Accuracy of \(p53\) Codon 72 Polymorphism Status Determined by Multiple Laboratory Methods: A Latent Class Model Analysis

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Abstract

**Introduction:** Studies of the association of a polymorphism in codon 72 of the \(p53\) tumour suppressor gene (rs1042522) with cervical neoplasia have inconsistent results. While several methods for genotyping \(p53\) exist, they vary in accuracy and are often discrepant.

**Methods:** We used latent class models (LCM) to examine the accuracy of six methods for \(p53\) determination, all conducted by the same laboratory. We also examined the association of \(p53\) with cytological cervical abnormalities, recognising potential test inaccuracy.

**Results:** Pairwise disagreement between laboratory methods occurred approximately 10% of the time. Given the estimated true \(p53\) status of each woman, we found that each laboratory method is most likely to classify a woman to her correct status. \(\text{Arg}/\text{Arg}\) women had the highest risk of squamous intraepithelial lesions (SIL). Test accuracy was independent of cytolgy. There was no strong evidence for correlations of test errors.

**Discussion:** Empirical analyses ignore possible laboratory errors, and so are inherently biased, but test accuracy estimated by the LCM approach is unbiased when model assumptions are met. LCM analysis avoids ambiguities arising from empirical test discrepancies, obviating the need to regard any of the methods as a "gold" standard measurement. The methods we presented here to analyse the \(p53\) data can be applied in many other situations where multiple tests exist, but where none of them is a gold standard.

Citation: Walter SD, Riddell CA, Rabachini T, Villa LL, Franco EL (2013) Accuracy of \(p53\) Codon 72 Polymorphism Status Determined by Multiple Laboratory Methods: A Latent Class Model Analysis. PLoS ONE 8(2): e56430. doi:10.1371/journal.pone.0056430

Discussion:

A polymorphism (rs1042522) in codon 72 of the \(p53\) tumour suppressor gene (Arg\(72\)Pro) has been extensively studied as a risk factor for cervical cancer and its precancerous lesions, but the results have been largely inconsistent [1,2]. Although a variety of laboratory methods for establishing \(p53\) status exist, they vary with respect to their reliability, in terms of inherent accuracy and under different assay and specimen conditions. Using more than one method can lead to discrepancies in the estimate of a subject's \(p53\) codon 72 genotype, which leads to irreolvable ambiguities about how one should best estimate the association between this genetic marker and cervical neoplasia. A recent editorial [3] has suggested that problems of this type are widespread in genetic testing. In the face of these ambiguities, it is not clear which method (if any) might be preferred to assess this association, or which might provide the best estimate of the disease odds ratio (OR). Furthermore, because empirical estimates of the association ignore the possibility of test errors, they are inevitably subject to bias. By using a latent class model (LCM) that incorporates information from multiple imperfect tests, we can evaluate the accuracy of alternative test methods, and improve the assessment of the association of the genetic marker with disease. In this paper, we develop a LCM approach to this type of data, and illustrate the results from a study of the relation between \(p53\) polymorphism and disease.

We previously investigated the variation between laboratories in determining \(p53\) genotype from split samples originating from the same sample of women enrolled in a case-control study [4]. In that work, three international laboratories used the same, standardised laboratory procedure to ascertain the genotype. Discrepancies between laboratory results for the same woman were not infrequent, leading to uncertainty about how to best represent the association of \(p53\) with cervical cancer. Our analytical approach was to use a LCM [5–11], which avoids the need to regard any of the laboratories as a gold standard or in any way superior to the others, but instead takes the inaccuracies of each laboratory into account. The sensitivity and specificity of each
laboratory’s data were estimated by the LCM, and the model also adjusted the disease OR for the laboratory measurement errors. LCMs have been widely used in many areas of medical research for situations in which a gold standard diagnosis of disease cannot be achieved, or where there are problems involved in measuring risk factors. The statistical estimation of the LCM model parameters is based on maximum likelihood, which implies some optimality in the results. Specifically, under a correctly specified model, maximum likelihood estimates are asymptotically unbiased and have maximum precision. The LCM also avoids the ambiguities caused by discrepancies in test results that occur when multiple tests are used.

