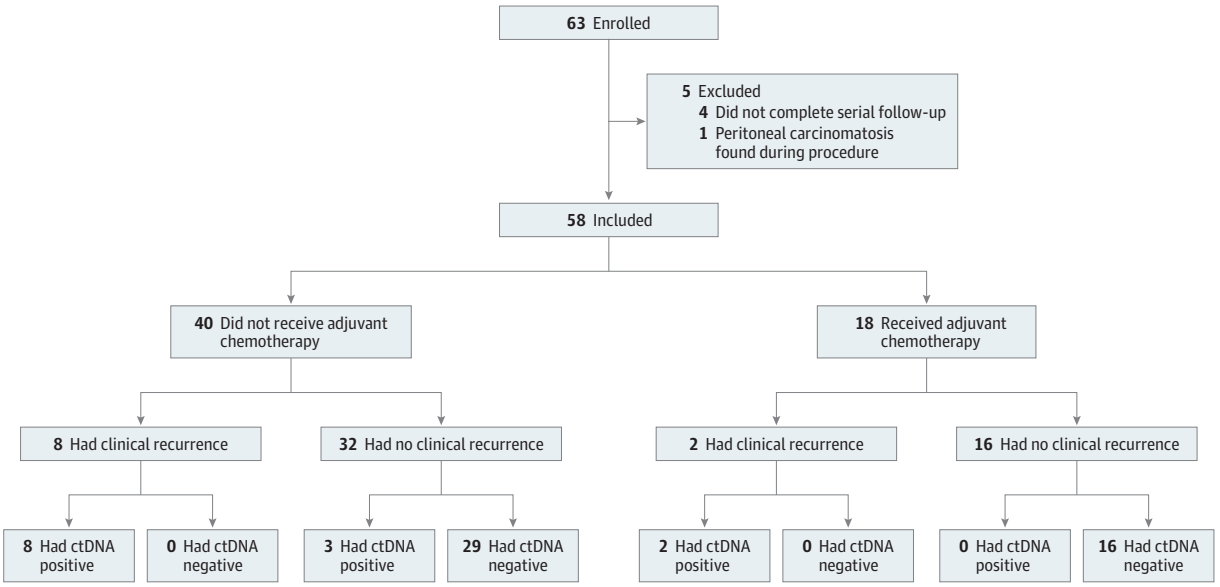
### Statistical Analysis

Safe-SeqS, an error-reduction technology for detecting low-frequency mutations, was used to detect mutations in plasma samples.[17] Circulating tumor DNA level was classified as detectable (ctDNA positive) or undetectable (ctDNA negative) on the basis of a permutation test that used the mutation frequencies in the experiment-specific controls. Under conditions as described and used in current studies, mutations present in more than 0.2% of template molecules could generally be reliably determined.[17,20] Additional details are included in the eMethods in the Supplement.

Each experiment-specific control consisted of DNA purified from white blood cells of healthy individuals without cancer. A total of 130 experiments were performed; in each experiment, the control and sample of interest were divided into multiple wells to increase sensitivity.

The mutant allele fraction (the ratio between the number of supermutants and the number of unique identifier sequences for the mutation of interest) was calculated for each well with more than 200 unique identifier sequences. The difference in the distributions of the mutant allele fraction between the sample of interest and the experiment-specific controls was then statistically evaluated with the permutation test, using the permTS function of the R package, version 3.2.3

Figure. Patient Enrollment, Follow-up, and Outcome




During follow-up, blood samples were collected 1 month after the surgical procedure, and then every 3 to 6 months. Computed tomography scans were performed every 6 to 12 months. Eighteen of the 58 patients received adjuvant chemotherapy at the discretion of their oncologist, who was blinded to the circulating tumor DNA (ctDNA) results. Each patient cohort (with or without adjuvant chemotherapy) was further divided by disease recurrence and ctDNA status.

(R Foundation for Statistical Computing). A 1-sided, rather than a 2-sided, test was used to avoid attributing significance to a ctDNA-negative sample that has fewer supermutants than the associated control. A 1-sided $P = .02$ was chosen as the threshold to classify a sample of interest as ctDNA positive ($P < .02$) or ctDNA negative ($P > .02$). Given the lack of a criterion standard, the choice of a $P < .02$ threshold was motivated by the specificity of at least 98% being desirable. Confidence intervals for sensitivities and specificities were calculated assuming binomial distributions, with the actual sensitivities and specificities set as the corresponding success probabilities.

