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Original Research

Prospective validation of a lymphocyte infiltration prognostic test in stage III colon cancer patients treated with adjuvant FOLFOX☆

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KEYWORDS
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Abstract ***Background:*** The prognostic value of lymphocyte infiltration (LI) of colorectal carcinoma (CC) has been demonstrated by several groups. However, no validated test is currently available for clinical practice. We previously described an automated and reproducible method for testing LI and aimed to validate it for clinical use.

Patients and methods: According to National Institutes of Health criteria, we designed a prospective validation of this biomarker in patients included in the PETACC8 phase III study. Primary objective was to compare percentage of patients alive and without recurrence at 2 years in patients with high versus low LI (#NCT02364024). Associations of LI with patient recurrence and survival were analysed, and multivariable models were adjusted for treatment and relevant factors. Automated testing of LI was performed on virtual slides without access to clinical data.

Results: Among the 1220 CC patients enrolled, LI was high, low and not evaluable in 241 (19.8%), 790 (64.8%) and 189 (15.5%), respectively. Primary objective was met with a 2-year recurrence rate of 14.4% versus 21.1% in patients with high and low LI, respectively ($p = 0.02$). Patients with high LI also had better disease free survival (DFS) and overall survival (OS). Tumour stage, grade, *RAS* status and *BRAF* status were with LI the only prognostic markers in multivariable analysis for OS. Subgroup analyses revealed that high LI had better DFS and OS in mismatch repair (MMR) proficient patients, and in patients without *RAS* mutation, but not in MMR deficient and *RAS* mutated patients.

Conclusion: Although this is the first validation with high level of evidence (IIB) of the prognostic value of a LI test in colon cancers, it still needs to be confirmed in independent series of colon cancer patients.

1. Introduction

Colorectal carcinoma (CC) is the 4th cause of cancer death worldwide [1]. After surgical resection of stage III colon carcinoma, up to 50% of patients develop recurrence and die from metastatic disease [2]. In 2004, adjuvant treatment with fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) was shown to be better than fluorouracil and leucovorin alone [3], and oxaliplatin benefit was confirmed in other trials [4,5]. FOLFOX4 is thus currently the standard adjuvant treatment for stage III patients and probably improves long term disease free survival (DFS) by 10–15% as compared to surgery alone [5,6]. The most recent clinical trials for stage III CC patients, investigated either intensifying adjuvant treatment by adding targeted therapies to FOLFOX [7–9] or shortening treatment duration to decrease adjuvant treatment toxicities (clinicaltrials.gov #NCT00958737). Obviously a better prognostic assessment of these patients using validated biomarkers, as well as biomarkers predictive for efficacy of a therapy, would help to select patients for either more or less intensive adjuvant therapy.

In 1986, lymphocyte infiltration (LI) of rectal carcinoma was reported to be associated with a better prognosis [10]. More than 300 publications investigating CC have confirmed this seminal publication. Lymphocytes were counted on haematoxylin and eosin or immunohistochemistry stained slides, manually or with image analysis software, on whole slides or on tissue arrays [11–14]. However despite all these publications and the need of biomarkers, no test has been validated today for clinical use. The lack of such test is due to a technical reason: immunostaining of lymphocytes is highly dependent on preanalytic conditions. It is also due to a biological reason: the density of lymphocytes within a given CC may vary from 1 to 50, depending on the counted areas [15]. Thus in all the previously published series, the cutoff of lymphocyte density to conclude the result as high or low was determined after analysing the whole retrospective series of patients.

We previously described a robust and reproducible test evaluating LI in CC [15]. This test is based on automated counting of CD3 lymphocytes within hundreds of small areas localised in both parts of the tumour margin. Each lymphocyte density is associated with the distance of the analysed area from the tumour margin, and used to generate a curve of variation of lymphocyte densities according to the distance from tumour margin. The local immune response (IR) to a given tumour is then interpreted on the curve, and does not depend on the preanalytic conditions.

The present work was designed to validate this test with a high level of evidence in a large pan-European prospective study of patients with stage III CC treated with adjuvant FOLFOX4 plus or minus cetuximab.

2. Materials and methods

2.1. Patient

Study design has been published on clinicaltrials.gov web site in February 2015 (#NCT02364024). Patients from