In this paper, we describe several LCMs used to examine the discrepancies between six competing laboratory methods for p53 determination, all conducted blindly by the same laboratory. The model strategy provides estimates of accuracy for each alternative test method, even though all of them are potentially subject to error. The strength of the method is that, in doing so, we are not required to incorrectly assume that any of the methods represents a preferred or ‘gold’ standard measurement.

As a secondary objective we examine the association of p53 with cervical abnormalities found on cytology, again taking inaccuracy of the data into account. Finally, we compare the results yielded by the latent class models with the empirical associations. The latter ignore the possibility of laboratory errors; again, this is obviously problematic, because the existence of discrepancies in test results for the same woman indicates that errors do in fact occur, and which therefore bias the empirical OR estimates.

The methods we propose are generalizable to other situations where multiple, imperfect tests exist and when it is unreasonable to assume a gold standard measure.

Materials and Methods

Epidemiological Study

The original study cohort consists of 2528 women aged 18–60 years (median = 33 years) from a maternal and child health program for low income families in Sao Paulo, Brazil. The racial composition of the study was comparable to that in the source population. Most women were of white (European), black, and mixed (mulata) ancestry, with less than 2% being of other ancestries. Cervical and blood samples were obtained, together with questionnaires, at the initial enrolment into the study, at follow-up visits every four months during the first year, and then twice yearly thereafter for at least eight years (with questionnaires at annual follow-up returns). This sampling was done on an unselected basis from the population of screened women, and hence is free from genetic selection bias. At the time the specimens were tested for this report there had been 24,545 visits from 2462 women, with a mean of ten visits per subject and 149,184 women months of follow-up (mean = 61 months, median = 76 months). We limited the dataset to women who had at least four out of six possible visits in the first two years of follow-up. More information on the study design and methods for the original study can be found elsewhere [12].

The outcome of interest in the Ludwig McGill study was two-year cumulative risk of squamous intraepithelial lesions (SIL). We chose to exclude women whose worst cytology outcomes were Atypical Squamous Cells of Undetermined Significance (ASCUS) or Atypical Glandular Cells of Undetermined Significance (AGUS), because their results lie somewhere between the case and non-case outcomes used in our analysis. We felt that there would not be a benefit to including ASCUS and AGUS because these are ambiguous cases in which cytology was equivocal. We believe that the best comparison to examine effects of the polymorphism is by contrasting the non-equivocal categories of lesion outcome. The polymorphism in question is not a marker of disease but potentially of susceptibility to HPV and in consequence to disease.

p53 Codon 72 Genotyping

We assayed DNA samples from 963 women, comprising those with long term follow-up and complete cytological and HPV DNA testing results at the end of 2002, after the exclusions described above. Details of the genotyping methods are described elsewhere [14]. In brief, we amplified a 279 base-pair fragment of exon 4 of p53 by a polymerase chain reaction protocol (PCR). P53 codon 72 genotyping of the amplified products was done in all samples by four methods: (i) denaturing high-performance liquid chromatography (DHPLC), (ii) dot blot hybridization (DBH) with sequence-specific oligonucleotide probes for p53Arg and p53Pro, (iii) restriction fragment length polymorphism (RFLP) analysis after cleavage of the PCR products with the BstUI endonuclease (RFLP-1) and (iv) with the BstUI endonuclease (RFLP-2). Two additional genotyping assays were also conducted in a subset of 144 women for whom sufficient DNA was available for extended testing. These include direct sequencing (DS) of the exon 4 amplified product and allele-specific PCR (AS-PCR).

Our analysis focused on the first four laboratory methods (DHPLC, DBH, RFLP-1, and RFLP-2). However, other analyses were carried out incorporating the additional data for the subset of women in which the DS and AS-PCR methods were also performed.

No research has been done on the determinants of error for these methods, however a separate paper by our laboratory colleagues describes the relative value of these methods from an assay performance perspective [14]. They each represent a balance between simplicity of the assay versus biological accuracy (i.e., sensitivity and specificity for the nucleotide sequences that identify the genotypes). Other than the usual work done to establish each technique in the laboratory to make sure that they work suitably based on performance standards and appropriate controls, there has been no systematic work on the repeatability of the assays in our lab or, to our knowledge, in other labs. That being said, these techniques represent legitimate alternative ways of analyzing p53 codon 72 polymorphism (rs1042522).

