Effect of thoracic epidural anaesthesia on serum vascular endothelial growth factor C and cytokines in patients undergoing anaesthesia and surgery for colon cancer

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Background. Serum vascular endothelial growth factor-C (VEGF-C), transforming growth factor-β (TGF-β), and interleukin (IL)-6 promote angiogenesis and metastases in colon cancer. We hypothesized that patients who received propofol–epidural anaesthesia (PEA) would exhibit decreases in VEGF-C, TGF-β, and IL-6 and an increase in IL-10 compared with patients who received general anaesthesia (GA).

Methods. Colon cancer surgery patients were randomly assigned to the PEA (n=20) or GA (n=20) group. Serum VEGF-C, TGF-β, IL-6, and IL-10 levels before surgery and 24 h after surgery were measured.

Results. Patients who received PEA showed decreases in VEGF-C [526 (261) vs 834 (304) pg ml⁻¹, P=0.001], TGF-β (P=0.027), and IL-6 (P=0.007) and an increase in IL-10 (P=0.001) 24 h after surgery compared with patients subjected to GA. The visual analogue scale scores at rest and during coughing at 2 and 24 h after operation were significantly lower in PEA patients (P<0.05).

Conclusions. PEA reduces serum concentrations of factors associated with angiogenesis during colon cancer surgery.


Keywords: anaesthesia, general; anaesthetic techniques, epidural; cancer, colorectal; surgery, colorectal

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Colon cancer is one of the five most prevalent cancers in the adult population.¹ Metastasis and recurrence after cancer surgery are major factors that affect survival. The reported rate of recurrence for colorectal cancer is 8–25%.² Local metastasis or recurrence largely depends on the balance between immune surveillance and the tumour’s ability to spread.³ ⁴

Angiogenesis can facilitate the delivery of oxygen, nutrients, and growth factors to tumour cells.⁵ Tumour angiogenesis plays an essential role in the growth, invasion, and metastatic spread of solid neoplasms. A number of molecular factors can stimulate and maintain angiogenesis. Vascular endothelial growth factor-C (VEGF-C) is an important factor for the promotion of tumour angiogenesis.⁶ ⁷

Regional anaesthesia has been postulated to have an effect on cancer outcome. Thoracic epidural anaesthesia and analgesia have commonly been used for the management of intra- and postoperative pain during colon cancer surgery. Because epidural anaesthesia blocks the afferent neural input, intra- and postoperative neuroendocrine stress responses can be decreased.⁸ Thus, the use of epidural anaesthesia may protect patients from postoperative tumour metastasis or recurrence. Studies on the outcome of colon cancer have been both positive and negative.⁹–¹²

Propofol may attenuate cancer cell migration, proliferation, and metastasis in vitro.¹³ Propofol also has cyclooxygenase (COX)-2 inhibitory activity.¹⁴ As such, we hypothesized that patients who receive propofol–epidural anaesthesia (PEA) would exhibit decreases in angiogenic factors compared with those who receive general anaesthesia (GA) and sufentanil analgesia. A similar study evaluated propofol-paravertebral anaesthesia vs GA alone in breast cancer patients and previously found VEGF increases after GA.¹⁵

The primary endpoint was the change in perioperative VEGF-C concentration. Secondary endpoints included levels
of transforming growth factor-β (TGF-β), interleukin (IL)-6, and IL-10 and also postoperative visual analogue scale (VAS) pain scores.

**Methods**

This randomized trial (ChiCTR.org ID ChiCTR-TRC-13003146) was conducted after approval from the Cancer Hospital, Fudan University Institutional Human Ethics Committee (Shanghai, China). After obtaining written informed consent, 40 ASA I–III patients aged 21–81 yr who underwent open colon cancer surgery were included in the trial. Patients with general contraindications for epidural anaesthesia, recent history (8 weeks) of chemotherapy or radiation, or any contraindication to the administration of midazolam, sufentanil, propofol, or sevoflurane were excluded.

