How to analyse dynamic MRI in oncology; advanced histogram analysis gives better statistical power & insight than simple averaging

Emma O'Connor[1] , Nick Fieller[1], Andrew Holmes[2], John Waterton[2]

[1]Department of Probability & Statistics, University of Sheffield, UK, [2]AstraZeneca, Alderley Park, UK

Email: stp03elo@sheffield.ac.uk

www.sheffield.ac.uk/paspgr/oconnor

Introduction

Currently, DCE-MRI tumour perfusion studies are usually analysed voxelwise, but the rich patterns of spatial and functional heterogeneity are often lost when the data are summarised for significance testing. Statistical analyses are often performed using a single (or small number of) measures summarising the distribution of a parameter over the ROI, such as mean or median K^{trans}. The substantial limitations of these histogram-based methods include:–

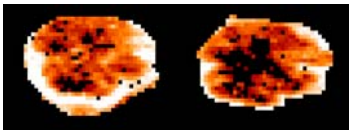


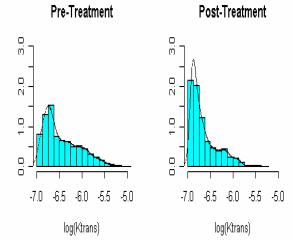
Figure 1: ***Maps of K^{trans} before and after treatment***

- the median might be zero pre & post if there is a large necrotic "core";
- there may be clear "rim" & "core" effects , as seen in Figure 1, but since rim and core are functionally defined, creating an element of circularity;
- spatial heterogeneity, which is also apparent in Figure 1;
- the mean is dominated by outliers from highly vascular voxels (with high K^{trans}), highlighted in Figure 2;
- multi-parameter "histogram" approaches raise multiple comparisons issues.

Histograms of the distribution of K^{trans} before and after treatment, Figure 2, indicate that summary by just mean values is too simplistic and misses clearly observable features in the distribution which could have biological interest. For example, changes in dose may be reflected in change in mean value but also by changes in spread or skewness.

Here we extend the methods to characterise and assess all of the changes in the distribution to provide additional insight and improved interpretation over and above the possibilities provided by crude histogram analysis in the situation where we cannot register images for a complete voxelwise "mapping" approach.

Figure 2: ***Kernel Density Estimates and histograms for one subject***



General Statistical Objective:

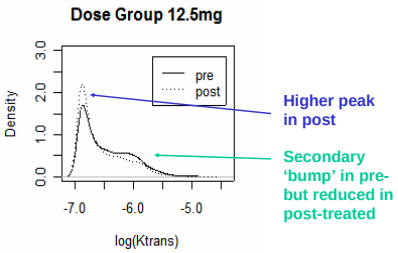
To develop flexible but rigorous statistical methodology to capture the observable features in distributions from images and provide an objective statistical assessment of their importance

Data

The data to be analysed arise from experiments (Checkley 2003) using DCE-MRI with gadopentate dimeglumine to monitor acute effects on tumour perfusion, following inhibition of vascular endothelial growth factor signalling. Mice bearing PC-3 human prostate adenocarcinoma xenografts, were treated with ZD6474, a novel anti-angiogenic that selectively inhibits both VEGF receptor-2 (KDR) tyrosine kinase and EGF receptor tyrosine kinase.

There were three experiments, comprising four doses 12.5, 25, 50, 100 and a control. DCE-MRI was performed twice, immediately before and 24 hours after treatment with ZD6474. The pharmacokinetic parameter $\mathbf{K^{trans}}$ was obtained for each voxel within the tumour region of interest, which reflects vascular permeability and perfusion. Figure 1 displays an example of corresponding maps of K^{trans} before and after treatment.

Figure 3: ***Average densities pre and post treatment for 12.5mg dose group***



Exploratory Analyses

Figure 2 compares the histograms and kernel density estimates of the distributions of K^{trans} before and after treatment. Simple summary statistics from the histograms capture changes in location and scale only and it is clear that treatment generally reduces the values of K^{trans} but more complex changes in distribution are apparent in the kernel density estimates in Figure 3. Comparison between average densities reveals two features common to all treated groups. The first being a higher primary peak post treatment and the second, the absence of a secondary bump post treatment.

Main Analyses

The standard approach of analysing this type of data is to model the distribution with a parametric model and then analyze changes in estimated parameters with change in dose. However, sometimes the distributional changes are too complex to capture with simple parametric models. For example, distributions which exhibit varying degrees of multimodality are very difficult to model and calculating sample moments such as means, variances is not sensible.

Here, the main analysis is based upon *functional principal component analysis* (FPCA). This is a multivariate technique that identifies components of variability and relates them to experimental structure to provide some insight into what causes the variation and whether this can be related to a separation between pre/post treatment groups and/or control and treated samples for example.

Method: The first step is to summarize the functions in a small number of parameters and this is achieved by discretization, which is explained in Figure 4. Each distribution can now be represented by a vector of 100 values enabling standard principal component analysis of the data. Sensitivity analysis revealed that using 50 or 200 values did not alter the results. The results however, need to be interpreted in terms of density functions. This method is based on ideas from Ramsay and Silverman (1997). The first few principal components account for most of the variation of the data and will therefore be the most interesting. Later principal components explain decreasing amounts of variation and can be regarded as 'noise' and so discarded.

