Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma

T Kantola1,7, K Klintrup2,7, JP Väyrynen1, J Vornanen1, R Bloigu3, T Karhu4, K-H Herzig4,5, J Näpäkangas4, J Mäkela2, Tj Kärttunen1,7, A Tuomisto1 and Mj Mäkinen*,1,6

1Department of Pathology, University of Oulu, POB 5000, Oulu FI-90014, Finland; 2Department of Surgery, Oulu University Hospital, Oulu, Finland; 3Medical Informatics Group, Faculty of Medicine, University of Oulu, Oulu, Finland; 4Institute of Biomedicine and Biocenter of Oulu, University of Oulu, Oulu, Finland; 5Department of Psychiatry, Kuopio University Hospital, Kuopio, Finland; 6Department of Pathology, Oulu University Hospital, Oulu, Finland

BACKGROUND: Inflammation contributes to the pathogenesis of colorectal cancer (CRC), and cytokine levels are altered during colorectal carcinogenesis.

METHODS: The serum levels of 13 cytokines and their relation to clinical and pathological parameters, and systemic inflammatory response (mGPS, CRP and neutrophil–lymphocyte ratio), were analysed from a prospective series of 148 CRC patients and 86 healthy age- and sex-matched controls.

RESULTS: CRC patients had higher serum platelet-derived growth factor, interleukin (IL)-6, IL-7, and IL-8 levels and lower monocyte chemotactic protein-1 (MCP-1) levels than the controls. A logistic regression model for discriminating the patients from the controls — including the five most predictive cytokines (high IL-8, high IL-6, low MCP-1, low IL-1ra, and low IP-10) — yielded an area under curve value of 0.890 in receiver operating characteristics analysis. Serum cytokines showed distinct correlation with other markers of systemic inflammatory response, and advanced CRCs were associated with higher levels of IL-8, IL-1ra, and IL-6. A metastasised disease was accompanied by an orientation towards Th2 cytokine milieu.

CONCLUSION: CRC is associated with extensive alterations in serum cytokine environment, highlighting the importance of studying relative cytokine level alterations. Serum cytokine profile shows promise in separating CRC patients from healthy controls but its clinical value is yet to be confirmed.

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Inflammation has a significant influence in the development of colorectal cancer (CRC) (Pages et al, 2010; Terzic et al, 2010). Patients with chronic inflammatory bowel disease, such as ulcerative colitis and Crohn’s disease, have an increased risk of developing CRC (Eaden et al, 2001; von Roosen et al, 2007). Preventive effect of NSAIDs on the development of CRC has been shown in epidemiological studies (Rothwell et al, 2010) and also in a randomised placebo-controlled trial in HNPCC (Burn et al, 2011). Immune cells also contribute to the immunosurveillance, and high-grade peri- and intratumoral inflammatory cell infiltration is an independent indicator of an improved survival in CRC (Klintrup et al, 2005; Galon et al, 2006; Roxburgh et al, 2009). Moreover, systemic inflammatory markers like modified Glasgow prognostic score (mGPS) and blood neutrophil/lymphocyte ratio have established prognostic value in CRC (Roxburgh and Glasgow, 2009). Moreover, systemic inflammatory markers have focused on the levels of inflammatory markers has been that they have focused on the levels of one or few cytokines at a time. However, relative alterations in cytokine levels can have substantial effects in the immune system, and changes in the cytokine environment can modulate tumour growth and microenvironment by mediating interactions between cancer cells and infiltrating inflammatory cells. On the basis of their function, cytokines can be grouped into anti- or proinflammatory cytokines, chemokines, and growth factors. Moreover, the classification into Th1 and Th2 cytokines is of importance. In general, Th1-type cytokines, e.g., interleukin (IL)-12, IL-15, and interferon gamma (IFN-γ), contribute to cellular immune reactions considered essential for an effective response against tumour cells, whereas Th2-type cytokines, e.g., IL-4, IL-5, IL-10, and IL-13, may suppress the tumour-specific immune response (Ellyard et al, 2007; Cui and Florholmen, 2008).

