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BAYESIAN DOSIMETRY II: CREDIBILITY INTERVALS FOR RADIATION DOSE

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SUMMARY

This paper is the natural sequel to Madruga *et al.* (*Environmetrics*, **5**, 47–56 (1994)) where data from literature were analysed. Here, three sets of experimental data were obtained at IPEN (Energetic and Nuclear Research Institute) from blood cultures from three patients with basal cellular carcinoma (1 male, age 68, and 2 females, ages 47 and 66), four healthy young subjects (2 males, ages 24 and 27, and 2 females, ages 20 and 27) and two older healthy subjects (1 male, age 50, and 1 female, age 46). These cultures were exposed *in vitro* to ^{60}Co radiation ranging from 0 to 500 cGy. The frequencies of cells with one or two nuclei, with or without micronuclei (MN), are the responses. Models for mono- and binucleated cells were obtained. As suggested in Madruga *et al.*, models for mononucleated are simpler than for binucleated cells. The novelty is that, based on the observed frequencies of micronucleated cells, we can generate credible intervals for unknown doses to which an individual was exposed.

KEY WORDS calibration; cytochalasin B; HPD intervals; *in vivo* experiments; *in vitro* experiments; logistic transformation; ^{60}Co radiation

1: INTRODUCTION

The purpose of this paper is to obtain calibration models that will be helpful in establishing credible intervals for unknown exposure doses of a given agent once we have the observed responses. The agent in our case is radiation and the responses are frequencies of mono- and binucleated cells with none, one and two or more micronuclei.

The underline probability distribution of the vector of these frequencies, $\boldsymbol{y}_i = (y_{i0}, y_{i1}, y_{i2})$, for each dose i, and for each type of cell (mono- and binucleated), is the trinomial distribution with parameter vector $\boldsymbol{\pi}_i = (\pi_{i0}, \pi_{i1}, \pi_{i2})$, where $\pi_{i0} + \pi_{i1} + \pi_{i2} = 1$. Recall that y_{i0} is the frequency of cells with no MN, y_{i1} with one, and y_{i2} with two or more MN.

The Bayesian approach is used taking the conjugate Dirichlet distribution as the prior. The

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Dirichlet posterior distribution is transformed into a bivariate normal distribution by considering the relation

$$\theta_j = \log \frac{\pi_j}{\pi_0}, \quad j = 1, 2, \tag{1}$$

known as the log-ratio transformation. The inverse transformation

$$\pi_j = \frac{\exp\{\theta_j\}}{1 + \exp\{\theta_1\} + \exp\{\theta_2\}} \tag{2}$$

is known as the logistic transformation. For more details, see Aitchison and Shen (1980). The dose response model is completely described in Madruga *et al.* (1994). As suggested, the model for mononucleated cells,

$$\pi_{ij} = \frac{\exp\left\{-\frac{\beta_j}{\gamma_j + D_i}\right\}}{1 + \exp\left\{-\frac{\beta_1}{\gamma_1 + D_i}\right\} + \exp\left\{-\frac{\beta_2}{\gamma_2 + D_i}\right\}}, \tag{3}$$

is simpler than for binucleated cells,

$$\pi_{ij} = \frac{\exp\left\{-\left(\alpha_j + \frac{\beta_j}{\gamma_j + D_i}\right)\right\}}{1 + \exp\left\{-\left(\alpha_1 + \frac{\beta_1}{\gamma_1 + D_i}\right)\right\} + \exp\left\{-\left(\alpha_2 + \frac{\beta_2}{\gamma_2 + D_i}\right)\right\}}, \tag{4}$$

where D_i is the ith dose level.

Section 2 presents our three sets of data and the adjusted dose-response models. Section 3 deals with the method used to build the credible intervals.

2. DOSE-RESPONSE CURVES

This analysis refers to data obtained from three groups of individuals as follows. 1,Three patients with basocellular carcinoma: 1 male, age 68, and 2 females, ages 47 and 66; 2, Four healthy young subjects: 2 males, ages 24 and 27, and 2 females, ages 20 and 27; and 3, Two healthy older subjects: 1 male, age 50, and 1 female, age 46.

Blood samples from each individual were exposed *in vitro* to ^{60}Co radiation with doses of 0, 20, 50, 100, 200, 300, 400 and 500 cGy (centigray). Lymphocyte cultures were prepared for the cytokinesis-block micronucleus assay using cytochalasin B (Fenech & Morley, 1985) and analysed for the presence of mono- and binucleated cells with none, one and two or more micronuclei (MN). The response in each of the three data sets is the vector of totals of cells of the individuals in the group. The response vector for the i-th dose level is denoted by $\mathbf{y}_i$, as described in Section 1. Tables I, II and III show the response vectors for each experimental group.

