Response to Park et al. reply to "Back to the future: routine morphological assessment of the tumour microenvironment is prognostic in stage II/III colon cancer in a large population-based study"

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Dear Editor,

We thank Park *et al* for their interest in our work and for bringing to our attention their alternative microenvironmental score for assessing colorectal cancer prognosis. [1-3] We are largely in agreement with many of the points raised. The scoring systems proposed by these two studies share many similarities, highlighting the importance of the non-epithelial tumour components and combining assessments of peritumoral inflammatory response and tumour stromal percentage to derive a prognostically valuable fibroinflammatory or microenvironmental score. Despite the overall similarity, a number of small, albeit clinically important, differences lie in the methodologies chosen for assessment of each individual parameter, and in the statistical approaches used.

As Park *et al* acknowledge, there is considerable evidence that it is the lymphoid

composition of the peritumoral inflammatory population (rather than general inflammation) that is of most prognostic influence for colorectal cancer, although reproducibility is likely inferior. [4-7] Their working group have previously reported assessment of both peritumoral lymphocytic inflammation (by Jass method) and general inflammation (by Klintrup-Makinen method) to have good reproducibility (inter-observer intraclass correlation coefficients 0.71 and 0.81 respectively). [8] Although we found good intraobserver reproducibility, we also observed poor interobserver reproducibility for assessment of peritumoral lymphoid infiltrates, and did not evaluate general peritumoral inflammation in our study. There are a growing number of translational studies highlighting that the prognostic value of the inflammatory microenvironment can be dictated by the relative abundance of specific immune-cell lineages. [9-11] We acknowledge that general inflammation may indeed be more easily and reproducibly assessed on haematoxylin and eosin (H&E)-stained slides and that immunohistochemistry, aided by digital image analysis, may be required for most accurate immunoscoring of lymphoid populations. This is deserved of further study.

A further distinction between the two studies is that, unlike the microenvironmental score proposed by Park *et al*, our score includes an assessment of peritumoral lymphoid aggregates, or Crohn's disease-like reaction (CLR). Supported by Klintrup *et al*, we consider this sufficiently independent of peritumoral diffuse lymphoid reaction to warrant inclusion as a separate parameter, adding prognostic value. [12] Our study employed the well-established semi-quantitative assessment proposed by Graham and Appelman, which we found to be reproducible. [13] The cited study by Kim *et al* advocates assessment of peritumoral lymphoid aggregates by size or density. [14] However this study methodology employed digital image analysis of scanned slides, to allow the required precise measurements for this classification, and is therefore not immediately applicable to routine histopathology practice at the current time. For this reason, this method of assessment was not considered in our study.

We adopted both global and focal assessments of tumour stromal percentage (TSP), as reported previously. [15-17] We found global, but not focal, assessment to be highly reproducible and prognostic. As discussed, we are aware this contradicts findings from two previous studies also highlighted by Park *et al*, both describing excellent reproducibility of

the refined focal method. [16,17] Key to the focal TSP method is identification of the most invasive point of tumour and, on post-study case discussion, this varied considerably between the four pathologists in our study, profoundly influencing assessments and reproducibility. This is likely to be an even greater problem in everyday reporting practice, with more tumour slides to evaluate per case. Also taking into consideration frequent heterogeneity of tumour morphology, we consider the global TSP method to be more valuable than the focal TSP method.

Finally, some differences in the statistical approaches to the two scores also exist. Park *et al* evaluated five year survival, whereas we evaluated prognosis up to ten years post-diagnosis, and retained confounders such as adjuvant chemotherapy in our multivariate model. They found TSP to be rare and not prognostic in a scenario of high general inflammation (Klintrup-Makinen) score. [3] Leaving aside the differences in assessment methodologies outlined above, we have now replicated this analysis, and also found a high global TSP to be of greater prognostic importance in the context of low peritumoral inflammation (unadjusted HR 1.63, 95% CI 1.11-2.40). However, as outlined in Table 5 of our results, taking into consideration all three measures (global TSP, peritumoral inflammation and CLR) resulted in the strongest association with colorectal cancer-specific survival. Perhaps of most importance, in multivariate models, we found a stronger association between colorectal cancer-specific survival and the highest fibroinflammatory score in our study (HR 2.44, 95% CI 1.56-3.81), than that noted for the highest Glasgow microenvironmental score (HR 1.93, 95% CI 1.36-2.73), although the confidence intervals do overlap. [3]

Despite differences in methodologies employed, the similar approaches adopted by the two groups draw similar conclusions and further highlight the potential utility of inflammatory and stromal assessments as valuable prognostic markers in colorectal cancer, alone or in some combination. We agree further independent studies are required for validation and determination of optimal methods of assessment.

Yours sincerely,

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