The development of CRC is accompanied by alterations in cytokine production, which is thought to polarise from Th1 to Th2 along the colorectal adenoma–carcinoma sequence (Cui and Florholmen, 2008). Increased serum cytokine concentrations, e.g., elevated levels of IL-8 (Ueda et al, 1994), IL-6 (Knupfer and Preiss, 2010), and platelet-derived growth factor (PDGF) (Belizon et al, 2009) have been reported in CRC patients compared with healthy individuals, and certain cytokines and chemokines, such as IL-6 and VEGF, are considered to have prognostic value (Chung and Chang, 2003; De Vita et al, 2004; Knupfer and Preiss, 2010). One of the limitations of the previous studies examining serum inflammatory markers has been that they have focused on the levels of one or few cytokines at a time. However, relative alterations in cytokine levels can have substantial effects in the immune system.
functions (Commins et al, 2010). Therefore, it is likely that an analysis of extensive set of cytokines would provide more accurate information on the tumour-related immunological responses, thus also bringing out the importance of individual cytokines on the immune response against CRC.

Tumour stage (TNM classification) is the most important prognostic factor in CRC (Puppa et al, 2010), and 5-year survival rate varies from 90% in stage I tumours to <10% in advanced stage IV tumours (O’Connell et al, 2004). To reduce mortality, it is essential to develop diagnostic tools for the early detection of cancer, which could be easily adapted to clinical use. At the moment, stool tests are neither very sensitive nor specific, and colonoscopy is a time-consuming and costly screening method (Sturgeon et al, 2008). Several serum markers have been assessed in the diagnostics of the disease, but none have been adapted to routine use so far (Sturgeon et al, 2008).

The aim of this study was to evaluate the pattern of alterations in the serum cytokine levels in CRC patients compared with controls. Accordingly, we analysed serum levels of 27 cytokines and chemokines from a series of 148 newly diagnosed CRC patients, and healthy controls matched for age and gender. An additional goal was to see whether the patterns of the serum cytokines in CRC could provide information of tumour stage.

MATERIALS AND METHODS

Patients and controls

This prospective study was introduced to all newly diagnosed CRC patients operated in Oulu University Hospital between April 2006 and January 2010 (n = 344), of which a total of 148 patients were both eligible for the study and had signed informed consent to participate. Control serum samples were obtained from age- and sex-matched healthy voluntary blood donors (Finnish Red Cross, Oulu, Finland; n = 36, age <65 years) and cataract surgery patients (Oulu University Hospital; n = 50, age ≥65 years). Patients and controls with earlier or simultaneously diagnosed other malignant diseases were excluded. Owing to the strict regulations in blood donation, other exclusion criteria for blood donor controls included, e.g., the absence of acute infections, trauma or operation during the preceding 4 months, chronic diseases like coronary artery disease, stroke or cancer, and organ transplantation. Study design was accepted by the Ethical Committee of Oulu University Hospital (58/2005, 184/2009).

Clinical details of patients and controls were acquired from the clinical records and by a questionnaire. Data on recurrences by the 24-month follow-up visit was collected in August 2012. Preoperative staging of CRC was done by whole-body CT-scan and the local staging of rectal cancer was done by MRI. Patients with T3 or T4 rectal tumours received preoperative radiotherapy or chemoradiotherapy (RT/CRT, n = 32). To avoid confounding and to model the situation before the diagnosis of the disease, only those CRC patients who did not receive preoperative RT/CRT (n = 116) were included in the analyses.

For the histopathological analysis, samples from the surgical specimens were fixed in 10% buffered formalin solution, embedded in paraffin, and 5-μm sections were stained with haematoxylin and eosin. TNM 6 (Sobin and Wittekind, 2002) was utilised in calculating the concentrations. BioPlex Manager Software 4.1 (Bio-Rad) was utilised in calculating the concentrations.