The patients were randomly assigned to receive PEA or GA according to a computer-generated random numbers table. In the PEA group, using the paramedian approach, an epidural catheter was inserted under sterile conditions through the T₉₋₁₂ interspace using the ‘loss-of-resistance’ technique. The catheter was advanced 4 cm cephalad. When the aspiration test results for blood and cerebrospinal fluid were negative, a test dose with lidocaine 1% (3 ml) was injected through the catheter. GA was induced by propofol plasma target-controlled infusion (TCI; a target plasma concentration of 3.5–4 μg ml⁻¹) using Marsh pharmacokinetic and Graseby 3500 TCI pump, and an i.v. midazolam 0.03 mg kg⁻¹, sufentanil 0.3 μg kg⁻¹, and cisatracurium 0.2 mg kg⁻¹. Anaesthesia was maintained with the TCI of propofol (a mean plasma concentration of 2.9 μg ml⁻¹). The loading dose of 0.375% ropivacaine was 6–8 ml, depending on the height and weight of the patient. Ropivacaine at 5 ml h⁻¹ was then infused using a microinfusion pump for the duration of surgery. The analgesic agent was composed of ropivacaine 0.15% and sufentanil 0.5 μg ml⁻¹. Patients received patient-controlled analgesia with a continuous infusion of 4 ml h⁻¹ and a 2 ml bolus on request with a 15 min lockout time. Analgesic regiments were supplied during 72 h.

The GA group had induction of balanced GA with midazolam 0.03 mg kg⁻¹, sufentanil 0.3 μg kg⁻¹, propofol 1–2 mg kg⁻¹, and cisatracurium 0.2 mg kg⁻¹. Anaesthesia was maintained with 1.0–1.5 minimum alveolar concentration sevoflurane. Intraoperative analgesia consisted of fentanyl 0.2–0.4 μg kg⁻¹ h⁻¹. Room temperature was adjusted to 22–25 °C. Oesophageal temperature was monitored and maintained above 36 °C throughout the operation. Patients in the GA group received patient-controlled i.v. analgesia with sufentanil (1 μg ml⁻¹, with a bolus 2 ml, lockout time of 15 min, and background infusion rate of 2.5 ml h⁻¹). The analgesia was maintained for 72 h. Pain intensity was assessed using a 10 cm VAS at rest and during coughing at 2, 24, and 48 h after operation.

Venous blood was withdrawn before the operation and 24 h after operation. Samples were centrifuged at 4000 g. Thereafter, the serum was stored at −20 °C for future measurement. Enzyme-linked immunosorbent assays were prepared for VEGF-C (Immuno-Biological Laboratories Co., Ltd, Japan) and TGF-β1 (DRG Instruments GmbH, Germany). Plasma levels of IL-6 and IL-10 were measured with commercially available quantitative sandwich enzyme-linked immunosorbent assay kits (Quantikine; R&D Systems, Minneapolis, MN, USA).

Previously published studies on VEGF suggested that its standard deviation (sd) in vivo is in the order of 200 pg ml⁻¹.¹⁶ ¹⁷ Fifteen patients would be required to detect a reduction of 1 standard deviation, 200 pg ml⁻¹, with an α-value of 0.05 and a power of 0.8. To compensate for potential dropouts, we enrolled 20 patients. The data were compared using an independent group t-test for parametric data and a Mann–Whitney U-test for non-parametric data. Differences in VAS scores were assessed using repeated-measures analysis of variance and corrected with a Tukey post hoc test. Categorical data were assessed using Fisher’s exact test. The data are presented as mean (sd). P<0.05 was considered statistically significant.

**Results**

All patients completed the study according to the protocol. All procedures were performed by the same team of anaesthetists and surgeons (Fig. 1).

The groups were similar with respect to the mean age, body weight, height, male/female ratio, functional status, anaemia, albumin, diabetes mellitus, β-blocker, COX-2 inhibitor, and statin therapy (Table 1). Intraoperative blood transfusion, blood loss, time of surgery, and tumour stage were similar in both groups (Table 2). The consumption of intraoperative sufentanil was significantly higher in the GA group than that in the PEA group [51 (5.5) vs 20 (3.2) μg, P<0.001] (Table 2).
At 24 h after surgery, VEGF-C levels were significantly higher in the GA group compared with the PEA group [834 (304) vs 526 (261) pg ml⁻¹, P = 0.001] (Fig. 2, Table 3). The GA group had a significantly higher TGF-β level than the PEA group during this time point [711 (174) vs 597 (135) pg ml⁻¹, P = 0.027] (Fig. 3, Table 3).