## Results

### Patient and Tumor Characteristics

This study included 319 blood samples from 58 patients with CRC who underwent surgical resection for stage I, II, or III disease (**Figure**). Among these patients, the median (range) age was 69 (47-83) years and there were 34 males (59%). DNA from the resected tumors was sequenced to identify at least 1 somatic mutation in each patient using a panel that queried regions from 15 genes that are commonly mutated in CRC (eTable 1 in the Supplement).[15] Patient demographics are summarized in eTable 2 in the Supplement, and the mutations identified are listed in eTable 3 in the Supplement.

For each patient, we designed the Safe-SeqS assay to interrogate the mutation of interest in plasma.[17] Blood samples were collected about 1 month postoperatively and at follow-up visits every 3 to 6 months. The median (range) follow-up time for patients who did not experience disease recurrence was 49 (11-70) months.

### ctDNA as a Diagnostic and Prognostic Marker of Recurrence

Forty (69%) of the 58 patients did not receive adjuvant chemotherapy. In this cohort, all of the 8 patients who relapsed (1 with stage I, 2 stage II, and 5 stage III; 100% [95% CI, 63%-100%]) had positive ctDNA during follow-up.

The recurrence rate among patients with positive ctDNA is 77% (10 of 13 patients). The median (range) time to recurrence since surgical resection was 9 (5-52) months. In all 8 patients, ctDNA was positive prior to radiographically evident recurrence on CT scans, with a median (range) lead time of 4 (2-31) months. In contrast, of the 32 patients who did not have a recurrence, 29 (91%; 95% CI, 75%-98%) had negative ctDNA throughout follow-up (median [range], 49 [11-70] months). The 3 with false-positives (patients 011, 130, and 139) included 1 with stage II and 2 with stage III. In all 3 patients, ctDNA levels eventually became undetectable during follow-up (eFigure 1 in the Supplement). In comparison to ctDNA level, the CEA level was positive (>5 μg/L) in 5 (63%; 95% CI, 24%-91%) of the 8 patients who had a recurrence and none (0%; 95% CI, 0%-11%) of the 32 patients without recurrence.

Eighteen (31%) of the 58 patients received chemotherapy at their clinicians' discretion without knowledge of the ctDNA analysis results. To avoid the potential confounding effect of treatment, we considered only postchemotherapy samples. In this cohort, 2 patients (11%) had positive postchemotherapy ctDNA (patient 138 had stage II and patient 146 had stage III disease), and both (100%; 95% CI, 16%-100%) of them

eventually relapsed. The ctDNA positivity, as in patients who did not receive adjuvant chemotherapy, preceded clinical recurrence according to radiographic evidence in both patients (eFigure 2 in the Supplement). The median (range) lead time was 1 month (4-5 weeks). In contrast, none (0%; 95% CI, 0%-21%) of the 16 patients without disease recurrence had positive ctDNA at any time during follow-up (median, 37 months). In patient 142, ctDNA was positive during chemotherapy but fell to undetectable levels after treatment. This patient remained clinically recurrence-free throughout the 37-month' follow-up. In comparison to ctDNA level, CEA level was positive in 1 (50%; 95% CI, 1.3%-99%) of the 2 patients who had a recurrence and 1 (6%; 95% CI, 0.16%-30%) of the 16 patients without recurrence.

Of the 45 patients who had negative ctDNA throughout follow-up (16 of whom received and 29 did not receive adjuvant chemotherapy), none (0%; 95% CI, 0%-7.9%) had a recurrence, with a median follow-up of 49 months. In comparison, CEA level was positive in 1 (2.2%; 95% CI, 0.06%-12%) of these 45 patients.

## Discussion

In this study, we showed that ctDNA is a sensitive marker of tumor burden. Serial ctDNA levels during follow-up can precede disease recurrence prior to routine radiographic imaging. In addition, ctDNA measurements had higher sensitivity in detecting recurrence compared with CEA levels (100% vs 60%). To our knowledge, this study is the first to demonstrate the potential utility of ctDNA in all 3 stages of nonmetastatic CRC.