Out of the 27 analytes, 13 cytokines (IL-1ra, IL-4, IL-6, IL-7, IL-8, IL-9, IL-12, IFN-γ, IP-10, MCP-1, MIP-1β, eotaxin, and PDGF-BB) with three (1.5%) or fewer values outside the assay working range were included in this study. The other 14 cytokines (IL-1β, IL-2, IL-5, IL-10, IL-13, IL-15, IL-17, fibroblast growth factor basic, PDGF, subtype BB (PDGF-BB), and vascular endothelial growth factor A. The assay conditions were controlled, standardized and pre-optimized to ensure optimal repeatability and reproducibility of the assays according to the manufacturer’s instructions. The assay kits were all from the same lot, which allows better control of inter-assay variability. For the analyses, the samples were diluted in the appropriate sample matrix 1:2. The beads were incubated overnight with the samples. A minimum of 50 events (beads) was normally collected for each analyte, and the concentrations calculated from the standard curves on the basis of median fluorescence intensities. BioPlex Manager Software 4.1 (Bio-Rad) was utilised in calculating the concentrations.

Statistical analyses

Normally distributed continuous variables are presented as mean (s.d.), whereas other continuous variables are presented as median (interquartile range). Statistical significances of the differences in serum cytokine levels between the different study groups and age, sex, stage, grade, inflammatory reaction, and BMI categories were analysed by Mann–Whitney U-test or Kruskal–Wallis test. Univariate correlations are presented as Pearson’s correlation coefficients after a logarithmic transformation of the variables with positive skewness. A logistic regression model was generated to evaluate the potential of serum cytokine profile in discriminating the CRC patients from the controls. For the model, the cytokine levels were logarithmically transformed to improve the goodness of fit. Backward stepwise approach was used to restrict the model to the five most predictive cytokines. The predictive power of the final model was evaluated by receiver operating characteristics analysis, and the goodness of fit of the model was tested with the Hosmer–Lemeshow statistic, where a nonsignificant probability value indicates good fit. The logistic regression model for the presence of nodal metastases was also constructed using the backward stepwise method. Multiple linear regression was used in stage-adjusted assessment of the associations between serum cytokines and patient age. The statistical analyses were carried out using the SPSS 19.0 software (SPSS, Chicago, IL, USA).
out using statistical analysis software PASW Statistics 18 (IBM, Chicago, IL, USA). In all the tests, a two-tailed, exact $P$ value < 0.05 was considered statistically significant.

**RESULTS**

**Univariate analyses**

The characteristics of 116 CRC patients and 86 healthy controls are shown in Table 1, and their preoperative serum cytokine levels are summarised in Table 2. The levels of IL-6, IL-7, IL-8, and PDGF-BB were increased, and the levels of MCP-1 were decreased in patients compared with controls. Also Th1 cytokines IL-12 and IFN-γ showed a tendency towards increased values in CRC.

**Age and gender**

For seven of the thirteen cytokines (PDGF-BB, IL-1ra, IL-7, IL-8, IL-9, IFN-γ, and MIP-1β), the serum levels were higher in patients younger than 65 years compared with older patients (Supplementary Table 2). Only IP-10 levels were higher in patients aged 65 or older. The associations were mainly similar when age was studied as a continuous variable and correlation coefficients were calculated (Supplementary Table 3). Serum levels of IL-6, IL-7, IL-8, and PDGF-BB shown in Table 1, and their preoperative serum cytokine levels are summarised in Table 2. The levels of IL-6, IL-7, IL-8, and PDGF-BB were increased, and the levels of MCP-1 were decreased in patients compared with controls. Also Th1 cytokines IL-12 and IFN-γ showed a tendency towards increased values in CRC.

**Clinicopathological parameters**

The characteristics of 116 CRC patients and 86 healthy controls are summarised in Table 2. The levels of IL-6, IL-7, IL-8, and PDGF-BB were increased, and the levels of MCP-1 were decreased in patients compared with controls. Also Th1 cytokines IL-12 and IFN-γ showed a tendency towards increased values in CRC.