The dose-response model for mononucleated cells is

$$-m_{ij} = \frac{\beta_j}{\gamma_j + D_i}, \tag{5}$$

where, for each i, (m_{i1}, m_{i2}) are the posterior means of the transformed normal parameters $\boldsymbol{\theta}_i = (\theta_{i1}, \theta_{i2})$ as given in Madruga *et al.* (1994). The estimates of β_j and γ_j of these dose-response models for the 3 groups are shown in Table IV.

The dose-response model for binucleated cells is

$$-m_{ij} = \alpha_j + \frac{\beta_j}{\gamma_j + D_i}. \tag{6}$$

The estimates of α_j, β_j and γ_j for these dose-response models for the 3 groups are shown in Table V.

Table VI presents the errors $\tilde{\varepsilon}_{i1}$ and $\tilde{\varepsilon}_{i2}$ due to the adjustment of the proportions π_{i1} and π_{i2} respectively for mononucleated cells of each group. Table VII presents these errors for binucleated cells.

Figures 1, 2 and 3 show the adjusted dose-response curves of the data sets for mononucleated

Table I. Frequencies observed for mono- and binucleated cells from patients with basal cellular carcinoma

		Mononucleated			Binucleated		
i	Dose (cGy)	y_{i0}	y_{i1}	y_{i2}	y_{i0}	y_{i1}	y_{i2}
0	0	20442	68	6	1492	13	6
1	20	25183	68	14	1478	45	4
2	50	27614	362	81	1504	150	35
3	100	27845	392	30	1305	125	13
4	200	13378	527	72	1231	203	38
5	300	6359	398	74	1156	286	111
6	400	6234	531	148	1038	305	126
7	500	3920	449	180	1001	392	222

Table II. Frequencies observed for mono- and binucleated cells from healthy young subjects

		Mononucleated			Binucleated		
i	Dose (cGy)	y_{i0}	y_{i1}	y_{i2}	y_{i0}	y_{i1}	y_{i2}
0	0	51237	28	7	2341	31	1
1	20	23891	81	28	2611	45	6
2	50	26688	172	32	1849	117	25
3	100	25916	465	56	1811	189	47
4	200	23482	926	141	2204	325	82
5	300	8523	681	140	1734	501	207
6	400	9808	799	204	1621	523	254
7	500	7684	842	288	1005	456	285

Table III. Frequencies observed for mono- and binucleated cells from healthy older subjects

		Mononucleated			Binucleated		
i	Dose (cGy)	y_{i0}	y_{i1}	y_{i2}	y_{i0}	y_{i1}	y_{i2}
0	0	15551	114	12	920	31	2
1	20	13953	96	20	989	41	8
2	50	16163	180	18	933	56	14
3	100	13319	291	38	939	114	32
4	200	6411	333	52	794	176	67
5	300	6699	366	75	683	209	59
6	400	4311	409	105	742	256	107
7	500	4689	370	152	771	327	143

Table IV. Parameter estimates for the dose-response model for mononucleated cells with one and two or more MN

	1 MN		2 or more MN	
Groups	β_1	γ_1	β_2	γ_2
Carcinoma	1732·40	312·87	2722·66	343·17
Young	1625·43	247·09	2866·95	368·85
Old	2225·71	469·58	3274·67	460·49

Table V. Parameter estimates for the dose-response model for binucleated cells with one and two or more MN

	1 MN			2 or more MN		
Groups	α_1	β_1	γ_1	α_2	β_2	γ_2
Carcinoma	0·89	165·69	44·57	−2·60	4282·26	530·69
Young	0·18	449·01	107·05	0·78	506·63	78·20
Old	−0·08	625·81	177·66	1·13	411·63	87·74

Table VI. Errors due to adjustment of the proportions of mononucleated cells with one and with 2 or more MN for each group

		Carcinoma		Young		Old	
i	Dose (cGy)	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$
0	0	−0·0005	−0·0001	−0·0008	−0·0002	−0·0013	0·0000
1	20	−0·0027	0·0000	0·0012	0·0006	−0·0035	0·0004
2	50	0·0046	0·0019	0·0023	0·0001	−0.0025	−0·0004
3	100	−0·0009	−0·0010	0·0084	0·0000	0·0018	0·0000
4	200	0·0050	−0·0012	0·0121	−0·0005	0·0145	0·0011
5	300	0·0034	−0·0025	0·0248	0·0022	−0·0005	−0·0020
6	400	−0·0020	−0·0015	0·0006	−0·0028	0·0147	0·0016
7	500	−0·0035	0·0055	−0·0035	0·0008	−0·0180	0·0002

Table VII. Errors due to adjustment of the proportions of binucleated cells with one and with 2 or more MN for each group