Furthermore, 24 h after the surgery, IL-6 levels were significantly higher in the GA group compared with the PEA group [33.60 (8.32) vs 26.75 (6.84) pg ml⁻¹, P = 0.007] (Fig. 4, Table 4); furthermore, IL-10 levels were lower in the GA group compared with those in the PEA group [17.2 (5.43) vs 24.3 (6.50) pg ml⁻¹, P = 0.001] (Fig. 5, Table 4).

The change in VAS scores of the PEA group differed significantly from that of the GA group (P < 0.05, Table 5). The VAS scores at 2 and 24 h after surgery were significantly lower in the PEA group (P < 0.05, Table 5).

**Discussion**

Our study found that serum levels of VEGF-C and TGF-β1 increased significantly in the GA group and remained unchanged in the PEA group. Meanwhile, patients who received propofol/epidural anaesthesia exhibited a decrease in IL-6 and an increase in IL-10, compared with those who received GA.

Previous studies have demonstrated that volatile agents and opioids enhance the ability of cancer cells to proliferate and invade. Volatile agents and opioids also impair the perioperative immune system and increase vascular permeability. Data support the use of i.v. propofol because of its anti-tumoural protective effects. By blocking the sympathetic outflow, epidural anaesthesia can attenuate the stress response and reduce the consumption of perioperative opioids. Thus, in our study, we used a PEA technique. All of our patients were given thoracic epidural analgesia. The thoracic epidural blocks the sympathetic nervous system and ameliorates immune suppression induced by surgical stress much better than does lumbar epidural anaesthesia.

The metastatically essential process of lymphovascular angiogenesis may be facilitated by surgery. Pro-angiogenic

**Table 1** Demographic and clinical data. Values are mean (range), mean (SD), or numbers. COX, cyclooxygenase

<table>
<thead>
<tr>
<th></th>
<th>General anaesthesia (n = 20)</th>
<th>Thoracic epidural (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [mean (range)]</td>
<td>59 (34 - 76)</td>
<td>55 (30 – 81)</td>
<td>0.22</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>12/8</td>
<td>13/7</td>
<td>0.74</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65 (8)</td>
<td>64 (12)</td>
<td>0.25</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 (6)</td>
<td>167 (8)</td>
<td>0.16</td>
</tr>
<tr>
<td>ASA I/II/III (n)</td>
<td>2/16/2</td>
<td>4/14/2</td>
<td>0.67</td>
</tr>
<tr>
<td>Functional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Independent/minimal assistance (n)</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Moderate assistance/nursing home (n)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Anaemia (n)</td>
<td>4</td>
<td>5</td>
<td>0.70</td>
</tr>
<tr>
<td>Albumin (g litre⁻¹)</td>
<td>41 (2.8)</td>
<td>40 (3.0)</td>
<td>0.79</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>5</td>
<td>4</td>
<td>0.70</td>
</tr>
<tr>
<td>β-Blockers therapy (n)</td>
<td>3</td>
<td>2</td>
<td>0.63</td>
</tr>
<tr>
<td>COX-2 inhibitor therapy (n)</td>
<td>2</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Statin therapy (n)</td>
<td>3</td>
<td>2</td>
<td>0.63</td>
</tr>
</tbody>
</table>