| Cytokine | CRC patients (n = 116) | Healthy controls (n = 86) | P-value |
|----------|------------------------|--------------------------|---------|
| IL-1ra   | 6.38 (3.79–9.82)       | 6.27 (4.38–7.59)         | 0.296   |
| IL-4     | 0.88 (0.75–1.13)       | 0.83 (0.72–0.99)         | 0.127   |
| IL-6     | 4.89 (3.44–8.92)       | 3.57 (2.62–4.54)         | 0.139   |
| IL-7     | 5.53 (3.99–7.46)       | 4.71 (3.43–5.61)         | 0.002   |
| IL-8     | 12.3 (9.13–58.1)       | 8.30 (6.94–10.5)         | 0.047   |
| IL-9     | 9.18 (5.57–13.9)       | 7.39 (6.06–12.0)         | 0.221   |
| IL-10    | 30.1 (15.7–40.9)       | 22.9 (14.3–34.6)         | 0.068   |
| IFN-γ    | 31.7 (24.1–43.4)       | 28.7 (23.1–34.4)         | 0.068   |
| Eotaxin  | 132.6 (91.0–181.4)     | 136.5 (108.8–211.3)      | 0.081   |
| MCP-1    | 918.5 (670.2–1212.3)   | 885.8 (694.0–1352.7)     | 0.869   |
| MIP-1β   | 16.6 (10.8–23.7)       | 22.3 (15.2–32.2)         | 0.002   |
| PDGF-BB  | 650.4 (502.8–819.7)    | 696.0 (558.8–871.1)      | 0.118   |

Abbreviations: BMI = body mass index; CRC = colorectal cancer; RT/CRT = radiotherapy or chemoradiotherapy; s.d. = standard deviation.

**Multivariate analyses**

We generated a logistic regression model to evaluate the potential of serum cytokine profile in discriminating the CRC patients from the controls and to assess the mutual relationships of the differences observed in the univariate analyses. Certain cytokine values from three subjects were unreadable, and the model was based on 115 of 116 CRC patients and 84 of 86 controls.

The final model is presented in Table 5. It consists of the five most predictive cytokines chosen using the backward stepwise method. As in the univariate analyses, high IL-8 and IL-6, as well as low MCP-1, were associated with CRC. Interestingly, also low IL-1ra and IP-10 were associated with CRC, although these cytokines showed no difference between CRC patients and healthy controls in the univariate analyses. Hosmer-Lemeshow test for

**Table 1** Characteristics of the patients and controls

| Clinicopathological parameters | CRC patients (n = 116) | Healthy controls (n = 86) |
|-------------------------------|------------------------|--------------------------|
| Age, mean (s.d.)              | 67.9 (11.2)            | 67.3 (10.6)              |
| BMI, mean (s.d.)              | 26.6 (4.5)             | 27.4 (3.6)               |
| White blood cell count, mean (s.d.) | 6.9 (2.1)             | 6.9 (1.3)               |
| Gender                        |                        |                          |
| Male                          | 58 (50%)               | 45 (52.3%)               |
| Female                        | 58 (50%)               | 41 (47.7%)               |
| Preoperative RT/CRT           |                        |                          |
| Yes                           | 0 (0%)                 |                          |
| No                            | 116 (100%)             |                          |
| Tumour location               |                        |                          |
| Proximal colon                | 49 (42.2%)             | 49 (55.3%)               |
| Distal colon                  | 27 (23.3%)             | 20 (23.3%)               |
| Rectum                        | 40 (34.5%)             | 17 (19.5%)               |
| WHO grade                     |                        |                          |
| Grade 1                       | 16 (13.8%)             | 16 (13.8%)               |
| Grade 2                       | 86 (74.1%)             | 86 (74.1%)               |
| Grade 3                       | 14 (12.1%)             | 14 (12.1%)               |
| TNM stage (n = 115)           |                        |                          |
| Stage I                       | 19 (16.5%)             | 19 (16.5%)               |
| Stage II                      | 46 (40.0%)             | 46 (40.0%)               |
| Stage III                     | 32 (27.8%)             | 32 (27.8%)               |
| Stage IV                      | 18 (15.7%)             | 18 (15.7%)               |

