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Recommendation to Use Exact P-values in Biomarker Discovery Research

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Abstract (250 words)

Background: In biomarker discovery studies, markers are ranked for validation using *P*-values. Standard *P*-value calculations use normal approximations that may not be valid for small *P*-values and small sample sizes common in discovery research.

Methods: We compared exact *P*-values, valid by definition, with normal and logit-normal approximations in a simulated study of 40 cases and 160 controls. The key measure of biomarker performance was sensitivity at 90% specificity. Data for 3000 uninformative markers and 30 true markers were generated randomly, with 10 replications of the simulation. We also analyzed real data on 2371 antibody array markers measured in plasma from 121 cases with ER/PR positive breast cancer and 121 controls.

Results: Using the same discovery criterion, the valid exact *P*-values lead to discovery of 24 true and 82 false biomarkers while approximate *P*-values yielded 15 true and 15 false biomarkers (normal approximation) and 20 true and 86 false biomarkers (logit-normal approximation). Moreover, the estimated numbers of true markers among those discovered were substantially incorrect for approximate *P*-values: normal estimated 0 true markers discovered but found 15; logit-normal estimated 42 but found 20. The exact method estimated 22, close to the actual number of 24 true discoveries. With real data, exact and approximate *P*-values ranked candidate breast cancer biomarkers very differently.

Conclusions: Exact *P*-values should be used because they are universally valid. Approximate *P*-values can lead to inappropriate biomarker selection rules and incorrect conclusions.

Impact: Rigorous data analysis methodology in discovery research may improve the yield of biomarkers that validate clinically.

Introduction

Biomarker discovery research has yielded few clinically useful biomarkers. Poor methodologies in the statistical design and evaluation of discovery studies may be contributing factors (1). Guidelines for statistical design of discovery studies have recently been discussed, including sources and numbers of biological samples (2). In this article we address a common and underappreciated issue in the evaluation of biomarker discovery studies.

The classic discovery study entails measuring many biomarkers, perhaps using array-based or other such high-throughput technology, on a set of biological samples from cases and controls. For each biomarker, one calculates a statistic and its *P*-value using the case and control data pertaining to that biomarker. The biomarkers are then ranked according to the *P*-values and top ranking candidates are considered for further development and validation. Thus statistical *P*-values play a fundamental role in the evaluation of biomarker discovery studies.

As an example, consider the "Colocare" study to discover and validate markers to predict colon cancer recurrence in patients diagnosed with stage 1 colon cancer (3). Tissue and blood samples taken at diagnosis from 40 cases with colon cancer recurrence and 160 controls without recurrence will be tested with approximately 3000 autoantibodies. As described in (2), the data analytic plan is to calculate the sensitivity corresponding to 90% specificity for each biomarker and to generate a corresponding standard *P*-value for no association between biomarker and case-control status. We simulated data for 3000 useless biomarkers not associated with case-control status and found that 69 (2.3%) had *P*-values less than 0.01 (see third row of Table 1 in (2)). Since one would expect that approximately 30 markers (i.e. 1% of markers) would attain *P*-values less than 0.01 if all 3000 biomarkers were useless, the data analysis suggests a positive result: it appears that 69-30 = 39 true biomarkers have been discovered. However this conclusion is incorrect since we generated the data in such a way that none of the 3000 markers are predictive of case-control status. The issue here is that standard *P*-value calculations that rely on asymptotic statistical theory are problematic and lead to an incorrect conclusion in this example.

In this paper we demonstrate this phenomenon in more detail and propose an alternative method for calculating *P*-values that is generally valid and robust to the vagaries of biomarker discovery data. This exact *P*-value approach is applicable regardless of the statistic used to rank biomarkers and it is computationally reasonable with modern computing capacities. Most importantly, we show that it leads to more reliable conclusions from biomarker discovery data than do standard methods.

Materials and Methods

Our study was designed to investigate if standard *P*-value calculations are potentially invalid in practice and if invalid *P*-value calculations can substantially affect the validity of conclusions drawn from biomarker discovery studies. To address these questions we simulated biomarker discovery data where the capacities of biomarkers to predict outcome were specified, allowing us to compare conclusions based on data analysis with the truth. The Colocare study provided a context to motivate the simulations.