Previous studies have shown that ctDNA can be an early marker of disease recurrence in patients with stage II,[15] locally invasive,[21] or metastatic CRC.[16] Our data provide further evidence that ctDNA is a dynamic and sensitive marker of tumor burden. The recurrence rate among patients with positive ctDNA (11 [79%] of 14) in a previous study of patients with stage II disease[15] is comparable to findings in the present study (10 [77%] of 13). Also consistent with the previous study is the finding that even patients with positive ctDNA could still be cured by chemotherapy, as demonstrated by patient 142.[15] In patients with negative ctDNA, the recurrence rate was 9.8% with a follow-up of 27 months in the stage II study.[15] The recurrence rate in the present study was 0% with a follow-up of 49 months. We attribute the higher specificity in this study to the more stringent cutoff for ctDNA positivity used ($P < .02$ vs $P < .10$).

This study also included 9 patients with stage I CRC. Only 1 patient (patient 145) relapsed, consistent with the low recurrence rate in this group. It was still encouraging that patient 145 had positive ctDNA preceding clinical recurrence (lead time: 1 month). This study demonstrates the potential role of ctDNA in all 3 stages of nonmetastatic CRC, including stage I, in which no consensus exists on the appropriate follow-up.

In terms of specificity, ctDNA was positive in 3 (6%; 95% CI, 1.3%-17%) of 48 patients who did not experience recurrence throughout follow-up. These 3 patients had ctDNA levels that eventually became undetectable during follow-up. One possible explanation is that these patients had minimal residual disease at the time that their ctDNA levels were positive, but this disease was cleared by the immune system. The ability of the immune system to destroy tumor cells in vivo has been dramatically accentuated by the introduction of immune checkpoint therapies in recent years. Although these patients were not treated with immune checkpoint inhibitors, we speculate that the immune system might have played a role in these 3 cases. However, we cannot exclude the possibility that these false-positives were the result of some unappreciated technical artifact.

One finding of this study was the low probability of relapse in patients with negative ctDNA throughout follow-up (0 of 45 patients; 0% [95% CI, 0%-7.9%]). This finding suggests the potential of ctDNA as a rule-out test, identifying patients in whom less frequent radiographic investigations or follow-up would be sufficient. This finding could also substantially minimize the radiation exposure and costs associated with unnecessary testing during postoperative surveillance.[22] Furthermore, an important part of surveillance is reassuring patients who are unlikely to experience recurrence. Our findings suggest that negative ctDNA result can provide prognostic information compared with a standard CEA test.

A larger, prospective study is needed to determine whether or not the lead time provided by ctDNA measurements is associated with a better clinical outcome and to validate the findings in our study. This would be particularly valuable as a recent meta-analysis found inconclusive advantage of intensive follow-up using traditional modalities.[23] Our findings provide further evidence of the potential value of ctDNA analysis in patients with early-stage cancer. Circulating tumor DNA measurements can be easily obtained from blood samples collected during routine follow-up. Unlike radiographic imaging interpretations, ctDNA results are quantitative and reader-independent. Thus, ctDNA test could, in principle, be easily incorporated into routine follow-up to complement conventional modalities, such as CEA test and radiographic imaging, to help stratify patients' risk for disease recurrence. Ideally, such a personalized surveillance strategy for each patient would allow for earlier detection of relapse while minimizing unnecessary testing.

### Limitations

The study was limited by the sample size, involving only 319 blood samples from 58 patients. Nevertheless, it is encouraging that the 10 patients who relapsed clinically had positive ctDNA levels that preceded radiographic evidence of recurrence. Although ctDNA positivity preceded recurrence by a median of 4 months in patients who did not receive adjuvant chemotherapy, the blood samples did not become ctDNA positive until a median of 9 months after surgical resection. This lead time would not be early enough to affect the decision on adjuvant chemotherapy, but it might still be sufficient to allow for earlier implementation of other curative or palliative strategies.

We suspect that the shorter lead time may, in part, be associated with the higher frequency of imaging than recommended by many guidelines for stage II or III disease. Two-thirds of the

patients in the study underwent CT imaging every 6 months, and the remaining one-third was imaged every 12 months.

## Conclusions

This study provides evidence of the potential value of ctDNA analysis in stratifying the risk of disease recurrence among patients with early-stage cancer. Because ctDNA measurements can be obtained from blood samples collected during routine follow-up, they may be easily incorporated into routine follow-up to complement a CEA test, radiographic imaging, and other conventional modalities to help stratify patients on the basis of the risk of disease recurrence. Such a personalized surveillance strategy may allow for earlier detection of relapse and minimize unnecessary testing.

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**Author Contributions**: Drs Vogelstein and Olsson had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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*Other*: Tie.
*Other - Method Development:* Dobbyn.

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