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The clinical utility of the local inflammatory response in colorectal cancer

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Abstract ***Background:*** The host immune response is important in the prevention of tumour progression in solid organ cancers. The aim was to evaluate the clinical utility of the local inflammatory response in patients with colorectal cancer.

Methods: Three hundred and sixty-five patients with primary operable colorectal cancer were included. The local inflammatory response was assessed using three different methods; (1) individual T-cell subtypes (CD3, CD8, CD45R0, FOXP3), (2) an immunohistochemistry-based immune score (Galon Immune Score) and (3) a histopathological assessment (Klintrup–Makinen grade). Relationships with tumour and host characteristics were established and the prognostic value of each method compared.

Results: A strong infiltration of tumour infiltrating lymphoctyes (TIL's) was associated with improved cancer-specific survival. When individual T-cell subtypes were considered, CD3-positive cells were the strongest predictor of survival at the invasive margin ($CD3^+$ IM) while CD8-positive cells were the strongest predictor in the cancer cell nests ($CD8^+$ CCN). Infiltration of T-cells was related to early tumour stage, expanding growth pattern and lower levels of venous invasion but was not influenced by host characteristics or degree of systemic inflammation. In summary, $CD3^+$ IM, $CD8^+$ CCN, The Galon Immune Score and the Klintrup–Makinen grade all exhibited similar survival relationships in both node-positive and node-negative colorectal cancer.

Conclusion: A coordinated adaptive immune response is an important factor in predicting outcome in patients with primary operable colorectal cancer. By comparing different methodologies we have provided a foundation on which to develop a standardised approach for assessing the local inflammatory response in these patients.

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1. Introduction

Colorectal cancer is the second most common cause of cancer death in Western Europe and North America [1]. Despite advances in operative and oncological treatments, only half of patients undergoing surgical resection with curative intent will survive up to 5 years [2]. The identification of patients at high risk of disease recurrence who may benefit from adjuvant chemotherapy currently relies on the histopathological assessment of the resected specimen [3]. Conventional staging (AJCC/UICC-tumour-node-metastasis (TNM) classification) therefore accurately summarises data on tumour burden but its prognostic value is limited and some stages, in particular node-negative disease, include patients with a wide range of clinical outcomes [4].

It is now recognised that the host immune response is an important determinant of outcome in human cancers [5]. A number of studies have demonstrated that infiltration of inflammatory cells in colorectal tumours is associated with improved survival, regardless of pathological stage [6]. It is generally assumed that the presence of these cells is a manifestation of an effective immune response although it is unclear whether this reflects distinct tumour biology or particular host characteristics.

Despite the potential to improve risk stratification for patients with colorectal cancer, a reliable measure of the local inflammatory response has yet to be incorporated into clinical practice. The reasons for this are likely to include the multitude of individual cell types and 'immune scores' proposed as prognostic as well as the inherent complexities of immunohistochemistry [7]. In particular, there is a need to clarify whether lymphocyte subtyping adds additional prognostic information beyond the evaluation of inflammatory cells on routine haematoxylin and eosin (H&E) stained sections [8].

The aims of the present study, therefore, were to evaluate the type, density and location of tumour infiltrating lymphocytes (TIL's) in patients with primary operable colorectal cancer and to examine their relationships with tumour and host characteristics. Furthermore, we sought to compare the prognostic value of individual T-cell subtypes, an immunohistochemistry-based immune score and a simple histopathological assessment of inflammatory cell infiltrate.

2. Patients and methods

2.1. Patients

Since January 1997, all colorectal cancer surgeries performed at Glasgow Royal Infirmary have been entered into a prospectively maintained database. The present study includes patients who, on the basis of preoperative staging and laparotomy findings, were considered to have undergone potentially curative resection of colorectal cancer (stage I–III) between January 1997 and December 2006. Exclusion criteria were (1) the presence of a co-existing inflammatory condition, (2) neoadjuvant chemo- or radiotherapy and (3) death within 30 days of surgery. Local ethics committee approval was granted.

Prospectively collected data included patient demographics, pathological characteristics and laboratory measurements; haemoglobin (Hb), white cell count (WCC), albumin, C-reactive protein (CRP), urea and electrolytes. Medical records were reviewed retrospectively to record deprivation index (Carstairs Deprivation Index) [9], American Society of Anesthesiologists (ASA) grade, smoking status and POSSUM physiology scores [10]. Preoperative systemic inflammatory response was assessed using three validated measures; (1) serum white cell count (WCC) [11]), (2) neutrophil to lymphocyte ratio (NLR) [12] and (3) the modified Glasgow Prognostic Score (mGPS) [13].

2.2. Histopathology and immunohistochemistry

Tumours were staged according to the fifth edition of the AJCC/UICC-TNM staging system [14]. Additional pathological features, including tumour differentiation and venous invasion, were taken from contemporary reports. Tumour necrosis was graded semiquantitatively as 'absent' (none), 'focal' (<10% of tumour area), 'moderate' (10–30%) or 'extensive' (>30%) according to published methodology [15].

The decision to only include specific T-cell subtypes in the analysis (namely CD3, a T-cell co-receptor expressed on all mature T-cells; CD8, a T-cell co-receptor predominantly expressed on cytotoxic T-cells; CD45R0, a transmembrane glycoprotein expressed largely on previously activated or memory T-cells; FOXP3, a protein regarded as a specific marker of natural and adaptive/induced T regulatory (Treg) cells) was made on the basis of a previous systematic review that suggested these subtypes were of primary importance in colorectal cancer prognosis [6]. Archived paraffin embedded blocks of the central tumour were retrieved to perform the immunohistochemistry. One block, representative of the point of deepest tumour invasion, was chosen per case. Consecutive blank 4 μm sections were cut and mounted on silanised slides before being dewaxed in xylene and rehydrated using graded alcohol washes. Heat-induced antigen retrieval was performed by microwaving under pressure using a citrate or Tris/ethylene diamine tetra-acetic acid (EDTA) buffer before endogenous peroxidase activity was blocked (5% normal goat serum in TRIS buffered saline (TBS)) and to the following cellular epitopes (CD), the following primary antibodies were applied; CD3 (Vector Labs, code VP-RM01, 1/100 dilution), CD8 (DakoCytomation, code M7103, 1/100 dilution), CD45R0 (DakoCytomation,