The purpose of Colocare is to find biomarkers predicting high risk of colon cancer recurrence in stage 1 patients treated with surgery. For each of 40 cases with recurrence and 160 controls without recurrence we simulated data corresponding to 3000 uninformative biomarkers that were uncorrelated with case-control status and for 30 informative biomarkers that were correlated with case-control status. We use the terminology “true biomarkers” for the 30 informative biomarkers. Each biomarker was generated as standard normal, mean=0 and standard deviation=1, for the 160 controls. The uninformative biomarker data was generated for the 40 cases in the same way as for controls. For the 30 true biomarkers we generated case biomarker data as normal with mean 0.536 and standard deviation 1. The mean was chosen so that true biomarkers would satisfy a performance criterion described below.

The key biomarker performance measure of interest in the Colocare study is the recurrence rate among biomarker positive patients. The biomarker positivity threshold is chosen as the 90^{th} percentile of the control values (i.e. the 16^{th} largest of the 160 control values) so as to guarantee the marker has 90% specificity. The recurrence rate among biomarker positive patients in the population (positive predictive value, *PPV*) will be estimated by Bayes formula as

$$logit\ (PPV) = logit\ (p) + log\ (sensitivity) - log\ (1\text{-}specificity) \quad (1)$$

where the *logit* function is $logit(x)=log(x/(1-x))$, p=10%=the overall recurrence rate in stage 1 patients (ie. prevalence), the *specificity* is set to 90% and *sensitivity* is the observed proportion of cases that are biomarker positive. The *sensitivity* is also known as the empirical estimate of ROC(0.1). Testing if a biomarker is uninformative is to test if biomarker-positive individuals have the same prevalence of the outcome as observed in the entire study population, i.e. H_0: PPV =10% . Given the above formula this is equivalent to testing H_0*: ROC(0.1)=0.1 (*ie. sensitivity=1-specificity, where specificity=0.90), so *P*-values will be based on testing the null hypothesis

$$H_0: ROC(0.1)=0.1$$

using the empirical ROC estimate, for which standard methods are available(4). Let obs-ROC_{emp} be the value of the empirical ROC estimate, denoted by ROC_{emp}, calculated with data on a biomarker from our study. The associated one-sided *P*-value is the probability that in repetitions of our study one would observe ROC_{emp} values as large as the one we found assuming that biomarker values for cases in the population have the same distribution as biomarker values for controls in the population.

$$P\text{-}value = Probability(ROC_{emp} >= obs\text{-}ROC_{emp} \mid cases\ same\ as\ controls).$$

Standard *P*-value calculations use the approximation that ROC_{emp} is normally distributed in large samples

$$standard\ normal\ P\text{-}value = 1 - \Phi(Z)$$

where $Z = (obs\text{-}ROC_{emp} - 0.1) / se(ROC_{emp}))$, Φ is the standard normal cumulative distribution function and the standard error, $se(ROC_{emp})$, is estimated by bootstrap resampling (4). We used 500 bootstrap samples, separately resampling 40 cases and 160 controls per the study design. Other methods for calculating the standard error are also possible but are more involved because they require estimating probability densities (5, 6). An alternative *P*-value calculation acknowledges that the sensitivity, $ROC_{emp}(0.1)$, can't really be normally distributed since the normal distribution is unrestricted in negative and positive directions while proportions such as $ROC_{emp}(0.1)$ are restricted between 0 and 1. Proportions are often more like normal variables after applying the *logit* transform. This gives rise to the *P*-value calculation

$$standard\ logit\text{-}normal\ P\text{-}value = 1 - \Phi(logit\text{-}Z)$$

where $logit\text{-}Z = (logit(obs\text{-}ROC_{emp}) - logit(0.1)) / se(logit(ROC_{emp}))$ and $se(logit(ROC_{emp}))$ is estimated by bootstrapping as above.