Statistical Analysis

We first examined the pattern of disagreements between the results of the alternative test methods. Crude and chance-corrected levels of agreement were calculated using weighted kappa statistics using Cicchetti and Allison weights [15,16]. In our primary analyses, we considered the genotype results as a variable with 3 levels (Arg/Arg, Arg/Pro, and Pro/Pro). The Arg/Arg genotype has been associated with increased risk of cervical neoplasia in many previous studies [1,2].
In our latent class models, a woman’s true \( p53 \) status is the latent (unobserved) variable, and the probability of being classified into the three possible levels of the \( p53 \) status by each method is estimated for each woman, conditional on her true status. These probabilities are then combined over the various possible levels of the actual status, according to the model specifications; this combination forms the contribution to the overall likelihood of the data from a particular woman. For instance, if the tests are assumed to be independent, the likelihood contribution is made up of a set of products of probabilities of the individual test results, with each set corresponding to the actual \( p53 \) status of a given woman; the sets are summed across levels of actual \( p53 \) status, using weights that correspond to the prevalences of the various \( p53 \) genotypes.

Finally, following standard maximum likelihood methods, the logarithms of the likelihood contributions are summed over study participants, and this sum is maximised numerically to obtain estimates of the model parameters. The model parameters include the probabilities of particular test results for a woman with given \( p53 \) status, and the prevalence of the various \( p53 \) genotypes in the sample. Optionally, correlations between the test errors for different methods can also be incorporated. Further technical details of the latent class model approach are given elsewhere [6,11].

Our Model 1 uses the results from the four main laboratory methods used in the study (DHPLC, DBH, RFLP-1, RFLP-2) without assuming any of them to be more or less accurate than the others. The sensitivity and specificity of each test is estimated, without assuming any of them to be more or less accurate than the methods used in the study (DHPLC, DBH, RFLP-1, RFLP-2) assumption is later relaxed in a further analysis, as described below.

Analyses of Robustness

To check the robustness of our model results and examine model assumptions, several secondary analyses were carried out. First, Model 1 was repeated after excluding cases of SIL from the dataset, and changes in the model estimates were noted. Second, Model 1 was repeated using all women in the study, so that they were not excluded based on their cytology status or number of visits.

Third, Models 1 and 2 were repeated to allow test accuracy to vary by cytology status, with likelihood ratio tests being used to assess the statistical significance of this variation. For completeness, we also fitted Models 1 and 2 with test accuracy constrained to be constant across tests; likelihood ratio tests were again used to test the hypothesis of constant accuracy.

Fourth, Models 1 and 2 assume that the tests are conditionally independent, given the true \( p53 \) status of an individual. To test this assumption, we fit extended LCMs that added correlation terms to represent the conditional associations of pairs of test results for women in each of the \( p53 \) groups. Likelihood ratio tests were used to evaluate if the model fit was significantly improved by the inclusion of these correlations.

Finally, two further sets of analyses were carried out with the \( p53 \) polymorphism being re-grouped into only two categories (first, into Arg/Arg vs. others, and second, into Pro/Pro vs. others), these being consistent with the expectation of risk associations as reported in the literature [2]. This was done because although the total sample size in this study was substantial, when polymorphism status was analysed at three levels for each test, some cross-classified cell frequencies were nevertheless small (particularly when the cytology result was included in the model), leading to some model instability and convergence problems. Aggregation of the data into fewer test levels alleviated many of these difficulties, while still providing useful results.

Results

Our analyses were conducted with the R statistical package [17] and the software program LEM [18]. \( R \) was used to examine test agreement, while LEM was used to run all of the models, including both the LCMs and the empirical models that do not allow for test inaccuracy.

Agreement of Test Results

Table 1 shows cross-tabulations of the \( p53 \) results from each pair of the 4 main laboratory tests, subdivided by cytology status, and with \( p53 \) taken as a variable with 3 levels for the test result. Although the majority of these pairwise test results are concordant, discrepancies were not uncommon.