Summary of process:

Measured response: **image**

⇩

Sample distribution of K^{trans}

⇩

Histogram

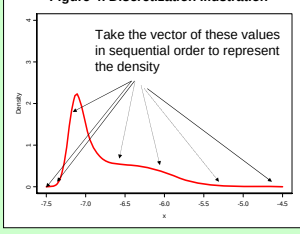
⇩

Kernel density estimate

⇩

Vector of density values

Figure 4: ***Discretization Illustration***



Results

Figures 5a and 5b display the effect of adding and subtracting a multiple of the first two principal components to the mean density of the entire data set. This allows interpretation of the PCs and aids understanding of the modes of variability between the images.

The first principal component can be identified as reflecting differences between a diffuse distribution and a less variable and more peaked one.

Low PC1 Score ⇔ High Primary Peak

High PC1 Score ⇔ Low Primary Peak

Figure 5a: ***Functional Principal Component One***

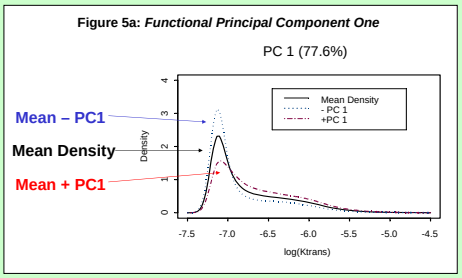
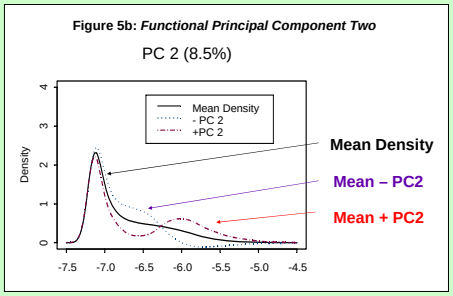


Figure 5b: ***Functional Principal Component Two***



The second component reflects the presence or absence of a secondary mode of higher values of K^{trans}.

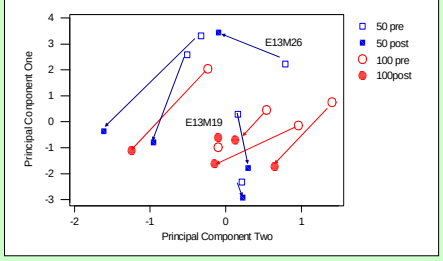
Low PC2 Score ⇔ No Bump

High PC2 Score ⇔ Bump

Note: Signs of PCs are arbitrary and have been chosen so that low values are 'desirable' in current context. Low scores on first two PCs corresponds to generally lower "permeability" & especially fewer fairly high values.

Scores for each probability density on the PCs can also be calculated to give principle component score plots of the samples such in Figure 6, which contains the score plot for Experiment 13. Individual pairings reveal a pre to post shift to the bottom left corner, low PC1 and PC2, corresponding to a post-treatment distribution with high primary peak (see Fig. 5a) and no secondary peak (see Fig. 5b). These features indicate a distribution with overall lower values of K^{trans} and no secondary subset of high values post-treatment: a positive treatment effect. Discarding low order PCs reduces random noise.

Figure 6: ***Score Plot and Individual Pairings for one experiment containing two dose groups***



Formal statistical testing of a difference between pre- and post-treatment densities is achieved by a randomisation test based on random relabelling of pre- and post-treated values. The test statistic is based on the sum of the differences in scores on PC1 and PC2 *(a city bock difference,* Figure 7) and equal weighting is given to the two PCs as it is not known whether one is of greater importance. This in turn means that there is increased weighting on the smaller mode of variation.

Figure 7: ***City-block distance***

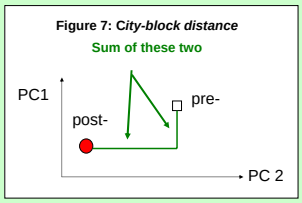


Figure 8: Results from the analysis of change in mean K^{trans} (A) and from FPCA (B)

Group	Number of Observations N	(A) p-value (t-test based on mean)	(B) p-value (rand. test based on FPCA)
Control	11	0.77	>> 0.5
12.5mg	6	0.2	< 0.1
25mg	6	0.07	< 0.05
50mg	10	< 0.01	< 0.01
100mg	11	< 0.01	< 0.01

The results of this analysis indicate that all four dose groups (but not controls) show clear evidence of effect of treatment. Figure 8 compares the p-values between the analysis of change in mean K^{trans} (A) and from FPCA (B). The results suggest that the latter statistic appears to be more sensitive for testing for a difference in the smaller groups. Furthermore, this statistic together with the score plots gives greater insight into where the changes are occurring.

Discussion

The analysis using FPCA has provided additional insight beyond the simple changes in mean values of K^{trans}. These results could not be obtained from commonly used 'histogram analysis' using a small number of summary measures. Our method could be used to locate where these changes occur within the tumour as the two different PC's are likely to represent different biology such as a well-perfused "rim" and poorly perfused "core", although these areas are not anatomically defined.

These methods are not only limited to tumour vitality studies but could be applied to other areas of imaging where we need to assess heterogeneous voxel values on an ROI basis. This technique is currently being validated using data from other research areas, where the responses are curves before refining the methodology.

The theory naturally extends to higher dimensions, thus a range of parameters can be analysed simultaneously. A focus of current work is to develop FPCA in two dimensions in order to analyse bivariate kernel density estimates of both K^{trans} and V_e, the volume fraction of extravascular extracellular space.

References:

Checkley D, Tessier J, Kendrew J, Waterton J & Wedge S. (2003). *Use of dynamic contrast-enhanced MRI to evaluate acute treatment with ZD6474, a VEGF signalling inhibitor, in PC-3 prostate tumour. Br J Cancer. Nov 17;89(10):1889-95*

Ramsey J. & Silverman B. (1997). *Functional Data Analysis*. Springer-Verlag, London.