**Table 2** Serum cytokine profile in colorectal cancer patients compared with healthy controls

Abbreviations: CRC = colorectal cancer; IFN-γ = interferon gamma; IL = interleukin; IP-10 = IFN-γ-induced protein 10 kDa; IQR = interquartile range; MCP-1 = monococyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; PDGF-BB = platelet-derived growth factor, subtype BB. P-values are for Mann–Whitney U-test.
goodness-of-fit of the model indicated a good calibration ($\chi^2 = 11.1, \text{probability value} = 0.195$). A receiver operating characteristics analysis for the model (Figure 1) yielded an area under curve of 0.890 (95% confidence interval, CI 0.845–0.934), which denotes an excellent discriminatory capability. Receiver operating characteristics curves were also obtained separately for all the cytokines included in the regression model, and the area under curves were the following: IL-1ra, 0.541 (95% CI 0.461–0.622); IL-6, 0.724 (95% CI 0.654–0.793); IL-8, 0.769 (95% CI 0.703–0.834); IP-10, 0.510 (95% CI 0.429–0.592); and MCP-1, 0.634 (95% CI 0.557–0.711).

Next, we generated a logistic regression model for discriminating patients with nodal metastases from patients without nodal metastases. Using the backward stepwise method, the model was restricted to the four most predictive cytokines because of a smaller number of positive cases than in the model used to separate patients from controls. In this model, low IFN-$\gamma$ (OR 0.09, 95% CI 0.005–1.65, $P = 0.105$) and IP-10 (OR 0.11, 95% CI 0.015–0.85, $P = 0.034$) and high IL-8 (OR 9.4, 95% CI 1.5–58.3, $P = 0.016$) and IL-1ra (OR 8.3, 95% CI 0.8–84.5, $P = 0.074$) were associated with lymph-node metastases. A small amount of M1 cases ($n = 18$) did not enable a construction of a sensible logistic regression model for the presence of distant metastases.

Regression models were also used to adjust the differences observed in univariate analyses for potential confounding factors. Several potential confounding factors were tested, and in our material, adjusting for BMI, sex, and the age of the patients did not influence the results of the univariate analyses (data not shown). Tumour grade correlated with stage, but small numbers of grade 3 ($n = 14$) tumours did not allow us to construct a regression model to confirm confounding by that. Most of the negative correlations between serum cytokine levels and patient age were found to be a
In univariate analyses, CRC patients had increased serum levels of PDGF-BB, IL-6, IL-7, and IL-8, and decreased levels of MCP-1. The increases in serum PDGF-BB, IL-6, and IL-8 levels in CRC have been reported earlier (Ueda et al, 1994; Belizon et al, 2009; Knupfer and Preiss, 2010). Increase of IL-7 levels and decrease of MCP-1 levels in CRC represent novel findings of this study. Previously, IL-7 levels have been reported to be increased in early-stage prostate cancer as compared with benign prostate hyperplasia (Mengus et al, 2011), and decreased MCP-1 levels have been described in gastric cancer (Tonouchi et al, 2002). Of these five cytokines, PDGF-BB has a prominent role in cell proliferation, cell migration, and angiogenesis (Hellberg et al, 2010). IL-6 is a pleiotropic cytokine with a variety of functions, including interaction between acute and chronic inflammation, inducing B-lymphocyte responses, and acute-phase functions like pyrogenic effect (Naugler and Karin, 2008), whereas IL-7 is considered to be an important haematopoietic cytokine having a role in the generation of both T and B lymphocytes (Mackall et al, 2011). The primary function of IL-8 is to recruit neutrophils during acute inflammation, while MCP-1 is chemotactic for monocytes (Wang et al, 2009).

The knowledge of the cancer-specific functions of cytokines and chemokines is still limited. In CRC, IL-6 has been reported to increase the proliferation and invasiveness of cancer cells and promote tumour angiogenesis (Knupfer and Preiss, 2010). Also IL-8 has been suggested to have a multifunctional role in CRC progression, which possibly involves enhancing the survival of cancer cells and regulating adhesion and invasion, in addition to recruiting neutrophils (Rubie et al, 2007; Waugh and Wilson, 2008). These suggested functions correspond to our observation of both IL-6 and IL-8 having an association with a metastasised disease. Higher levels of IL-7 in CRC seen in this study is an interesting finding, as IL-7 enhances the responses to immunisation, especially to weak or low-affinity antigens common for cancer (Melchionda et al, 2005; Capitini et al, 2009). Monocyte chemottractant MCP-1 has been shown to be overexpressed in several solid tumours, and it is thought to be responsible for acquiring tumour-associated macrophages, which in most tumours present with an anti-inflammatory phenotype (Mantovani et al, 2008).

