Brief Communication

Circulating Epithelial Cells in Patients with Benign Colon Diseases

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BACKGROUND: Detection of circulating tumor cells (CTCs) in the peripheral blood is a rapidly developing research field with clear clinical implications for the staging and monitoring of cancer patients. Current CTC assays, including the US Food and Drug Administration–cleared CellSearch® system (Veridex/Johnson & Johnson) and the CTC chip are based on the enrichment and subsequent identification of CTCs via the use of antibodies against epithelial markers (e.g., the transmembrane glycoprotein EpCAM [epithelial cell adhesion molecule], cytokeratins [CKs]) that are expressed on both normal and malignant epithelial cells (2). The specificity of this widely accepted approach is derived from the fact that blood cells usually lack detectable expression of epithelial markers because of their mesenchymal origin. It is unclear, however, whether trafficking of normal epithelial cells could occur in the blood circulation under certain circumstances, which might contribute to false-positive findings in current assays unless unambiguous criteria for the malignant nature of the marker–positive cells are used. Thus far, only 1 report has suggested the presence of circulating epithelial cells in some patients with benign adenomas (3 of 30 patients, 10%) or benign inflammatory diseases (4 of 34 patients, 12%) (3), suggesting that cells from nonmalignant colonic epithelium may also gain entry into the bloodstream in patients with benign bowel diseases. This reverse-transcription PCR–based study, however, included rather unspecific epithelial markers: mucins, which are frequently produced in blood cells (3), and CK20, which can also be detected in normal granulocytes (4).

METHODS: We enrolled 53 patients with benign colon diseases (e.g., diverticulosis, benign polyps, Crohn disease, ulcerative rectocolitis, colonic endometriosis) and analyzed their peripheral blood with 2 previously validated CTC assays: the epithelial immunospot (EPISPOT) assay and the CellSearch system. The EPISPOT assay detects only viable, CK19-releasing CTCs that were enriched by depletion of CD45+ leukocytes, whereas the CellSearch system detects CK-positive CTCs after positive EpCAM-based immunomagnetic enrichment.

RESULTS: In patients with benign colon diseases, positive events that met the criteria for "tumor cells" were detected with both the CellSearch system (11.3%) and the CK19-EPISPOT assay (18.9%), whereas no positive events were detected in samples from healthy volunteers. Positive events were detected most frequently in patients with diverticulosis and Crohn disease. All positive events lacked expression of CD45, a common leukocyte antigen.

CONCLUSIONS: These results indicate that patients with benign inflammatory colon diseases in particular can harbor viable circulating epithelial cells that are detectable with current CTC assays. This finding points to the need for further molecular characterization of circulating epithelial cells and has important implications for the use of CTC testing.

During the past decade, researchers have developed ultrasensitive assays that allow the detection of circulating tumor cells (CTCs) at the single-cell stage in the peripheral blood (1). Most of these assays [including the US Food and Drug Administration (FDA)-cleared CellSearch® system (Veridex/Johnson & Johnson) and the CTC chip] are based on the enrichment and subsequent identification of CTCs via the use of antibodies against epithelial markers [e.g., the transmembrane glycoprotein EpCAM (epithelial cell adhesion molecule), cytokeratins (CKs)] that are expressed on both normal and malignant epithelial cells (2). The specificity of this widely accepted approach is derived from the fact that blood cells usually lack detectable expression of epithelial markers because of their mesenchymal origin. It is unclear, however, whether trafficking of normal epithelial cells could occur in the blood circulation under certain circumstances, which might contribute to false-positive findings in current assays unless unambiguous criteria for the malignant nature of the marker–positive cells are used. Thus far, only 1 report has suggested the presence of circulating epithelial cells in some patients with benign adenomas (3 of 30 patients, 10%) or benign inflammatory diseases (4 of 34 patients, 12%) (3), suggesting that cells from nonmalignant colonic epithelium may also gain entry into the bloodstream in patients with benign bowel diseases. This reverse-transcription PCR–based study, however, included rather unspecific epithelial markers: mucins, which are frequently produced in blood cells (3), and CK20, which can also be detected in normal granulocytes (4).