Table 2 summarises the pairwise test data in terms of crude and chance corrected agreement levels. The crude agreement was 90% or better in all cases, while the weighted kappa statistics ranged between 0.84 and 0.92. While this pattern is encouraging overall, the disagreements between pairs of tests that occurs for approximately 10% of women is clearly problematic. Furthermore, when more than two tests are done, the chance of disagreement between any two tests increases substantially.

Accuracy of Laboratory Test Methods

Table 3 shows the results of the latent Model 1 fitted to the available data from four main tests (DHPLC, DBH, RFLP-1, and RFLP-2). Each cell in the table shows the estimated classification probability for each of the three possible genotype test results (i.e., Arg/Arg, Arg/Pro, Pro/Pro), for each laboratory test and for each of the three possible genotype status levels. For instance, women who are truly Arg/Arg are estimated to have a 96.4% chance of being classified as Arg/Arg by the DHPLC method, a 2.8% chance of being classified as Arg/Pro, and a 0.8% chance of being classified as Pro/Pro.

For each of the tests, the model suggests that the most likely classification of any given woman is to her correct \( p53 \) status. To clarify, the LCM estimates the probability that a woman’s true status is each of: Arg/Arg, Arg/Pro and Pro/Pro. The estimate with the highest probability, conditional on her true status, is the most likely test classification for a woman with that true status. We found that across the entire sample, the estimated true status is equal to the most likely test classification. Furthermore, the overall accuracy is reasonably high. The accuracy is high for all three \( p53 \) genotypes for tests DHPLC and DBH. For test RFLP-1,
performance is slightly inferior to the other tests for women who are truly Pro/Pro; they have approximately a 10% chance of being incorrectly classified as Arg/Pro, and an accuracy of only 89% with this method. For test RFLP-2, the classifications are less accurate for Arg/Arg women, for whom there is an accuracy of only 84%; women in this group may be classified as Arg/Pro with probability approximately 14%. Note that all of the standard errors for these estimated probabilities are small, mostly in the range of 1–2%. However, some caution should be exercised in interpreting these results, because a few model parameters were on a boundary, with an associated zero probability of misclassification for certain data combinations (e.g. the incorrect classification of Arg/Pro women into the Pro/Pro category by test DHPLC).

It is reassuring that the most likely classification of a given woman by a laboratory test is to her true p53 genotype status. Moreover, it is interesting to note that the least likely classification for homozygous Arg/Arg women is to its most dissimilar result (Pro/Pro), and vice versa, with intermediate probabilities for the heterogeneous Arg/Pro group.

**Genotype – Cytology Relationship**

Table 4 shows estimates of the cumulative two-year risk of SIL for each level of p53, based on Model 2. Also shown are the associated ORs and standard errors from this LCM, for the Arg/Arg and Arg/Pro groups against the Pro/Pro reference category. The results indicate that the homozygous Arg/Arg women have the highest risk, with an OR of 1.89, while women in the heterozygous