**Table 2** Intraoperative factors. Values are mean (SD) or numbers

<table>
<thead>
<tr>
<th></th>
<th>General anaesthesia (n = 20)</th>
<th>Thoracic epidural (n = 20)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Blood transfusion (n)</td>
<td>4</td>
<td>5</td>
<td>0.70</td>
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<tr>
<td>Blood loss (ml)</td>
<td>240 (104)</td>
<td>250 (115)</td>
<td>0.80</td>
</tr>
<tr>
<td>Time of surgery</td>
<td>118 (38)</td>
<td>102 (29)</td>
<td>0.78</td>
</tr>
<tr>
<td>Tumour stage</td>
<td></td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Sufentanil (μg)</td>
<td>51 (5.5)</td>
<td>20 (3.2)</td>
<td>&lt;0.001</td>
</tr>
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</table>
factors and anti-angiogenic factors regulate the process of angiogenesis. Pro-angiogenic factors include VEGF and TGF-β. VEGF-C belongs to the VEGF family, which includes VEGF-A, B, C, D, and E and three tyrosine kinase receptors, VEGFR-1, VEGFR-2, and VEGFR-3. VEGF-C is a specific factor that promotes lymphangiogenesis. The proliferation and migration of lymphatic endothelial cells can be specifically promoted by VEGF-C. VEGF-C increases lymphatic permeability and plays an important role in tumour growth, spread, and metastasis. In colorectal cancer, VEGF-C expression has been found to correlate with lymphatic invasion and lymph node metastasis. Studies have reported that an elevated level of plasma VEGF-C is associated with deeper invasion, and more severe venous and lymphatic invasions in colorectal cancer. Therefore, if the anaesthetic technique can decrease VEGF-C concentrations, it may also reduce metastatic risk in colorectal cancer by reducing tumour angiogenesis. The mechanism by which the anaesthetic technique affects VEGF concentrations is described in the following tables and figures.
is unknown. VEGF may be secreted from host cells in the body. These host cells include platelets, muscle cells, and tumour-associated stromal cells. Thus, the use of thoracic epidural anaesthesia and propofol may alter immunological or surgical stress responses and render the serum milieu less conducive to angiogenic factor production.15

TGF-β, a 25 kDa polypeptide, can act as a potent angiogenic factor. This growth factor regulates cell growth and differentiation in both normal and transformed cells. TGF-β has dual functions as a tumour promotor and as a tumour suppressor.24 Recent studies suggest that the role of TGF-β might change from a tumour suppressor to a tumour promoter in colorectal cancer. TGF-β is closely related to the invasion and metastasis of colorectal cancer, and it may be used as a possible biomarker. TGF-β serves as a tumour promoter, which promotes colorectal cancer cell growth in vitro and in vivo.25 26 Our study found increased TGF-β levels after GA, and the reduced concentrations after PEA could be consistent with the hypothesis that the PEA anaesthetic technique might reduce the risk of metastasis in colon cancer.

Multiple cytokines can modulate the immune system. Cancer progression may be paradoxically hindered or assisted by cytokines. Pro-inflammatory cytokines may favour tumour progression. Cytokines play an important role in immune function, inflammation, and general tissue homeostasis. The inflammatory milieu provides a continuous supply of cytokines, chemokines, enzymes, and growth factors, which lead to immunosupression, tissue remodelling, and loss of tissue architecture. The inflammatory milieu also provides an environment that supports tumour progression and metastasis.27–29

IL-6, a multipotent cytokine, is produced by many cell types. IL-6 acts on a wide range of tissues and cell lines. IL-6 can induce cell growth and differentiation, production and expression of other cytokines, and acute-phase protein synthesis. IL-6 also promotes growth arrest and angiogenesis through the induction of VEGF expression. Another mechanism linking angiogenesis to malignancy is the IL-6 pathway.7 30 In the present study, lower serum concentrations of protumourigenic IL-6 were detected in patients who received PEA 24 h after surgery. As such, epidural anaesthesia and analgesia might protect the body’s immune function. This may help to explain why epidural anaesthesia is associated with decreased recurrence rates and prolonged survival in cancer patients.