Our proposal is to calculate the *P*-value exactly without approximation. This is in fact an old concept for rank statistics such as the Wilcoxon rank sum statistic where published tables have long been available for use with data from studies involving very small sample sizes (7). Modern computing power now makes the approach feasible for studies with larger sample sizes and for any statistic. The idea is to enumerate all the possible values of the statistic for the setting where cases have biomarker values with the same distribution as controls. For example, in the Colocare study we will have a total of 200 subjects and suppose the cases are labelled as subjects 1-40. If cases have biomarker values with the same population distribution as controls, the study data will be comprised of a random enumeration of ranks for 200 individuals. We calculate the corresponding ROC_{emp} statistic for a large number of random enumerations (or all 200! possible enumerations) and tabulate the results. Because there are 40 cases, there are at most 40 possible values for ROC_{emp}, so it is easy to tabulate the distribution of ROC_{emp} (Table 1) and report the exact *P*-value corresponding to an observed value of ROC_{emp}

$$exact\ P\text{-}value = proportion\ of\ enumerations\ with\ ROC_{emp} >= obs\text{-}ROC_{emp}.$$

For example if the empirical ROC estimate calculated for a biomarker is 0.20, the corresponding exact *P*-value is 0.059175 (Table 1). We selected to use 40,000 enumerations at random with replacement since this required far fewer than all 200! enumerations and yet provided reasonably precise *P*-value calculations. In particular the standard errors of the *P*-value estimates are 0.001, 0.00063 and 0.00045 when the *P*-values are 0.05, 0.02 and 0.01, respectively.

To demonstrate that the method used to calculate *P*-values in real data analysis can have a substantial effect on conclusions drawn, we reanalyzed data from an ER/PR positive breast cancer biomarker discovery study reported in (8). The study sought to discover early detection biomarkers that might be used to encourage women who do not have easy access to

mammography to go for mammography screening. Markers that maximize sensitivity while maintaining at least 90% specificity are preferred for this clinical context. As described in detail in (8), preclinical plasma samples from 121 cases and 121 controls from the WHI observational study were interrogated with an array of 3290 antibodies. There were 2467 biomarkers reported in (8) after removing technical controls and imposing quality control filters based on coefficient of variation across triplicate spots and a criterion for percent of observations missing data. We only included the subset of 2371 biomarkers where at least 100 controls and at least 100 cases have data. As noted above, and similar to the Colocare study, we focused on the sensitivity corresponding to 90% specificity as the biomarker performance measure of interest. Our analysis approach differed in many respects from that previously reported in (8) because our goals were different and more limited. For example, since we just wanted to investigate if different *P*-value calculations provided different rankings and selections of biomarkers, we did not need to split the data into training and test sets as was done in (8).

Results

Reference Distribution for Calculating Exact P-values

Table 1 shows the reference distribution for ROC_{emp} based on 40 cases and 160 controls when a biomarker is not informative about case-control status (a false biomarker). This table will be used to calculate exact *P*-values when biomarker data are available from the Colocare study. Possible values for the ROC_{emp} are 0/40, 1/40, 2/40, 3/40, etc. because there are 40 cases and the estimated ROC is the fraction of those 40 cases whose biomarker values exceed the 90^{th} percentile of control values (i.e. exceed the 16^{th} largest control value). We see that among the 40,000 simulated studies of uninformative markers, in only 1 study did the estimated ROC reach a value of 0.40. Therefore the exact *P*-value corresponding to an ROC of 0.40 is 1/40,000 = 0.000025. Correspondingly, in 5 simulations the estimated ROC reached a value of 0.375 or more, so the *P*-value corresponding to 0.375 is 5/40,000 = 0.000125. Even though it is theoretically possible to observe an estimated ROC greater than 0.40 for a false (uninformative) biomarker, we see that the probability of that occurring is essentially zero.

