Pancreatic cancer (pancreatic ductal adenocarcinoma [PDAC]) is a dismal disease with a 5-year survival of less than 5%.1 Even after curative resection, most patients develop metastatic disease within 18 months, which is a major cause of mortality.2-4 The last few years have seen a number of studies documenting circulating tumor cells (CTCs) in the peripheral blood from patients with most types of epithelial cancers.5 Circulating tumor cells may be released from the primary tumor and have the potential to spread to other sites and develop into micrometastatic and subsequently macrometastatic deposits.6 We and others have shown that the routine detection of CTCs can be used in the follow-up of patients with breast,7 pancreatic,8-11 hepatobiliary,12 and colorectal13 cancers at high risk for relapse. It is possible that handling the tumor at the time of surgery may cause a release of CTCs, hence contributing to future disease recurrence. No-touch isolation (NT) pancreaticoduodenectomy (PD) for pancreatic head resection has been described previously.14 Although it has been shown to be a safe method for PD, it is not widely accepted among pancreatic surgeons owing to its technical difficulties. Our aim was to assess the impact of 2 different surgical resection techniques, standard (ST) PD and NT-PD, on tumor cell spread and patient survival by using portal venous (PV) CTCs as biomarkers. To our knowledge, this is the first report of its kind.

Methods

This study was limited to patients who underwent PD for PDAC between September 6, 2010, and April 30, 2012. The main inclusion criteria were those patients undergoing potentially curative resection for PDAC who consented for intraoperative blood sampling. Patients were randomized into 2 groups: ST-PD and NT-PD. All operative procedures were performed by a single surgeon (L.R.J.). Our method for ST-PD has been previously detailed.2 In comparison, the concept of NT-PD is that the tumor must not be manipulated before the vascular and lymphatic drainage vessels are completely isolated; it was performed as previously described.14 This is further explained in the eAppendix in the Supplement. Following identification and exposure of the PV, 7.5 mL of blood was drawn directly using a 21-gauge butterfly needle before dissection and immediately after removal of the PD specimen. Blood samples were taken at each point in a CellSave (Veridex) preservative tube. The samples were then processed in our laboratory within 24 hours using the bead-based fluorescence CellSearch system (Veridex) according to the manufacturer's protocol. Samples were then scanned on the CellTracks Analyzer II fluorescent microscope (Veridex) for analysis. The cells were evaluated for CTCs independently by 2 operators (eAppendix in Supple-
ment and Figure 1). Statistical analyses were performed using IBM SPSS Statistics version 20 software (IBM Corp). Approval was obtained from a UK national research ethics committee and the Imperial College Healthcare NHS Trust. Written informed consent was obtained from the patients.

Results

During the 2-year period, 91 patients underwent PD, 30 of whom had a histological diagnosis of PDAC. Based on the availability of the CellSearch system to perform the assay, 12 patients with preoperative suspicion of PDAC were randomized into this study (ST-PD, n = 6; NT-PD, n = 6). All patients had stage II disease (potentially resectable) and none had received neoadjuvant chemotherapy. There were no differences between the 2 groups with regard to age, operative time, length of hospital stay, complication rate, metastatic to examined lymph node ratio, or tumor characteristics (Table). Only 1 patient (in the ST-PD group) required a perioperative blood transfusion (2 units). For pancreatic duct reconstruction, 5 patients in each group had a longitudinal ventral pancreaticojejunostomy anastomosis and 1 in each group had a pancreaticogastrostomy. Major complications included 1 biliary leak in the ST-PD group and 1 pancreatic leak (pancreaticogastrostomy anastomosis) in the NT-PD group. Both cases were treated conservatively. All tumors were confirmed histologically as PDAC and American Joint Committee on Cancer stage IIB (pT3N1M0). An R1 resection was defined as cancer cells within 1 mm of a circumferential or transactional margin. A negative resection margin (R0) was achieved for 6 patients (50%), with 3 in each group having R1 medial resection margins. Prior to resection of the pancreatic head, there was no difference in the number of CTCs between the 2 groups (range, 0–4 CTCs in the

Table. Clinicopathological Characteristics of Patients Undergoing Pancreatoduodenectomy for Pancreatic Cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ST-PD (n = 6)</th>
<th>NT-PD (n = 6)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean, y</td>
<td>66.3</td>
<td>65.6</td>
<td>.92</td>
</tr>
<tr>
<td>Operative time, mean (range), h</td>
<td>4.0 (3.0-6.0)</td>
<td>3.3 (2.5-4.5)</td>
<td>.27</td>
</tr>
<tr>
<td>Length of stay, mean (range), d</td>
<td>25.2 (15-62)</td>
<td>16.2 (10-37)</td>
<td>.32</td>
</tr>
<tr>
<td>Major complications, No.</td>
<td>1</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Pathological tumor stage</td>
<td>pT3</td>
<td>pT3</td>
<td>...</td>
</tr>
<tr>
<td>Tumor size, mean, mm</td>
<td>35</td>
<td>37</td>
<td>.81</td>
</tr>
<tr>
<td>Metastatic to examined lymph node ratio, mean</td>
<td>0.29</td>
<td>0.38</td>
<td>.41</td>
</tr>
<tr>
<td>R0 resection margins, No.</td>
<td>3</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td>Perineural invasion, No.</td>
<td>6</td>
<td>6</td>
<td>...</td>
</tr>
<tr>
<td>Lymphovascular invasion, No.</td>
<td>6</td>
<td>6</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: NT-PD, no-touch isolation pancreatoduodenectomy; ST-PD, standard pancreatoduodenectomy; ellipses, not applicable.