| Table 1. Cross tabulation of genotyping test results across four laboratory methods and by cytology status. |
| --- |
| **Cytology Status** |
| **Negative Cytology (SIL = 1)** | **Any Grade SIL (SIL = 2)** |
| **First Test Rating** | **Second Test Rating** | **First Test Rating** | **Second Test Rating** |
| Arg/Arg | Pro/Arg | Pro/Pro | Missing | Arg/Arg | Pro/Arg | Pro/Pro | Missing |
| First test: DHPLC | | | | | | | |
| Arg/Arg | 308 | 5 | 8 | 0 | 31 | 0 | 1 | 0 |
| Second test: DBH | Pro/Arg | 9 | 365 | 9 | 0 | 1 | 21 | 1 | 0 |
| Pro/Pro | 3 | 4 | 139 | 0 | 0 | 0 | 6 | 0 |
| First test: DHPLC | Arg/Arg | 299 | 15 | 7 | 0 | 30 | 1 | 1 | 0 |
| Second test: RFLP-1 | Pro/Arg | 18 | 360 | 5 | 0 | 1 | 21 | 1 | 0 |
| Pro/Pro | 5 | 15 | 126 | 0 | 0 | 1 | 5 | 0 |
| First test: DHPLC | Arg/Arg | 258 | 49 | 13 | 1 | 29 | 1 | 2 | 0 |
| Second test: RELP-2 | Pro/Arg | 9 | 361 | 13 | 0 | 0 | 22 | 1 | 0 |
| Pro/Pro | 4 | 2 | 140 | 0 | 0 | 0 | 6 | 0 |
| First test: DBH | Arg/Arg | 302 | 16 | 2 | 0 | 31 | 1 | 0 | 0 |
| Second test: RFLP-1 | Pro/Arg | 16 | 355 | 3 | 0 | 0 | 21 | 0 | 0 |
| Pro/Pro | 4 | 19 | 133 | 0 | 0 | 1 | 7 | 0 |
| First test: DBH | Arg/Arg | 262 | 50 | 7 | 1 | 29 | 2 | 1 | 0 |
| Second test: RFLP-2 | Pro/Arg | 7 | 354 | 13 | 0 | 0 | 21 | 0 | 0 |
| Pro/Pro | 2 | 8 | 146 | 0 | 0 | 0 | 8 | 0 |
| First test: RFLP-1 | Arg/Arg | 266 | 46 | 9 | 1 | 28 | 2 | 1 | 0 |
| Second test: RFLP-2 | Pro/Arg | 3 | 365 | 22 | 0 | 1 | 21 | 1 | 0 |
| Pro/Pro | 2 | 1 | 135 | 0 | 0 | 0 | 7 | 0 |

**Table 2. Pairwise comparison of agreement among laboratory methods using crude agreement percentages and kappa coefficients.**

| Laboratory methods compared | Crude Agreement (%) | Kappa Coefficient (ASE) |
| --- | --- | --- |
| | | Unweighted | Weighted |
| DHPLC and DBH | 0.95 | 0.93 (0.01) | 0.92 (0.04) |
| DHPLC and RFLP-1 | 0.92 | 0.88 (0.01) | 0.88 (0.04) |
| DHPLC and RFLP-2 | 0.90 | 0.84 (0.02) | 0.84 (0.04) |
| DBH and RFLP-1 | 0.93 | 0.89 (0.01) | 0.9 (0.04) |
| DBH and RFLP-2 | 0.90 | 0.84 (0.02) | 0.86 (0.04) |
| RFLP-1 and RFLP-2 | 0.90 | 0.85 (0.02) | 0.85 (0.04) |

It is reassuring that the most likely classification of a given woman by a laboratory test is to her true p53 genotype status. Moreover, it is interesting to note that the least likely classification for homozygous Arg/Arg women is to its most dissimilar result (Pro/Pro), and vice versa, with intermediate probabilities for the heterogeneous Arg/Pro group.
Arg/Pro group have an OR that is elevated only slightly above 1. It is important to note again that these parameters are maximum likelihood estimates, and take the possibility of laboratory errors into account. These ORs avoid the ambiguity associated with different empirical OR values from different methods, as discussed below. In addition to the parameters shown in Table 4, this model also gave estimates of test accuracy. Those results were very similar to those from Model 1, so details are not shown here.

Table 5 shows the empirical prevalences of each level of p53 in the case and non-case groups, and empirical ORs, for four laboratory tests.

Table 6 shows the Model 1 results for the two additional laboratory tests (AS-PCR and DS) that were used less frequently in this study (only for the subset of cases with sufficient DNA material for expanded genotyping assays). The data for the four earlier tests were also included in this analysis, giving a total of six possible tests for a given woman. The accuracy estimates for the four earlier tests were very similar to those shown already, so these numerical details are omitted from Table 6.

The results of Table 6 show that, like the previous four tests, AS-PCR and DS have their highest classification probabilities associated with the actual p53 value for a given woman, but their accuracy appears to be somewhat lower than for the other four tests. For example, a woman who is Arg/Arg has a 92% probability of correct classification by the AS-PCR test, and a 90% probability with the DS test, compared to 96%, 97%, and 96% associated with DHPLC, DBH and RFLP-1 respectively. Similarly, the correctness rates for Pro/Pro women (79% for AS-PCR and 90% for DS) are inferior to the earlier tests. As seen with the other tests,
AS-PCR and DS also have intermediate misclassification probabilities associated with the heterogeneous group, and the smallest misclassification probabilities for Arg/Arg women being classified into Pro/Pro and vice versa. Overall, the results suggest that AS-PCR and DS are somewhat less accurate than any of the four previous methods, but in light of the restricted dataset in which these comparisons were made it would not be prudent to make generalizations concerning assay accuracy. Note that some of the estimated probabilities in Table 6 are zero, indicating that further boundaries have been encountered. This may imply that the model parameters and their standard errors may be somewhat inaccurate, especially considering the smaller number of women with six test results available, and the correspondingly larger number of parameters required by this extended model.