Normal P-values can be Incorrect

Table 2 and Figure 1 demonstrate that *P*-values calculated with the standard normal approximation methods can be substantially different from the correct exact *P*-values. The data were simulated for a biomarker discovery study that included 30 true biomarkers and 3000 false biomarkers with all 3030 biomarkers evaluated on 40 case and 160 control samples. Figure 1 shows *P*-values calculated with the two standard normal approximation methods versus the *P*-value calculated with the exact method. Data are displayed for the 106 biomarkers where the exact *P*-value was less than 0.05. Values above the line are larger than the exact *P*-value. We see that *P*-values based on the normal approximation without logit transformation are generally larger than the exact *P*-value, sometimes very substantially so. For example there are 3 biomarkers for which the exact *P*-values were 0.0119 while the normal *P*-values were 0.103, 0.124 and 0.136. *P*-values based on the normal approximation to the logit transformed statistic, while closer to the exact *P*-values, have a tendency towards being smaller than the exact values. The magnitudes of the differences are easier to see in Table 2 where the actual values

are listed for the 30 true markers in the study. Although *P*-values are often of similar magnitudes that would lead to the same decisions about efforts to validate or not, there are multiple instances where the differences could lead to different decisions with use of logit-normal versus exact *P*-values (see highlighted biomarkers 3, 14 and 27).

Impact of Invalid P-Values in the Simulated Colocare Study

Differences in *P*-value calculations had a substantial effect on the numbers of biomarkers discovered in the simulated study. The top panel of Table 3 shows the numbers of biomarkers that passed the discovery criterion: *P*-value <=0.0277. We chose this odd threshold since among the finite set of attainable *P*-values that are possible (Table 1) it is closest to 0.02. If we had chosen say the threshold 0.02, the actual threshold for the exact *P*-value would have been 0.0121 and the comparison between *P*-value methods would have been flawed. Observe from Table 3 that use of different *P*-value algorithms leads to substantially different numbers of markers discovered: 106 for exact, 30 for normal and 126 for logit-normal. Use of the exact *P*-value led to discovery of 24 true biomarkers and 82 false biomarkers. Consistent with the overly conservative property of the normal *P*-values, fewer true and false biomarkers were discovered by applying the same criterion to the standard normal *P*-values, while the anti-conservative logit normal *P*-values led to 20 true discoveries and 106 false discoveries.

We next examined if the incorrect normal *P*-value calculations impacted the validity of conclusions drawn from the discovery study. Discovery data analyses typically report estimates of the numbers of true and false biomarkers among those that meet the selection criterion. A simple way to estimate these numbers is as follows: assuming that the vast majority of 3030 markers are false (i.e. uninformative), one estimates that there are 3030 × 0.0277 = 84 false discoveries among the markers discovered. Comparing this number with the actual numbers of false discoveries in Table 3, we see that the estimate is very close for *P*-exact (84 versus 82), but a substantial under estimate (84 versus 106) for the logit-normal *P*-value and a substantial over estimate (84 versus 15) for the normal *P*-value. The number of markers estimated to be true discoveries is calculated as the number of discoveries less the estimated false discoveries. With logit normal we estimate that 126–84 = 42 (33%) of the 126 discoveries are true discoveries. However, only 20 (16%) of the discoveries are true. Therefore, with logit-normal *P*-values we believe we are doing much better than we actually are. For the untransformed normal *P*-value, we estimate that none (0%) of the discovered biomarkers are true while in fact 15 (50%) are true discoveries. We are led to believe we are doing worse than we actually are. In contrast, the exact *P*-value method discovers 24 true biomarkers (23% of total discoveries) and this is in line with the estimated number of true discoveries, namely 106–84 = 22 (21%). In summary, estimates of numbers of true and false discoveries made are much closer to the actual numbers of true and false discoveries when the exact *P*-values are used. In this sense, conclusions drawn from the study are more valid for the exact *P*-value method than for the normal approximation *P*-values.

Results with use of a more stringent *P*-value threshold criterion, namely 0.0121, shown in the lower panel of Table 3 are similar. We repeated the simulation study 10 times to determine if the observations made from the study shown in Table 3 were found in general. We see from Table

S.1 in the Supplementary materials that there is a consistent tendency for the normal approximation *P*-values to provide poor estimates of the numbers of true and false discoveries made and that the exact *P*-value method leads to more reliable conclusions.