The role of tumour-associated macrophages in CRC is controversial. Most studies have indicated that peritumoral macrophages have a favourable effect on CRC prognosis, suggesting their polarisation towards M1 phenotype (Klintrup et al, 2005; Erreni et al, 2011). On the contrary, intratumoral macrophages in CRC have been shown to be correlated with invasion and lymph-node metastases, and in general with more aggressive behaviour, which allows a suggestion that intratumoral macrophages in CRC are polarised towards M2 phenotype (Pancione et al, 2009; Kang et al, 2010). In colon, increased MCP-1 expression has been observed in colorectal adenomas in relation to normal epithelium, and it has also been related to an increased tumour-associated macrophage accumulation within tumour tissue and to an advancing stage (Tanaka et al, 2006; Bailey et al, 2007). It should be noted, however, that the increase of MCP-1 in colorectal adenoma and CRC tissue has been observed in non-neoplastic mucosa of CRC or adenoma patients, not to the normal mucosa obtained from healthy controls. In our study, serum MCP-1 concentrations are increased by advancing stage, yet in all CRC patients, we detected lower mean serum concentrations of MCP-1 than in controls. In an earlier report examining serum MCP-1 levels, 14% of CRC patients had lower MCP-1 value than the minimum value of any of the healthy volunteers, although no statistically significant difference between these two study groups was observed (Tonouchi et al, 2004).

According to our results, the absolute levels of cytokines generally increase in CRC, but there are also alterations in the relative cytokine levels indicating a shift in the balance of the immune system. Our logistic regression model revealed a relative DISCUSSION

Cytokines and chemokines form a complex network of regulatory proteins, and often several cytokines are required to synergize to bring about an optimal effect (Commins et al, 2010). To our knowledge, this is so far the most extensive study on serum cytokine and chemokine alterations in CRC. The method used in this study offers an opportunity to study relative changes of cytokine levels, allowing analysing the shifts in the immune balance occurring in patients with CRC. In this study, we generated a logistic regression model that showed an excellent ability to discriminate patients from the controls, and yielded information that may prove useful for the development of clinical applications for screening, diagnosis, staging, and even for the follow-up of CRC.

Recurrence analysis

Owing to the short follow-up at the moment, we were not able to perform a complete 60-month survival analysis. Instead, we carried out a 24-month recurrence analysis, which included 79 (68.1%) of 116 patients. Of the 116 patients, 22 (19.0%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available. Recurrences were observed in 17 patients (12.9%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available. Recurrences were observed in 17 patients (12.9%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available. Recurrences were observed in 17 patients (12.9%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available. Recurrences were observed in 17 patients (12.9%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available. Recurrences were observed in 17 patients (12.9%) were excluded because they underwent a palliative operation, and 15 (12.9%) were excluded because they did not have the 24-month follow-up data available.

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According to our results, the absolute levels of cytokines generally increase in CRC, but there are also alterations in the relative cytokine levels indicating a shift in the balance of the immune system. Our logistic regression model revealed a relative
decrease in the levels of serum IL-1ra and IP-10 in CRC, besides the increases in serum IL-6 and IL-8 and the decrease in serum MCP-1, which were also seen in univariate analyses. In this model, decreased IL-1ra/IL-6 ratio in CRC might indicate a decreased systemic anti-inflammatory response against the tumour. Similarly, increased levels of neutrophil chemokine IL-8 relative to monocyte chemokine receptors IP-10 and MCP-1 might depict an orientation towards a granulocyte response, although mono-nuclear cells are considered to be the main contributors to restricting cancer growth (Klintrup et al, 2005). However, the actions of most cytokines and chemokines are pleiotropic (Commans et al, 2010), and, e.g., IP-10, a chemokine secreted by several cell types in response to IFN-γ, is antiangiogenic besides possessing a leukocyte-directed chemotactic function (Luster and Leder, 1993; Strieter et al, 1995).