* Two-tailed t test.
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Comparison between groups was made by log-rank test (P = .33).

ST-PD group vs 1-6 CTCs in the NT-PD group; P = .31). Following resection, an increase in the number of CTCs was seen in 5 of 6 patients (83%) in the ST-PD group but 0 of 6 patients in the NT-PD group (P = .003) (eTable in Supplement). The median follow-up period for the entire cohort was 14.6 months (range, 10.5-27.9 months). At the end of follow-up, there were 7 deaths and 5 survivors. Median overall survival (OS) was 13.0 months (95% CI, 10.1-15.9) in the ST-PD group and 16.7 months (95% CI, 12.6-20.8) in the NT-PD group (P = .33) (Figure 2). Three patients in the ST-PD group and 2 patients in the NT-PD group developed disease recurrence in the liver (n = 2) and lung (n = 3). There was no difference in disease-free survival (DFS) between the 2 groups (P = .42). However, correlations with outcomes become unstable with small numbers of patients.

Discussion

To our knowledge, this is the first study to examine CTC numbers in the PV circulation during PD for PDAC. The NT-PD technique has been adopted as the standard for pancreatic head resection in our patients with PDAC. There was a significant increase in the number of CTCs following ST-PD but not following NT-PD. Although the NT-PD group trended toward better OS and DFS, there was no significant difference. The log-rank test performs poorly with small sample sizes, so this may be a type II error. The NT technique was first described as a way of preventing the spread of cancer cells in colorectal and eye cancer by limiting the handling of the tumors. In colorectal cancer, a tendency for a reduction in the number and time to liver metastases over 5 years has been seen using the NT technique compared with conventional resection. More recently, the NT technique has been shown to be safe for PD and distal pancreatectomy, with comparable operative morbidity and mortality. In a small pilot study, carcinoembryonic antigen messenger RNA (mRNA) was identified in PV blood samples from 5 of 10 patients (50%) undergoing ST-PD compared with only 1 of 8 patients (13%) having NT-PD. The latter also had reduced disease recurrence and a longer OS. This study provided the first evidence for the biological benefit of this technique, even though mRNA levels were not isolated from a specific cell type.

The detection of CTCs is extremely difficult, as they are rare and samples are often contaminated by leukocytes. However, the ability to detect, enumerate, and characterize CTCs is important for the study of the metastatic cascade and improved clinical management of patients with cancer. To date, most studies have used the CellSearch system, the only one with US Food and Drug Administration approval. In recent years, other reproducible methods of CTC detection and analysis have been developed. However, the CellSearch system has been shown to be the most reliable tool in clinical trials using CTCs as a prognostic marker. Limitations to this method and the functional significance of detected CTCs are further discussed in the eAppendix in the Supplement.

Peripheral CTCs have been detected using a low cutoff (>1 CTC/7.5 mL) in 11% of patients with locally advanced PDAC and 40% to 80.5% with metastatic disease, compared with none in healthy individuals and those with chronic pancreatitis. While we did not assess CTCs in the peripheral circulation, it appears that CTCs are easier to detect in the PV circulation, even in earlier-stage disease, as 11 of our 12 patients (92%) had 1 or more CTCs per 7.5 mL in the PV prior to PD. The clinical implications of detecting CTCs in PDAC are under investigation; however, reports are divided regarding the association between enumeration and survival outcomes. Indeed, Kurihara et al17 found that in stage III to IV disease, CTC-positive patients (11 of 26 patients) had worse OS compared with those who were CTC negative (15 of 26 patients). Similarly, Bidard et al31 showed that in patients with stage III disease, CTC positivity at any point (baseline or after 2 months of chemotherapy) was associated with shorter OS (hazard ratio = 2.5; 95% CI, 1.2-5.4), although CTC detection did not influence DFS. Conversely, Khoja et al34 and Negin et al25 failed to correlate CTC number with OS or DFS in metastatic PDAC. Plausible explanations for these findings include small sample sizes or low statistical power of many of these studies, different detection methods and markers used, and heterogeneous patient groups. However, most studies do show a trend toward an association between CTC positivity and poor survival; thus, their role in metastatic development is still debated.

Conclusions

Our pilot study, albeit in a small number of patients, showed that NT-PD is comparable to ST-PD with regard to perioperative morbidity and long-term outcome. In addition, we provide further evidence that CTCs can be detected in patients with PDAC in the PV circulation and that the count is increased following tumor manipulation. This may affect disease recurrence and survival, but larger studies are needed for a definitive answer with regard to outcome.
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ARTICLE INFORMATION
Accepted for Publication: July 8, 2013.
Published Online: March 5, 2014.

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Conflict of Interest Disclosures: None reported.

Funding/Support: This work was supported by the Alliance Family Foundation.

Role of the Sponsor: The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES