A note on Poisson goodness-of-fit tests for ionizing radiation induced chromosomal aberration samples

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\section*{ABSTRACT}

\textbf{Purpose:} To present Poisson exact goodness-of-fit tests as alternatives and complements to the asymptotic \(u\)-test, which is the most widely used in cytogenetic biodosimetry, to decide whether a sample of chromosomal aberrations in blood cells comes from an homogeneous or inhomogeneous exposure.

\textbf{Materials and Methods:} Three Poisson exact goodness-of-fit test from the literature are introduced and implemented in the R environment. A Shiny R Studio application, named GOF Poisson, has been updated for the purpose of giving support to this work. The three exact tests and the \(u\)-test are applied in chromosomal aberration data from clinical and accidental radiation exposure patients.

\textbf{Results:} It is observed how the \(u\)-test is not an appropriate approximation in small samples with small yield of chromosomal aberrations. Tools are provided to compute the three exact tests, which is not as trivial as the implementation of the \(u\)-test.

\textbf{Conclusions:} Poisson exact goodness-of-fit tests should be considered jointly to the \(u\)-test for detecting inhomogeneous exposures in the cytogenetic biodosimetry practice.

\section*{KEYWORDS}
Exact tests; dispersion index; frequency of zero counts; homogeneity; chromosomal
1. Introduction

Counts of dicentric chromosomes in peripheral blood lymphocytes allow distinguish between homogeneous and non-homogeneous irradiations in biological dosimetry. Chromosome alterations are produced randomly after an homogeneous irradiation. It is typically assumed, for low linear energy transfer exposures, the distribution of dicentrics per blood cell follows a Poisson distribution (Edwards et al. 1979) and in this sense the detection of overdispersion (variance greater than the mean) indicates non-homogeneous irradiations. The so-called $u$-test (IAEA 2011), which is a normalized unit of the dispersion index (the ratio of the variance to the mean), is the classical way to check whether a sample of dicentrics follows a Poisson distribution and was initially introduced for biological dosimetry studies by (Savage 1970). The Poisson distribution assumes the variance is equal to the mean and consequently the value of the $u$ statistic is 0. The capacity to determine the absorbed dose by the irradiated fraction have been assessed both in partial irradiation simulations (Lloyd et al. 2000; Barquinero et al. 1995) and in dose reconstruction after radiation accidents (Beinke et al. 2015). It is considered that a correct estimation of an absorbed dose requires at least the analysis of 500 blood cells or to score at least 100 dicentrics (IAEA 2011). However, after an accident with multiple victims the ability of blood samples analysis of a laboratory may be affected. One strategy to face this problem is to decrease the number of analyzed cell for each individual and consequently reduce the time involved to a first categorization. This categorization is based on a not very accurate dose estimation. However, it allows to make a useful initial threshold to optimize the clinical response to the radiation accident (IAEA 2011). To analyze less blood cells implies to score less dicentrics. This decreasing of the scored dicentrics implies a higher difficulty to correctly detect over-dispersion. Goodness-of-fit tests for Poisson samples with small counts was initially analyzed by (Fisher 1950; Rao and Chakravarti 1956).

There are several goodness-of-fit tests to assess whether a count sample follows a Poisson distribution. These tests are based on different properties of the Poisson
model, as the equi-dispersion (the variance equals to the mean) and the frequency of zeros. The general asymptotic goodness-of-fit test (the classical $\chi^2$-test) for the Poisson distribution is not used here because it has been proved to be inaccurate for small samples, particularly for the case of chromosomal aberration counts (Rao and Chakravarti 1956; Savage 1970; Merkle 1981). The $u$-test is asymptotic, because it is not based on the exact distribution of a measure (for instance the dispersion index) and it is based on the normal approximation of an exact distribution. Concretely, (Merkle 1981) claimed about the usage of exact test for samples with a small total of counts for the analysis of dicentric chromosomes, in contrast to the approximate tests, like the $u$-test, due to the discrepancy of values. Exact tests and their asymptotic versions about different properties of the Poisson distribution (equidispersion or the probability of the number of zeros given the sample sum) are referred in this work. Application examples will be shown based on radiation induced chromosome aberration from radiological accidents and clinical applications of biological dosimetry. The first step here is to know the probability of a Poisson realization, given the sample sum.

2. Probability of a Poisson realization conditional to the sample sum

Given $n$ Poisson realizations $X = \{x_1, x_2, \ldots, x_n\}$ represented by its frequencies $F = \{f_0, f_1, f_2, \ldots, f_m\}$, i.e. $f_0$ is the number of 0’s, $f_1$ is the number of 1’s and so ($m$ is the maximum observed count). The probability of the frequencies $F$ given the sample sum $S = \sum_{i=1}^{n} x_i$ was studied by (Fisher 1950),

$$P(F|S) = \frac{n!}{\prod_{i=0}^{m} f_i!} \cdot \frac{S!}{\prod_{i=1}^{m} i^{f_i} \cdot n^S}.$$  

(1)

For instance, the probability of a sample of 50 Poisson realizations of all 0’s except one with 2 counts following Equation (1) is

$$P(\{f_0 = 49, f_2 = 1\}) = \frac{50!}{49! \cdot 1!} \cdot \frac{2!}{2! \cdot 50^2} = \frac{1}{50} = 0.02.$$
This means that for a Poisson sample with sample size 50 and sum 2, the probability of a sample with 49 realizations equals to 0 and 1 equals to 2 is 0.02. The other choice of frequencies is 2 realizations equals to 1 and the rest of them 0, and consequently its probability is \( P(\{f_0 = 48, f_1 = 2\}) = 1 - P(\{f_0 = 49, f_2 = 1\}) = 0.98 \).

3. Exact tests for Poisson distribution

The exact \( D \)-test proposed by (Fisher 1950) is derived from the classical \( \chi^2 \)-test, and consists on the cumulative exact probability of the so-called \( D \)-statistic, \( D = \sum (x_i - \bar{x})^2 / \bar{x} \). Note \( D = (n-1) \cdot \text{var}(X) \), and both the variance and the the sample square sum (\( D' = \sum x_i^2 \)) are equivalent statistics. The \( u \)-statistic is the asymptotic typified normal approximation of the \( D \)-statistic, and was applied by (Papworth 1983) to chromosomal aberrations frequencies. For the purpose to compare with the \( D \)-test in checking over-dispersion, here the \( u \)-test is going to be used as right-tailed, in contrast to the most used two-tailed in biodosimetry.

Later (Rao and Chakravarti 1956) proposed the frequency of zero observations and the statistic \( L' = \sum x_i \log x_i \) for exact tests. The frequency of zero test checks for reliable zero proportion and the \( L' \)-test checks the homogeneity of data, i.e. the Poisson distribution means of all realization are equal. The \( L' \)-test is based on the likelihood ratio test and in (Rao and Chakravarti 1956) it is stated that “is preferable to \( D \) for discriminating a compound Poisson distribution from a simple Poisson”. The \( f_0 \) test has been recently studied in depth by (Fernández-Fontelo et al. 2017).

Fisher designed the exact test considering all the possible samples with equal size and total. For instance, if we observe 3 chromosomal aberrations in 50 blood cells, there are three different samples with size 50 which sum 3, see Table 1.

Each distribution has an associated statistic \( D \) (or equivalently \( D' \)), \( L' \) and \( f_0 \), and following Expression 1 each distribution has an associated probability. To contrast (under-) over-dispersion for \( X \), it is calculated the cumulative probability of the realizations with (lower) greater or equal \( D \). Analogously, to contrast zero-inflation (-deflation), it is calculated the cumulative probability of the realizations with greater (lower) or equal \( f_0 \). And to test heterogeneity, it is calculated the cumulative prob-
ability of the realizations with greater or equal \( L' \). Note \( L' \) test only makes sense as right-tailed to check for heterogeneity. In Table 1 it is showed the associated probabilities for \( f_0 \), \( D' \) and \( L' \) are the same, but this is not a general rule.

4. Application of the exact \( D' \) and \( L' \) tests to chromosomal aberrations data

The exact \( D \) test was applied to samples of chromosomal aberrations by (Papworth 1983) to validate the p-values obtained from the \( u \)-test, (Savage 1970). Another application of the exact \( D \)-test was performed by (DuFrain et al. 1980) to over-dispersed data of chromosomal aberrations in human lymphocytes exposed to \( \alpha \)-particles. (Merkle 1981) applied the exact test to investigate the behavior of several test for the Poisson goodness of fit. A computer program for the exact test was published in (Papworth 1983) based on the test proposed by (Fisher 1950) and the existence of the program was mentioned in (Merkle 1981) as a note added in proof. The program was written in FORTRAN language to obtain the exact probability values (Papworth 1983). This program has the particular characteristic of flexibility that allows the user to change the hypothesis direction and to use a different statistic instead of \( D \). More recently, in (Fernández-Fontelo et al. 2017) the authors studied the properties of the exact test based on the \( f_0 \) statistic for the cytogenetic biodosimetry practice. This paper provides a software support in form of a Shiny RStudio application called GOF Poisson. This application, free available at http://asapps.bcamath.org:5053/, has been updated for supporting this publication too. It includes the one-tailed exact tests for the \( f_0 \), \( D' \) and \( L' \) statistics, among others.

5. Asymptotic \( u \)-test in the case of small counts of aberrations

In the Appendix authored by D. G. Papworth in (Savage 1970) is suggested as a minimum requirement for the \( u \) test to be valid that at least one of the quantities the yield \( y = S/n \) or the sample size \( n \) to be large. The case of \( y \ll 1 \) is usual at low doses in cytogenetic samples, and then the most likely outcome is that the total number of
aberrations observed is made up exclusively of cells with only one aberration in it, even for large $n$. For small counts the true distribution of $u$ is therefore heavily skewed to the right and does not approach the normal distribution for any practically feasible $n$ (Merkle 1981). Graphically, the assumption of normal distribution of $D$-statistic (the $u$-statistic is based in this asymptotic approximation) can be appreciated in the Figure 1 for $n = 500$ and in the Figure 2 for $n = 50$. The $y$ values lower than 0.1 represent samples of chromosomal aberrations from 0 to 50 aberrations in the conventional scoring of 500 metaphases. In relation to radiation doses, $y << 1$ represent doses lower than 1 Gy for the case of Co-60 or X-rays irradiation. When $y > 0.1$ some deviations from normality are appreciated.

6. Applied examples

The right-tailed test analyzed in this work are applied in data from recent literature and unpublished data (samples 4, 5, 6 and 7 in Table 2). Those p-values lower than 0.001 are represented by the lowest power of 10 which is bigger than the p-value.

6.1. Cases in accidental and clinical radiation exposures

Table 2 shows several cytogenetic examinations of overexposed individuals’ blood lymphocytes.

Sample 1 is the dicentric distribution from the accidental $\gamma$-radiation exposure of an industrial radiography described by (Beinke et al. 2015). Samples 2 and 3 are the dicentric plus ring distribution of the same patient analysed by two different laboratories of the 2011 radiation accident suffered in Stamboliyski (Bulgaria) (Grégoire 2013).

Samples 4 and 6 come from patients with differentiated thyroid cancer (DTC) treated with $^{131}I$therapy, within the therapeutic scheme following thyroidectomy, for the ablation of thyroid remnants and treatment of metastatic disease. The aim of this therapy is to achieve a lethal dose in the tumor tissue, without exceeding the dose of tolerance in healthy tissues (doses > 2 Gy in bone marrow could lead to myelotoxicity). Absorbed dose to the whole body and bone marrow by applying biological dosimetry
techniques, including the evaluation of dose distribution in the body, contributed to optimize the $^{131}$I therapeutic administration.

Sample 5 comes from the biodosimetric evaluation of a radiological accident occurred in Argentina, with an industrial gammagraphy source of $^{192}$Ir (32 Ci).

Sample 7 corresponds to the data of the Biological Dosimetry of a Nuclear Power Plant worker in Argentina due to an incident of internal contamination with tritium.

The p-values of the right-tailed $f_0$, $u$, $D'$ and $L'$ statistics are also shown. Although most $f_0$, $u$, $D'$ and $L'$ p-values agree in rejecting or not the Poisson assumption (for a 5% significance level), the difference between the asymptotic test $u$ and the exact tests $f_0$, $D'$ and $L'$ is notorious except for samples 2 and 3 (note they have mean greater than 1).

The distribution of $u$ is simulated for samples 1 (Figure 3) and 2 (Figure 4) in Table 2 in three ways. In all them the $u$-statistic is calculated from samples which simulate the dicentric distribution of both samples. These samples are simulated by:

- $n$ Poisson draws with intensity $y$;
- a multinomial draw for $S$ trials within $n$ elements with equal event probabilities;
- $n$ resamples with substitution from the original sample.

In figure 3, the three histograms present a significant departure from the typified normal distribution. This indicates the normal asymptotic approximation of the $D$-test is not appropriate. The histogram of the resampling draws looks different to the other, which are based on the Poisson assumption and its maximum likelihood estimator (the sample mean) and the Poisson assumption conditional to its sufficient statistic, the sample sum. This difference is also indicating that the Poisson assumption is not appropriate for this sample.

In figure 4, the two first histograms are closer to the the typified normal distribution. Again, the histogram of the resampling draws looks different to the other, which is indicating that this sample is not over-dispersed Poisson, but it is under-dispersed, but this is not analysed in this work.

Attending sample 1 in Table 2, the probability of observing a Poisson sequence of size 2048 and a total count of 7 with $D' \geq 9$ is 0.01. This rejects the null hypothesis of
equi-dispersion, for a 5% significance level. The \( u \)-test also rejects this null hypothesis, but is not approximating accurately to the distribution probability of the statistic \( D \).