Our findings corroborate earlier observations that serum cytokine levels in CRC have only minor association with age and gender (Kaminska et al, 2005; Sharma et al, 2010). In our study, the levels of PDGF-BB, IL-1ra, IL-7, IL-8, IL-9, IFN-γ, and MIP-1β were slightly higher and IP-10 levels were lower in younger patients in univariate analysis, but younger patients more often presented with a metastatic disease. After adjusting for disease stage, the only significant correlations between serum cytokines and patient age were lower IP-10 and higher PDGF levels in the younger patients. The only difference we observed between males and females was higher eotaxin levels in the male patients. Biology of this difference is unknown at present, but it could offer a new aspect for studying gender-differentiated differences of CRC.

Proximal and distal CRCs are different regarding their pathogenesis and many clinical and pathological characteristics, as well as in their behaviour. Proximal CRC is more frequent in female patients, presents more often with MSI and increased levels of both Th2 (IL-4, IL-13, and MIP-1β) and Th1 cytokines. Instead, a Th2-oriented cytokine milieu – evidenced by increased levels of both Th2 (IL-4) and Th1 (IL-12 and IFN-γ) cytokines. Instead, a Th2-oriented cytokine milieu – evidenced by an increase in IL-4 in the absence of significant alterations in IL-12 and IFN-γ - was associated with a metastatic disease. Thus, rather than being characteristic to CRC, shift of Th1/Th2 balance towards Th2 seems to be related to the progression of cancer. In tissue level, local Th1-type immune response typically shows high densities of tumour-infiltrating lymphocytes and is associated with better survival (Ropponen et al, 1997; Naito et al, 1998). An association of Th1 response with prognostically significant antitumour activity is further supported by our regression model, where high IFN-γ associated with the absence of nodal metastases. Therefore, we believe that assessment of Th1/Th2 balance may help to identify CRC patients with a shift of cytokine levels towards Th2, resulting in an ineffective immune response and unfavourable prognosis. Such patients could be reasonable subjects for more intensive or tailored treatment, including immunomodulatory therapy (Pages et al, 2010). However, at this point, the follow-up time in our prospective material is not long enough, and it remains to be seen, whether serum cytokine profile could add prognostic information to other systemic inflammation-based markers like neutrophil/lymphocyte ratio or modified Glasgow prognostic score (Roxburgh and McMillan, 2010).

Majority of CRC deaths could be prevented by sensitive screening methods. At the moment, stool tests are neither very sensitive nor specific, and colonoscopy is a time-consuming and costly method (Sturgeon et al, 2008). Carcinoembryonic antigen is a widely used serum biomarker for CRC follow-up, but its sensitivity and specificity in the detection of CRC is low (Duffy, 2001; Sturgeon et al, 2008). As cytokines and chemokines are involved in most systemic and local inflammatory diseases, it is unlikely that determination of any single cytokine could be used as a diagnostic or a follow-up marker for CRC. Instead, by examining changes in multiple cytokines, it may be possible to detect more specific ‘cytokine footprints’ for different inflammatory and neoplastic diseases. For example, a promising result based on a multiplexed cytokine immunoassay in identifying lung cancer has been reported recently (Lee et al, 2010). In our study, measurements covering several essential cytokines enabled us to generate a logistic regression model – a serum cytokine profile – which achieved an excellent accuracy in discriminating CRC patients from healthy controls with an area under curve of 0.890 in the receiver operating characteristics analysis. This observation supports the potential of cytokine determinations as a screening or diagnostic tool. Still, our study has also limitations, as we did not have comparison groups of patients with inflammatory or infectious diseases and the logistic regression model was specifically fitted to our material. Therefore, our results and approach need to be confirmed by subsequent studies with independent study populations, and in studies including subjects with inflammatory conditions and gastrointestinal symptoms.

In conclusion, CRC is characterised by broad and complex alterations in serum cytokine and chemokine levels, including increased PDGF-BB, IL-6, IL-7, and IL-8 and decreased MCP-1. Our study highlights the benefits of analysing the serum cytokine levels as a group to observe relative expression level changes. Future studies are needed to compare the alterations between serum cytokine levels and individual inflammatory cell response to reveal immune reaction patterns in CRC, as well as to evaluate the specificity of the alterations of the cytokine profile in CRC in relation to other inflammatory disorders and neoplasms.

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Conflict of interest
The authors declare no conflict of interest.

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