Robustness of Results

Given the difficulty of obtaining reliable estimates of accuracy specific to the SIL cases, we ran additional models after excluding them, to assess the robustness of the main results. These additional models yielded test accuracy values that were very similar to those when the SIL cases were included, such as in Table 1, and we therefore conclude that the inclusion of the SIL cases made no material difference to the results. Similarly, when all women were included, regardless of cytology status or number of visits, the results were again very similar, indicating no important impact of these exclusion criteria.

When Model 1 was extended by additionally allowing the test accuracy parameters to depend on cytology status, it failed to converge with the available data, and thus it did not provide estimates for the accuracy parameters. However, we do not expect genotype test accuracy to depend on cytology, especially when considering that one’s genotype may have been measured before abnormal cytology results were documented during follow-up. When Models 1 and 2 were constrained to have constant accuracy parameters across test methods, there was a significant deterioration in the fit to the data (relative to the corresponding unconstrained model), indicating that an assumption of equal test accuracy was untenable.

The extended models that allowed for possible correlations between test errors typically showed no significant improvement in the model fit. The one exception was the suggestion of an interaction between the DHPLC and DBH methods. However, we had no a priori reason to expect a correlation between this particular pair of tests. Bearing in mind that there are 6 pairwise correlations between the 4 tests, a multiple comparisons adjustment to the p-value of the DHPLC-DBH association renders it non-significant. Overall we conclude that there is no strong evidence for correlated test errors, and that the results from models assuming conditional independence are valid.

When the analyses were repeated using the p53 latent variable at only 2 levels (Arg/Arg vs. Arg/Pro and Pro/Pro combined, or Pro/Pro vs. Arg/Arg and Arg/Pro combined) the corresponding estimates of classification probabilities were very similar to those shown in Table 3, but with the advantage that no parameters hit their boundaries of an estimated zero probability. This suggests that although several of the parameters fall on the boundary in the Model 1 results with three levels for p53, the other estimates are trustworthy, despite the greater demands on the data by a model with three levels for p53 as opposed to only two.

Discussion

Since 1998 [19], there have been numerous studies examining the association between p53 codon 72 genotype (rs1042522) and risk of cervical cancer or precancer. Although the association is biologically plausible because of the different binding affinities to the E6 gene of HPV by the resulting Arg and Pro products, there has been considerable heterogeneity in risk estimates across studies [1,2]. At least some of the heterogeneity may have been due to variations in accuracy and reproducibility of the different assays available to genotype this polymorphism, which prompted us to examine the issue using a data modelling approach in a study in which multiple assays were used to genotype p53. Our work in examining the variability in genotyping methods was prompted by the state of uncertainty in the literature at the time. More recent work indicated that although plausible the association may not be real or may be too small to be of clinical relevance [2]. Furthermore, the issue of population stratification, in which race confounds the relationship between p53 status and the outcome, could bias our effect estimates. As previous studies have found that allele frequencies vary across ethnic groups and that the risk of cervical cancer varies by ethnicity it could be the case that population stratification may have biased the effect estimate. Among the three primary racial groups, we found very little difference in the risk of the outcome (risk varied between 8 and 10%) and preliminary analyses of the same data showed that race and age-adjusted effect estimated did not vary importantly from crude estimates (data not shown). However, irrespective of the true nature of the relation between p53 codon 72 polymorphism and cervical neoplasia, we believe that the LCM methodology reported here is a useful tool to examine the relation between disease and a biomarker that is measured with error.

