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Histological intratumoral heterogeneity in the pre-treatment oesophageal cancer biopsies predicts survival benefit from neoadjuvant chemotherapy – results from the UK MRC OE02 trial

Running title

Heterogeneity in oesophageal cancer

Naser Davarzani*[1,2], Lindsay C. Hewitt*[1,3], Matthew D. Hale[3], Veerle Melotte[1,4], Matthew Nankivell[5], Gordon G. A. Hutchins[3], David Cunningham[6], William H. Allum[7], Ruth E. Langley[5], Shahab Jolani[#8], Heike I. Grabsch[#1,3]

1. Department of Pathology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands.
2. Biosystems Data Analysis, Swammerdam Institute for Life Sciences, Amsterdam University, Amsterdam, The Netherlands
3. Division of Pathology and Data Analytics, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK
4. Department of Clinical Genetics, University of Rotterdam, Erasmus University Medical Center, Rotterdam, The Netherlands
5. Medical Research Council Clinical Trials Unit at University College, London, UK
6. Gastrointestinal and Lymphoma Unit, Royal Marsden Hospital, London, UK
7. Department of Surgery, Royal Marsden Hospital, London, UK.
8. Department of Methodology and Statistics, CAPHRI, Maastricht University, Maastricht, The Netherlands.

* joint first authors

joint last authors

Corresponding author:

Heike I Grabsch MD PhD FRCPath

Professor, Department of Pathology

Maastricht University Medical Center+

P. Debyelaan 25

6229 HX Maastricht

The Netherlands

Phone: +31433874610

Fax: +31433874616

Email: H.Grabsch@maastrichtuniversity.nl

Supplementary items

Supplementary text

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Text S2: Simulation study methodology measuring the error in intratumour heterogeneity of the proportion of tumour (IHPoT) estimation using mixed effect model (MEM)

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Text S1: Statistical methodology for mixed effect model (MEM)

For the ith biopsy piece we define Y_{ij} as a binary outcome variable with a value of 1 if the jth point is tumour, otherwise the value is zero. Define P_{ij} as the probability that the jth point is tumour, that is $Y_{ij} = 1$. Then the MEM is defined as follow:

$$\log\left(\frac{P_{ij}}{1-P_{ij}}\right) = \mu+\tau_i,$$

where, μ is the model intercept and τ_i is called the random effect which is a function of heterogeneity between the biopsy pieces. For the ith biopsy piece, τ_i is assumed to have a normal distribution with mean zero and variance of σ^2 where σ^2 is defined as the intratumour heterogeneity between biopsy pieces of the given patient. Implementing MEM, using R package "lme4", we can estimate the σ^2 as the intratumour heterogeneity between the *K* biopsy pieces of the given patient.

Text S2: Simulation study methodology measuring the error in intratumour heterogeneity of the proportion of tumour (IHPoT) estimation using mixed effect model (MEM)

We performed a simulation study to measure the error of estimating IHPoT using a MEM in R (version 3.5.1). This was based on the assumption that the real heterogeneity is 0.16 (close to the median of heterogeneity in our data), the number of biopsy pieces ranges from 2 to 12 while each biopsy piece has 450 points. For a given number of biopsy pieces, based on 1000 simulated data sets, the corresponding mean squared error (MSE) of the IHPoT estimates (using MEM model) was obtained.

Table S1: Layout of dataset for applying mixed effect model

Patient ID	Biopsy piece ID	Point	Y_{ij}
1	1	1	1
1	1	2	0
1	1	3	0
.	.	.	.
.	.	.	.
.	.	.	.
1	1	n_1-1	1
1	1	n_1	0
1	2	1	0
1	2	2	1
1	2	3	0
.	.	.	.
.	.	.	.
.	.	.	.
1	2	n_2-1	1
1	2	n_2	0
.	.	.	.
.	.	.	.
.	.	.	.
1	K	1	0
1	K	2	1
1	K	3	1
.	.	.	
.	.	.	
.	.	.	
1	K	n_k-1	0
1	K	n_k	0

Figure Legends

Figure S1. Plot showing the number of biopsy pieces and corresponding mean squared error (MSE) of IHPoT estimates using MEM model. Regardless of the number of biopsy pieces MEM model has a very small error (close zero) in estimating IHPoT.

Figure S2. Five year overall survival of patients treated with chemotherapy plus surgery (CS) versus surgery (S) alone group with low and high IHPoT and PoT<40% or PoT>70%.

(A) Patients with low IHPoT index and PoT<40%: There was no significant difference in survival between CS patients and S patients (HR=1.001, 95%CI: 0.329-3.047, *P*=0.999).

(B) Patients with high IHPoT index and PoT<40%: There was no significant difference in survival between CS patients and S patients. (HR=1.448, 95%CI: 0.454-4.615, *P*=0.532).

(C) Patients with low IHPoT index and PoT>70%: There was no significant difference in survival between CS patients and S patients (HR=0.636, 95%CI: 0.257-1.575, *P*=0.328).

(D) Patients with high IHPoT index and PoT>70%: There was no significant difference in survival between CS patients and S patients (HR=0.357, 95%CI: 0. 093-1.372, *P*=0.134).