European Journal of Cancer (2014) **50**, 544–552

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Comparison of visual and automated assessment of tumour inflammatory infiltrates in patients with colorectal cancer

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Available online 11 December 2013

KEYWORDS
Tumour inflammatory cell infiltrate
Automated assessment
Colorectal cancer

Abstract ***Background:*** Cancer-associated inflammation is increasingly recognised to be an important determinant of oncological outcome. In colorectal cancer, the presence of peritumoural inflammatory/lymphocytic infiltrates predicts improved survival. To date, these infiltrates, assessed visually on haematoxylin and eosin (H&E) stained sections, have failed to enter routine clinical practice, partly due to their subjective assessment and considerable inter-observer variation. The present study aims to develop an automated scoring method to enable consistent and reproducible assessment of tumour inflammatory infiltrates in colorectal cancer.

Methods: 154 colorectal cancer patients who underwent curative resection were included in the study. The local inflammatory infiltrate was assessed using the method described by Klintrup–Makinen. H&E tumour sections were uploaded to an image analysis programme (Slidepath, Leica Biosystems). An image analysis algorithm was developed to count the inflammatory cells at the invasive margin. The manual and automated assessments of the tumour inflammatory infiltrates were then compared.

Results: The automated inflammatory cell counts assessed using the freehand annotation method ($p < 0.001$) and the rectangular box method ($p < 0.001$) were significantly associated with both K–M score ($p < 0.001$) and K–M grade ($p < 0.001$). The inflammatory cell counts were divided using quartiles to group tumours with similar inflammatory cell densities. There was good agreement between the manual and automated scoring methods (intraclass correlation coefficient (ICC) = 0.82). Similar to the visual K–M scoring system, the automated K–M classification of the inflammatory cell counts, using quartiles, was significantly associated with

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http://dx.doi.org/10.1016/j.ejca.2013.11.003

venous invasion ($p < 0.05$) and modified Glasgow Prognostic Score (mGPS) ($p \leqslant 0.05$). On univariate survival analysis, both automated K–M category ($p < 0.05$) and automated K–M grade ($p < 0.005$) were associated with cancer-specific survival.

Conclusion: The results of the present study demonstrate that automated assessment effectively recapitulates the clinical value of visual assessment of the local inflammatory cell infiltrate at the invasive margin of colorectal tumours. In addition, it is possible to obtain an objective assessment of tumour inflammatory infiltrates using routinely stained H&E sections. An automated, computer-based scoring method is therefore a workable and cost-effective approach to clinical assessment of local immune cell infiltrates in colorectal cancer.

1. Introduction

Colorectal cancer is the second most common cause of cancer death in both men and women in the United Kingdom (UK) with 16,000 deaths per year (Cancerstats, UK, 2010). Despite improvements in treatment, outcomes remain poor with approximately half of those undergoing curative resection dying from the disease [1].

In recent years it has become increasingly clear that cancer-associated inflammation, in the form of local and systemic inflammatory responses, is a key determinant of progression and survival in colorectal cancer. In particular, there is consistent evidence that the presence of a high grade local inflammatory cell infiltrate both within the tumour and in the immediate microenvironment predicts survival independent of tumour stage in colorectal cancer [2–4]. Many studies have reported that increasing density of inflammatory cells in and around the tumour is associated with improved outcome in patients with colorectal cancer and this is thought to represent the host anti-tumour response [4]. Further, there is good evidence that the immune classification of tumours has independent and superior prognostic value when compared to traditional staging methods [3].

Despite the strong evidence supporting the prognostic value of inflammatory cell infiltrates, and the existence of well-described methods for the semi-quantitative assessment of inflammatory cell infiltration [2,3], the extent of the local inflammatory cell infiltrate is not routinely considered in clinical practice and conventional staging systems such as tumour-node-metastasis (TNM) stage remain the mainstay in clinical practice. Reasons for this include the complexity and lack of reproducibility of scoring the inflammatory cell infiltrate caused by differences in immunohistochemical staining methods between different units, the different cell types present and importantly, the subjectivity of assessing the tumour inflammatory cell infiltrate.

Therefore a reliable and accurate measure of the tumour inflammatory cell infiltrate may be useful in the refinement of staging the host inflammatory response in clinical pathological practice.

There is now image analysis software capable of point-scoring cells in routinely processed haematoxylin and eosin (H&E) tumour sections. Recent studies have reported that computer-aided analysis has significant advantages over manual scoring methods including: objectivity, accuracy and reproducibility [5–8]. Therefore, this modality may offer a method of standardising the assessment of the local inflammatory cell infiltrate in patients with colorectal cancer.

The aim of the present study was to compare visual and automated assessment of tumour inflammatory cell infiltration in patients with colorectal cancer.

2. Patients and Methods

Patients with colorectal cancer who, on the basis of pre-operative staging and laparotomy findings, were considered to have undergone an elective, potentially curative resection of colorectal cancer between 1997 and 2006 in a single surgical unit at Glasgow Royal Infirmary were included in the study. Tumours were staged using the conventional tumour-node-metastasis (TNM) staging system, 7th Edition, 2010 [9]. Patients with conditions known to elicit an acute or chronic systemic inflammatory response were excluded. These were namely (i) pre-operative chemoradiotherapy, (ii) clinical evidence of active pre-operative infection, or (iii) chronic active inflammatory diseases such as rheumatoid arthritis. The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary, Glasgow.

2.1. Visual assessment of tumour inflammatory cell infiltration

Assessment of the tumour inflammatory cell infiltrate was performed on original haematoxylin and eosin-stained full-sections of the tumour, considered to be representative of the specimen. The local inflammatory response was evaluated previously in this cohort (GJKG and CSDR) using the method described by Klintrup et al. [2].

Briefly, K–M criteria is a four-point scale, a score of 0 indicates no increase in inflammatory cells at the invasive margin, a score of 1 indicates presence of a mild/patchy increase in inflammatory cell reaction at the invasive margin but no destruction of invading cancer cell islets, a score of 2 indicates observation of a band-like inflammatory reaction at the invasive margin and