When the numbers of cases and controls available are small, investigators often resort to use of more global measures of biomarker performance such as AUC, although pitfalls of using such clinically irrelevant measures are well documented(9, 10). Table 4 investigates performance of exact, normal and logit-normal *P*-values for the AUC statistic when 20 cases and 20 controls are included in the discovery study. Here normal *P*-values tend to be too small and logit-normal *P*-values tend to be too large. Interestingly, this is opposite to results for ROC_{emp}. Most importantly, we see again that estimates of true and false discoveries are much closer to the actual numbers of true and false discoveries when using exact rather than normal approximation *P*-values.

Application to Real Data for Receptor Positive Breast Cancer Biomarker Candidates

ROC statistics and *P*-values were calculated with antibody array data from the ER-PR positive Women's Health Initiative breast cancer study (8). Of the two normal approximation *P*-value methods, for brevity we focus on one here, the logit-normal method. The top panel of Table 5 shows the top 40 candidates ranked according to exact *P*-value and the bottom panel shows the top 40 candidates ranked according to logit-normal *P*-value. One gets very different impressions of the results depending on which *P*-value method is used. For exact *P*-value, the number of true biomarkers in the top 40 is estimated to be 15.7 and the estimated false discovery rate is 63%. In contrast, the logit-normal *P*-value method estimates 33.8 true markers in the top 40 and a false discovery rate of only 15%. Given the previous simulation results, we believe that the estimates based on the exact *P*-values are more reliable. Note that we estimated the false discovery rate here using the Benjamini-Hochberg method (13) implemented with the qqvalue command and Simes option in the Stata software package (14, 15). The simple intuitive calculation method noted in previous tables gave very similar results (data not shown) but that method does not restrict the FDR to increase with increasing *P*-value as does the Benjamini-Hochberg method.

Another interesting observation in Table 5 is that the ROC values align pretty well with the *P*-values when using the exact method, i.e. the highest ROC estimates are at the top of the list corresponding to the smallest *P*-values (see also Figure S.1 in the Supplementary Materials) . In contrast, the logit-normal *P*-value method does not align ROC estimates with *P*-values very well. For example, the highest ROC estimate, 0.339, is way down the list at rank 38 according to the logit normal *P*-value, just above a biomarker with estimated ROC = 0.198.

Considering the biomarker selection criterion 'p<0.05' for which 0.05 X 2371 = 118.5 false biomarkers are expected to be identified, the estimated numbers of true biomarkers selected is 11.5 based on exact *P*-values (130 markers selected in total) and 75.5 with logit-normal *P*-values (194 markers selected in total). These are very different estimates. The biomarker selection criterion p<0.02, for which 47.4 false biomarkers are expected to be identified, yields estimated numbers of true biomarkers selected of 11.6 with exact *P*-values (59 markers selected in total) and 73.6 with logit-normal *P*-values (121 in total). Again, given the simulation

results above, we have more trust in the estimated numbers of true and false biomarkers based on exact *P*-values than in those based on logit-normal *P*-values.

Discussion

Exact *P*-value calculations are the most valid approach possible to calculating *P*-values because they exactly calculate (according to the definition of *P*-value) the probability of observing a statistic as extreme as that observed in the study when case biomarker values are derived from the same distribution as controls. Approximation *P*-values are typically used in practice. Our results show that approximations can be substantially off and can lead to less reliable conclusions drawn from discovery data compared with exact calculations.

Our analyses used nonparametric rank statistics, in particular the sensitivity at fixed 90% specificity (a simple function of the positive predictive value when prevalence is fixed as shown in equation (1)) and the area under the ROC curve (a simple function of the Wilcoxon rank-sum statistic). The issues concerning exact versus approximate *P*-values also apply to parametric statistics, including the t-test for example. However, the null hypothesis reference distribution for the t-test that is needed to calculate exact *P*-values requires estimating the control distribution and repeatedly generating random case and control simulated study data from it. This can be a complicated exercise particularly in the context of discovery research where automated procedures are needed to deal with data on large numbers of biomarkers. We cannot assess parametric assumptions for each biomarker. Moreover, outliers and non-standard distributions are common in discovery research. Therefore, rank based nonparametric statistics are preferred, and those were the focus of our study.