The \( u \)-test p-values for samples 2 and 3 are closer to the \( D' \)-test ones, despite sample sizes are not big, they have sample mean over 1. This agrees with (Merkle 1981), where it is indicated the \( u \)-test approximates better for higher yield.

Sample 4 is the unique case of the table with discrepancy between the significance of both \( u \)- and \( D' \)-test, for a 5% significance level. The \( D \)-test does not reject the Poisson assumption, but the p-value is low, 0.055, whereas the \( u \)-test p-value clearly rejects the assumption, \((<10^{-4})\).

In samples 5, 6 and 7 the Poisson assumption is clearly rejected by all tests, but again, the differences between the \( u \)-test with the three exact tests p-values are huge.

6.2. RTGene project data

In the study (Moquet et al. 2018), dicentric assay data was analyzed from 20 radiotherapy patients. Table 3 shows the dicentric distributions of these patients and the p-values of \( f_0 \), \( u \), \( D' \) and \( L' \) tests. Again, those p-values lower than 0.001 are represented by the lowest power of 10 which is bigger than the p-value.

In this example all \( f_0 \), \( u \), \( D' \) and \( L' \)-test p-values agree in rejecting or not the Poisson assumption (for a 5% significance level), the differences between the asymptotic one and the three exact are notorious in all samples except sample 6.

7. Final remarks

For small sample sizes, \( i.e. \ y < 0.1 \), the exact and asymptotic p-values can be quite different and can lead to different conclusions about the hypothesis of interest. Moreover the normal distribution assumption of \( u \) statistic if not meet when \( y < 0.1 \). In the case of very small counts of aberrations the simulation performed confirms the Merkle concerns about the behavior of \( u \)-statistic and the use of exact p-values for the Poisson equi-dispersion hypothesis is highly recommended.

In the duality of the exact \( D \) test and its approximation, the \( u \)-test, authors recommend to use both. Of course, the \( D \) test is more accurate, but the \( u \)-test tends to
be more sensitive in detecting inhomogeneous exposures, and it has been applied for decades in biodosimetry, so the experience of cytogenetic experts using this test, is an added value for it. The $f_0$ and $L$ tests are respectively focused on the zero proportion (for detecting partial body irradiation) and the homogeneity of data (for detecting inhomogeneous exposure), so they should be considered too. Over-dispersion, zero-inflation and heterogeneity are closely related, so in many scenarios, the three exact tests lead to the same conclusion.

These methodologies provide information that may be useful to guide medical treatment in cases of inhomogeneous exposures since it would allow correlating this information with residual hematopoiesis in cases of victims who manifest the haematopoietic form of the acute radiation syndrome.

The R files which reproduce the results in this work are available under request to the corresponding author.

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Disclosure statement

The authors report no conflicts of interest.

8. References

References


Figure 1. Cumulative probabilities of the normalized (solid line) and the simulated ($10^5$ draws) exact (circles) D-statistic for sample size $n = 500$ and different yield values $y = \{0.06, 0.1, 0.2, 0.5, 1, 3\}$. 
Figure 2. Cumulative probabilities of the normalized (solid line) and the simulated (10^6 draws) exact (circles) D-statistic for sample size n = 50 and different yield values y = \{0.06, 0.1, 0.2, 0.5, 1, 3\}.
Figure 3. Histogram of $10^4$ simulations of $u$-statistic by three different methods of drawing the true sample in (Beinke et al. 2015). Dashed lines represent the typified Gaussian density.
Figure 4. Histogram of $10^5$ simulations of $u$-statistic by three different methods of drawing the true sample. 3. Dashed lines represent the typified Gaussian density.
Table 1. Possible distributions of counts for sample of size 50 and total 3.

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<tr>
<th>$f_0$</th>
<th>$f_1$</th>
<th>$f_2$</th>
<th>$f_3$</th>
<th>$D'$</th>
<th>$L'$</th>
<th>Probability</th>
<th>Cumulative probability</th>
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Table 2. Chromosomal aberrations distributions of different in vivo accidental and clinical exposures.

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<th>$f_1$</th>
<th>$f_2$</th>
<th>$f_3$</th>
<th>$f_4$</th>
<th>$f_5$</th>
<th>$S$</th>
<th>$n$</th>
<th>$y$</th>
<th>$u$ (p-value)</th>
<th>$D'$ (p-value)</th>
<th>$L'$ (p-value)</th>
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<td>250 (0.604)</td>
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<td>1.386 (0.055)</td>
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<td>1000</td>
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<td>59.93 ($&lt;10^{-323}$)</td>
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