In the present study, we enrolled 53 patients [20 (37.7%) women and 33 (62.3%) men, mean age, 56.7 years; range, 18–84 years] with benign colon diseases, including diverticulosis, benign polyps, Crohn disease,
ulcerative rectocolitis, colonic endometriosis, and others (Table 1) at the Gastrointestinal Surgery Department, Hospital Saint-Eloi, University Medical Centre, Montpellier, France. None of these patients had a history of solid cancer. Twenty-five healthy control individuals were tested in parallel. The bioethics committee approved the study protocol (biobank no. DC2008830), and all patients provided written informed consent. For the detection of epithelial cells in blood, we used 2 independent CTC assays, both of which are based on the use of CKs for detecting tumor cells. Currently, the CellSearch system is the only technology that has been cleared by the FDA for the detection of CTC in patients with metastatic breast, prostate, or colorectal cancer (5–10). This system is based on positive selection for CTCs with antibodies to EpCAM and the staining of EpCAM-positive cells for CKs with 4’,6-diamidino-2-phenylindole and for CD45. Interestingly, isolation and detection of CTCs with immunomagnetic-enrichment methods is critically dependent on the EpCAM clone used (11). More recently, we introduced a new functional test, the epithelial immunospot (EPISPOT) assay, which allows the detection of only viable tumor cells (12–15). Leukocytes in the blood sample are depleted with a cocktail of antibodies (not only anti-CD45, but also anti-CD4, anti-CD8, and anti-CD19), and the CD45− cells are cultured ex vivo. Of this cell subpopulation, only CK19-releasing cells are considered CTCs (16, 17). Even when the first 5 mL of peripheral blood were not discarded, blood analyses of healthy controls previously demonstrated the specificity of both assays (7, 17, 18); however, data for age-matched controls with benign diseases were lacking.

Remarkably, we found circulating epithelial cells in our patient cohort with both the CellSearch system and the CK19-EPISPOT assay, but we found no positive events in the group of healthy volunteers. The diagnosis of positively testing patients was based on the detection of positive events according to the strict criteria defined by both technologies and after comparisons with internal positive controls. The CellSearch system detected CK-positive cells in the blood of 6 (11.3%) of 53 patients, and the CK19-EPISPOT assay found CK19-releasing cells in 10 (18.9%) of 53 patients. Only 1 sample was positive with both CTC as-

### Table 1. Distribution of patients with benign colon diseases related to the presence of CTCs and counts of CTCs detected by the EPISPOT and CellSearch assays.

<table>
<thead>
<tr>
<th></th>
<th>EPISPOT assay</th>
<th>P</th>
<th>CellSearch assay</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC+ patient age, yearsa</td>
<td>52.5</td>
<td>0.37</td>
<td>65.8</td>
<td>0.13</td>
</tr>
<tr>
<td>CTC+ patients, n/total (%))b</td>
<td>10/53 (18.9)</td>
<td></td>
<td>6/53 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3/20 (15)</td>
<td>0.72</td>
<td>2/20 (10)</td>
<td>1.0</td>
</tr>
<tr>
<td>Male</td>
<td>7/33 (21.2)</td>
<td></td>
<td>4/33 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Disease type</td>
<td></td>
<td>0.86</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Diverticulosis</td>
<td>5/23 (21.7)</td>
<td></td>
<td>3/23 (13)</td>
<td></td>
</tr>
<tr>
<td>Benign polyps</td>
<td>1/12 (8.3)</td>
<td></td>
<td>1/12 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Crohn disease</td>
<td>2/7 (28.6)</td>
<td></td>
<td>1/7 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Ulcerative rectocolitis</td>
<td>1/5 (20)</td>
<td></td>
<td>0/5 (0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1/6 (18.7)</td>
<td></td>
<td>1/6 (18.7)</td>
<td></td>
</tr>
<tr>
<td>CTC countc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.4 (0–41), 0</td>
<td></td>
<td>1.1 (0–37), 0</td>
<td></td>
</tr>
<tr>
<td>Per disease</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diverticulosis</td>
<td>3.5 (0–41), 0</td>
<td></td>
<td>2 (0–37), 0</td>
<td></td>
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<tr>
<td>Benign polyps</td>
<td>0.5 (0–6), 0</td>
<td></td>
<td>0.2 (0–3), 0</td>
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<tr>
<td>Crohn disease</td>
<td>1 (0–6), 0</td>
<td></td>
<td>0.8 (0–5), 0</td>
<td></td>
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<tr>
<td>Ulcerative rectocolitis</td>
<td>6.4 (0–32), 0</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.4 (0–2), 0</td>
<td></td>
<td>0.4 (0–3), 0</td>
<td></td>
</tr>
</tbody>
</table>

a Data are presented as the mean.  
b Data are presented as the number of CTC+/patients/total number of patients in the group (percent).  
c Data are presented as the mean (range), median.
Fig. 1. Detection of circulating epithelial cells with the CellSearch system [Cohen et al. (8)] and the CK19-EPISPOT assay [Alix-Panabières et al. (17)] in patients with a benign colon disease.