Our study showed that IL-10 levels increased 24 h after surgery in the PEA group compared with that in the GA group. The effects of IL-10 on tumour growth are complex and incompletely understood. The release of proinflammatory cytokines, including IL-1, IL-6, IL-8, and TNF-α, is notably inhibited by IL-10. IL-10 exhibits anti-tumour and anti-metastatic activity by enhancing NK cell lysis of tumour cells.31–33

So far, the potential beneficial effect of regional anaesthesia to the improvement of long-term outcome after cancer surgery has been attributed mainly to the inhibition of the neuroendocrine stress response to surgery and to the reduction in the requirements of volatile anaesthetics and opioids.34 Another interesting potential mechanism, based on the results of our study, is the possibility that systemic absorption of local anaesthetics may influence cytokine responses. Local anaesthetics have been associated with cytotoxic effects on T-lymphoma cells in vitro. Apoptosis was observed at lower concentrations, while necrosis was seen at higher concentrations. Eight local anaesthetics were studied in total and each exhibited varying cytotoxic effects, which appeared to correlate with their lipophilicity and potency.35 Studies demonstrated in vitro that amide local anaesthetics can block tumour necrosis factor (TNF)-α-induced Src activation and intercellular adhesion molecule-1 (ICAM-1) phosphorylation.36 Local anaesthetics also attenuate tumour cell migration and signalling pathways (B-cells inhibitor (IκB)-nuclear factor-κB-ICAM-1) that enhance tumour growth and metastasis.37 This has provided a molecular mechanism by which regional anaesthesia might inhibit or reduce cancer metastases. However, these are in vitro results, and it is still unknown whether they exist in vivo.

There are several limitations of the current study. First, we did not measure plasma levels of ICAM-1 and TNF-α. Because ICAM-1 is associated with a variety of cancer types and has a role in cancer metastases, it can also be used as a biomarker for tumour prognosis and a target for therapeutic interventions.38 TNF-α can increase the expression of ICAM-1.36 We intend to study the effect of epidural anaesthesia on serum ICAM-1 and TNF-α levels in the future. Secondly, we did not measure plasma levels of ropivacaine. We were unable to determine whether the change in VEGF-C was caused by ropivacaine use, its plasma concentration, or sympathetic block. Thirdly, our study was limited to data collected 1 day after surgery. Perioperative immune suppressive phenomena may be observed 7–30 days or more after surgery.39 40 We wish to address this limitation in our future research work. Thus, whether epidural anaesthesia combined with GA might change long-term outcomes in patients undergoing colon cancer surgery cannot be clearly concluded from the current study. Thus, our findings should be interpreted with caution. Several reviews have reported that a number of perioperative factors may influence metastatic progression or resistance.3 41–43 These factors include β-blocker, COX-2, and statin therapy. Our findings could theoretically be affected by these factors. However, our study was a randomized controlled trial. Preoperative medications (β-blocker, COX-2 inhibitor therapy, and statin therapy) were similar in both groups. For 24 h after surgery, none of our patients received β-blocker, COX-2, or statin therapy. Thus, the only difference between the groups was the received anaesthetic technique.

In conclusion, this randomized controlled study in colon cancer patients demonstrates that the combination of thoracic epidural anaesthesia and propofol causes a reduction in serum VEGF-C, TGF-β1, and IL-6 levels, and an increase in IL-10 compared with GA. The result is consistent with the hypothesis that the anaesthetic technique may influence colon cancer outcome. Additional large-scale prospective trials involving colon cancer recurrence and metastasis as the endpoints are required to determine the significance of these observations.

Thoracic epidural anaesthesia and colon cancer angiogenesis

BJA
Authors’ contributions

Y.J.X.: study design, data analysis, and writing up of the first draft of the paper; W.K.C.: study design, patient recruitment, data collection, and data analysis; Y.Z.: patient recruitment and data collection; C.H.M.: study design, data analysis, and writing up of the first draft of the paper.

Declaration of interest

None declared.

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References

5 Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990; 82: 4–6
7 Kwon KA, Kim SH, Oh SY, et al. Clinical significance of preoperative serum vascular endothelial growth factor, interleukin-6, and C-reactive protein level in colorectal cancer. BMCCancer 2010; 10: 203
12 Cummings KC, Xu F, Cummings LC, Cooper GS. A comparison of epidural analgesia and traditional pain management effects on survival and cancer recurrence after colectomy. Anesthesiology 2012; 116: 797–806
16 Shaheen RM, Davies DW, Liu W. Antiangiogenic therapy targeting the tyrosine kinase receptor for VEGF receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. Cancer Res 1999; 59: 5412–6


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