		Carcinoma		Young		Old	
i	Dose (cGy)	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$	$\tilde{\varepsilon}_{i1}$	$\tilde{\varepsilon}_{i2}$
0	0	0·0000	0·0004	0·0013	0·0001	0·0032	0·0002
1	20	0·0002	−0·0022	−0·0062	0·0000	−0·0023	0·0019
2	50	0·0233	0·0133	0·0141	0·0047	−0·0066	−0·0002
3	100	−0·0261	−0·0036	0·0079	−0·0006	0·0066	−0·0013
4	200	−0·0285	−0·0045	−0·0281	−0·0269	0·0097	0·0048
5	300	−0·0062	0·0131	0·0064	−0·0009	0·0118	−0·0171
6	400	0·0081	−0·0094	−0·0119	0·0005	−0·0121	0·0046
7	500	0·0425	−0·0024	0·0106	0·0435	−0·0074	0·0147

cells with 0, 1 and 2 or more MN respectively. They show that there is no difference among the groups studied, which is what should be expected because, in this technique, the population of mononucleated cells represents cells that either have not entered division or that escaped the action of cytochalasin B, that is, divided and gave origin to two daughter-cells. Therefore, the analysis of this type of cells is not informative.

Figures 4, 5 and 6 show the adjusted curves for binucleated cells. To obtain the curves for π_{ij} we use relations (3) and (4). Note that, for the cases of no MN, the numerator of these relations is the unit. Binucleated cells are the ones that in fact have suffered the action of the drug. That is, they had a nuclear but not a cytoplasm division. Figure 4 shows that, in the three groups, the

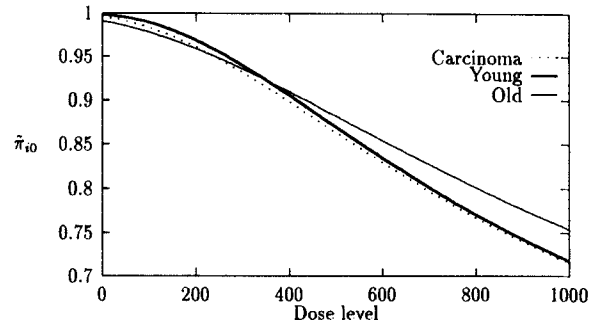

Figure 1. Proportion of mononucleated cells with zero MN adjusted for each group

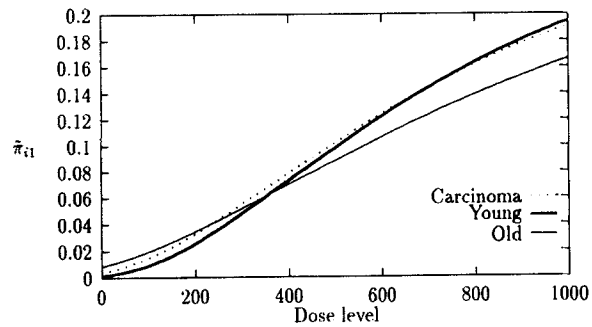

Figure 2. Proportion of mononucleated cells with one MN adjusted for each group

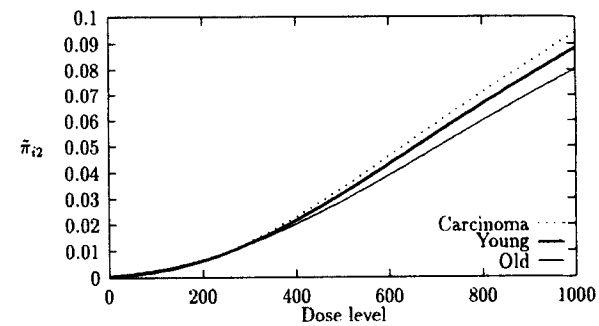

Figure 3. Proportion of mononucleated cells with two or more MN adjusted for each group

proportions of cells not affected by radiation, having no micronuclei, are not different. Figures 5 and 6 show that the carcinoma group differs from the other two which are similar. Note that, for the carcinoma group, while the proportion of cells with one MN decreases drastically, the proportion of the ones with two or more MN increases with the same intensity.

The conclusion is that the proportions of damaged cells are similar in the healthy and in the carcinoma patient groups. The extent of the damage, however, is different: in the carcinoma group the proportion of cells with one MN decreases more rapidly with the dose than in the two groups of healthy individuals. Concomitantly, for two or more MN, the situation is the reverse.