The main advantage of applying the LCM approach to this dataset is that it provides detailed estimates of the accuracy of each laboratory method. In particular, the models yield estimates of the probability of correct or incorrect classification of the p53 genotype, from each level of the actual genotype variable to each possible observed value, for each test. We are therefore able to assess and compare the performance of each method, but without incorrectly assuming any one of the tests to be a reference or gold standard.

In this study we found that each method had the highest probability of classifying a given woman into her actual p53 category; the least likely misclassifications were from actual Arg/Arg into apparent Pro/Pro, and vice versa. However, the tests did vary significantly in their accuracy. In our analysis, the DHPLC and DBH laboratory methods were very accurate across levels of p53 status, while the RFLP-1 and RFLP-2 methods were slightly inferior as they misclassified women of a particular p53 status more than 10% of the time.

We also tried to investigate if test accuracy depended on whether a woman had abnormal cytology or not. The number of SIL cases was relatively small, which limited our capability to demonstrate such an effect, if it exists; however, it is reasonable to assume that test accuracy does not depend on cytology, especially considering that abnormalities may not have been present when the genotype was measured.

The LCM analyses also provide estimates of the OR between the p53 genotype and cervical abnormalities, taking possibility of genotype measurement errors into account. These ORs are based on the ensemble of available test information for each woman, and, being based on maximum likelihood principles, enjoy asymptotic unbiasedness and optimally maximal precision for a correctly specified model. In contrast, the empirical estimates of the OR are based on the subset of data from each method in turn. The empirical test-specific estimates inevitably vary (e.g. the DHPLC test shows substantially higher ORs than the other frequently used tests), and so it is not immediately obvious which
test would be preferred in order to convey the risk associated with the *p53* genotypes. The empirical OR estimates were slightly less precise than the corresponding LCM values, because the former are based on a more limited portion of the data than the LCM, which exploits the information from all available tests simultaneously.

Failure of the model to converge when test accuracy was allowed to depend on the cytology result probably reflects the very limited number of SIL cases, because a much larger number of parameters is then required in the extended model. While we are unable to say unequivocally if test accuracy depends on cytology, given the type of specimens used in this study (DNA extracted from exfoliated cervical cells of asymptomatic women with at most low volume lesions) and its cohort design (lesions detected mostly at time points that were different than when the genotyping specimen was obtained), we believe it is reasonable to assume that test accuracy is actually independent of cytology.

The methods we have employed on the Ludwig-McGill study data are quite general, and could be applied in other situations where a gold standard measurement of a risk marker (genetic or otherwise) is not possible. Application of the basic LCM requires that three or more independent tests be available for each subject [6,20]; in this study we had at least four tests per woman. The basic model assumes that the test errors are conditionally independent, given the true (but unobserved) status of an individual, which is a plausible expectation. If more than three tests are available, the assumption can be tested by additionally including correlation terms in the LCM, and then assessing the improvement in fit through a likelihood ratio test. Although the assumption of conditionally independent errors has been raised as a concern in the application of the LCM method, a number of investigations [21–23] have shown that the assumption may sometimes actually be a reasonable one, or that the model is robust to misspecification in this regard. In the hypothetical situation of a general pattern of positive correlation among tests, the analysis would overestimate test accuracy both in terms of sensitivity and specificity. However, in our earlier work on *p53* [4] we found no evidence of such correlations between the test results for the same woman, using the same method in different laboratories. The likelihood of such correlations between different laboratory methods is presumably smaller, and indeed the analysis in this paper revealed no convincing evidence of correlated errors.

Overall, latent class models are useful for assessing the accuracy of various test procedures without assuming a gold standard. In addition, these methods can provide an adjusted, unbiased estimate of the odds ratio. While each test on its own is imperfect, the adjusted OR estimated by the LCM accounts for this test inaccuracy by taking advantage of the information presented by having an ensemble of testing methods upon which to base its result.

**Acknowledgments**

We are grateful to Maria L. Baggio, Lenice Galan, Silvaneide Ferreira, Joao S. Sobrinho, and Jose´ M. Prado for managing patients and testing specimens, and for Helen Trottier for preliminary data management in the Ludwig-McGill cohort study.

**Author Contributions**

Conceived and designed the experiments: ELF LLV. Performed the experiments: TR. Analyzed the data: CAR SDW. Wrote the paper: SDW CAR ELF.

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