CellSearch system: Blood samples were drawn into 10-mL CellSave tubes (Veridex/Johnson & Johnson) and processed within 96 h of collection, as recommended by the manufacturer and confirmed in our previous study [Riethdorf et al. (7)]. Circulating epithelial cells were enriched via detection of EpCAM expression and defined as EpCAM-isolated intact cells staining positively for CKs (CK8, CK18, CK19) and negatively for CD45 (a specific marker of hematopoietic cells). Representative examples of CK-positive cells detected with the CellSearch system are shown in this photo gallery (cases 1 and 2).

CK19-EPISPOT assay: Blood samples (10-20 mL) were drawn into EDTA-containing tubes, maintained at room temperature, and processed within 24 h of collection for detection of viable circulating epithelial cells. Functional circulating epithelial cells were first enriched by depleting hematopoietic CD45⁺ cells [Schwarzenbach et al. (15)]. These cells were CK19-releasing cells, as defined according to our previous work [Alix-Panabières et al. (17)]. One CK19-releasing cell (on the left) was detected in the blood of a patient with a diverticulosis (case 3), compared with the positive control (colon cancer cell line HT-29, on the right). DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate.
In conclusion, our analysis suggests that epithelial cells from nonmalignant colonic epithelium may enter the bloodstream under certain conditions, such as inflammation. This finding is consistent with the fact that inflammatory cytokines can stimulate the migration of epithelial cells (19). With respect to bowel diseases in particular, the potential background of nonmalignant epithelial cells in blood may be an important confounding factor in cancer patients with very low “CTC” counts and may lead to false-positive findings in CTC diagnostics unless strict morphologic criteria are applied. Unambiguous morphologic identification of each marker-positive cell in a given blood or bone marrow sample is difficult (20), however, and most cells detected in our study met the “tumor cell” criteria of the FDA-cleared CellSearch device.

We cannot exclude the possibility that some of the false-positive events detected in our study were actually tumor cells already present in some of the benign lesions or in the adjacent colon, even if such cells were not detected by endoscopic visual inspection and/or via routine histopathologic analysis of the resected samples. Because tumor cell dissemination appears to be an early event in tumor progression, CTCs may appear at very early stages of tumor development, and their detection may have potential for use in the early diagnosis of colon cancer. Large-scale epidemiologic studies with long-term follow-up are required to test this provocative hypothesis. Moreover, additional genetic characterization for mutations in oncogenes [e.g., KRAS7 (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog)] or tumor suppressor genes [e.g., TP53 (tumor protein p53)], and fluorescence in situ hybridization–based detection of numerical chromosomal aberrations at the single-cell level may add specificity to current CTC assays. Carcinoma cells are genetically heterogeneous, however, a fact that points to the need for complex technologies for multiplexing single CTCs. Moreover, hundreds of patients with benign disease need to be analyzed, because we assume that only a small fraction of CK-positive cells (if any at all) would be tumor cells, given that most of our patients with benign diseases have an excellent prognosis and will not develop cancer or metastasis. Thus, a large effort is required to prove the nature of circulating CK-positive cells in these patients, but the morphology of these cells suggests that most might not be CTC.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: Grant from the European Commission (DISMAL project, contract no. LSHC-CT-2005-018911); K. Pantel, European Research Council (ERC) Advanced Investigator Grant (no. 269081).
Expert Testimony: None declared.
Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: We are grateful to Nathalie Pätzzer and Delphine Gueroult in Montpellier, as well as to Cornelia Cohr and Oliver Mauermann in Hamburg, for their expert technical assistance.

7 Human genes: KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; TP53, tumor protein p53.
References


Previously published online at DOI: 10.1373/clinchem.2011.175570