These results are expected since the purpose of using radiation for cancer therapy is to induce

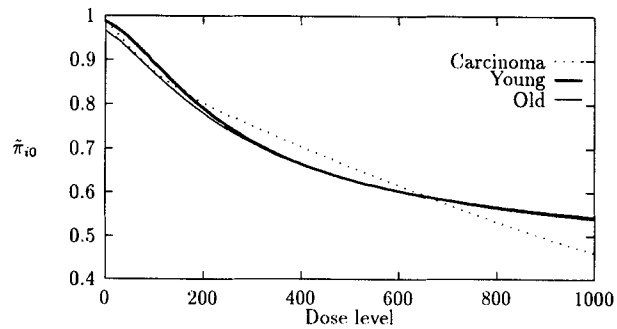

Figure 4. Proportion of binucleated cells with zero MN adjusted for each group

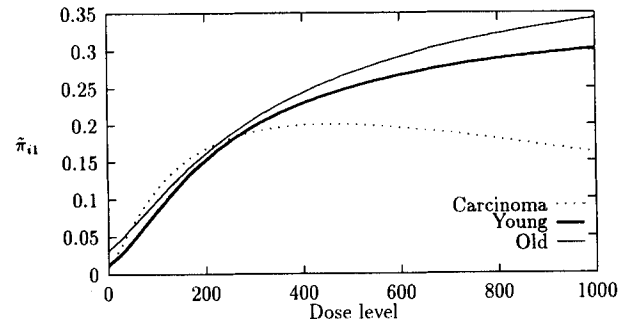

Figure 5. Proportion of binucleated cells with one MN adjusted for each group

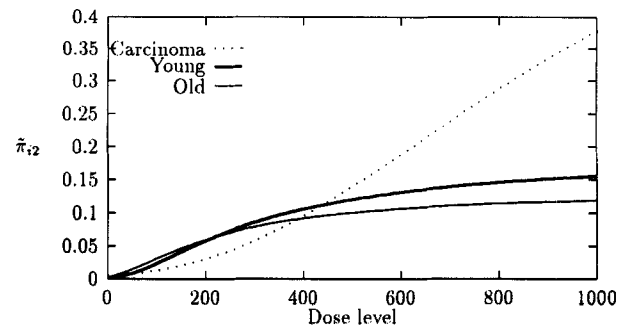

Figure 6. Proportion of binucleated cells with two or more MN adjusted for each group

cell death and prevent damage to pass from generation to generation of cells, with the development of the tumor. Cells which have been less damaged, with one MN, have a higher chance to survive than the ones with more damage, with two or more MN.

3. CREDIBILITY INTERVALS

The data analysed in Section 2 result from *in vitro* experiments where blood samples received controled radiation doses. In a more realistic situation, an individual is exposed to an unknown dose $\boldsymbol{d}$. To estimate this dose and define the appropriate medical conduct, a calibration experiment is designed. Blood cultures from other workers of the same environment, but not suffering the accidental exposure, are treated *in vitro* to known controlled doses. Responses $\boldsymbol{y}_d$ for each dose level are then obtained. With these experimental calibration data, a dose-response model, as described in Section 2, is adjusted and used in the estimation of $\boldsymbol{d}$. Only binucleated cells are considered as response vectors because, as mentioned before, they are more informative than mononucleated ones.

Let us consider three examples, each one is similar to the ones in the groups previouly analysed. The response for these individuals, one with carcinoma, one young healthy, and one old healthy, are, respectively, $\boldsymbol{y}_{dc} = (1408,412,607)$, $\boldsymbol{y}_{dy} = (1335,728,364)$, and $\boldsymbol{y}_{do} = (1310,801,316)$, with posterior parameters $\mathbf{A}_{dc} = (1413,415,608)$, $\mathbf{A}_{dy} = (1340,731,365)$, and $\mathbf{A}_{do} = (1315,804,317)$. Clearly, for the three cases, the prior distribution considered for parameter $= (\pi_0, \pi_1, \pi_2)$ is the Dirichlet with parameters (5,3,1). Due to the large number of cells observed, this prior can be considered non-informative for the problem.

Using now the posterior distribution of $\theta = (\theta_1, \theta_2)$, which is normal (as shown in Madruga *et al.*, 1994), and standard methods, we construct, for the three cases, the HPD regions, also known as credibility sets. Using relations (6), we obtain, from transformation, the corresponding doses for each point of this credibility set. This set will form an interval for the unknown doses. Clearly, there are some thechnical dificulties since one goes from the space to the real line, but this can be overcome by using well known procedures.

With the data described here, we have obtained the following credibility intervals for doses: 1, carcinoma subject (685;748), with credibility 0·9319; 2, young subject (830;1200), with credibility 0·9411; and 3, old subject (815;1210), with credibility 0·9539.

Note that the calibration experiment is made *in vitro* and the cells are exposed directly to radiation. In the case of a nuclear accident, there are several biological barriers to be crossed before radiation actually reaches the blood. Thus, the numbers presented above may, in fact, be an under-estimate of the real dose of exposure. The estimated doses are an idealization of what might have occurred if the individual had suffered an *in vitro* exposure. The next challenge then is to build a function that relates dose-response *in vitro* to dose-response *in vivo*. In this way we will be able to generalize the results from laboratory experimental results to actual life.

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