We found two additional advantages derived from use of exact *P*-values. The first is that biomarker performance measures align well with exact *P*-values in the sense that markers with the best estimated performances have the smallest *P*-values. This inverse relationship holds by definition when the same numbers of case and control data points are available for each biomarker. In addition we found the inverse relationship was mostly true in the analysis of the breast cancer data set where data were sporadically missing. However, the analysis that used approximate normal *P*-values led to some major inconsistencies between estimated performance and *P*-value. A second and unexpected advantage to use of exact *P*-values concerns computational effort. When there is no missing biomarker data, the number of case and control data points is the same for each biomarker, and only one reference distribution must be calculated for the entire analysis. For example, exact *P*-values were calculated for each of the 3030 biomarkers in our simulated study using only Table 1. The computation involved to calculate exact *P*-values is therefore very fast once the reference table is created. In contrast, the normal approximation *P*-values required calculation of standard errors for each biomarker separately, a process that was time consuming with use of bootstrap resampling.

We considered two different types of statistical criteria for selecting biomarkers. One approach demonstrated in Tables 3 and 4 was of the form "*P*-value < threshold". Another approach demonstrated in Table 5 was to select the "top K markers" where K was set to 40 in Table 5, similar to the number of biomarkers selected in previous analyses of the same data (8). Yet another criterion is to select markers for which the false discovery rate among markers ranked at or above is below a specified threshold (13). One can see from the breast cancer results in Table 5 that the different *P*-value calculations would lead to very different biomarker selections

based on a false discovery rate criterion. For example “false discovery rate<10%” would lead to two markers selected with exact *P*-values but 30 markers selected with the logit-normal *P*-values. Since false discovery rates are functions of *P*-values, it is important to use valid *P*-value calculations when calculating false discovery rates.

We recommend use of exact *P*-value calculations in the analysis of biomarker discovery data because they are always valid regardless of sample sizes and biomarker distributions. We showed that exact *P*-values can provide more reliable conclusions than standard approximate *P*-values. Specifically, they provide better estimates of true and false discovery rates, key parameters reported in discovery research. Robust valid calculation of *P*-values is crucial in discovery research where interest is often focused on small *P*-values, sample sizes are often small and the numbers of biomarkers tested often preclude evaluating data for distributional assumptions. Nevertheless, it may also be prudent to use exact rather than approximate calculations in biomarker validation research.

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https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf

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Table 1: Reference distribution for the sensitivity corresponding to 90% specificity estimated with the empirical ROC when calculated with data for 40 cases and 160 controls[a]. The reference distribution is used to determine exact *P*-values and was generated by 40,000 randomly chosen enumerations[b] of ranks for 200 subjects with the first 40 labelled as cases.

r	Probability that the estimated sensitivity ≥ r
0.000	1.000000
0.025	0.976575
0.050	0.892075
0.075	0.739125
0.100	0.549825
0.125	0.367025
0.150	0.218200
0.175	0.119050
0.200	0.059175
0.225	0.027675
0.250	0.012025
0.275	0.004850
0.300	0.001750
0.325	0.000550
0.350	0.000225
0.375	0.000125
0.400	0.000025

[a] Smallest increment for realized values of the estimated sensitivity is 0.025 = 1/40 where 40 is the number of cases.

[b] Smallest increment for probability is 0.000025 = 1/40000 where 40000 is the number of random rank enumerations.

Table 2: P-values calculated for the 30 true biomarkers in the simulated biomarker study of 40 cases and 160 controls. Calculations use standard normal approximations to the distributions of the estimated sensitivity at 90% specificity with or without logit transformation or use exact methods. For at least 3 markers, *P*-values are substantially different.

Biomarker	Exact *P*-value	Normal *P*-value	Logit-Normal *P*-value
1	0.001750	0.0065580	0.0002190
2	0.012025	0.0161432	0.0016413
3	0.059175	0.1601699	0.0986310
4	0.004850	0.0183031	0.0017292
5	0.000550	0.0029499	0.0000414
6	0.000225	0.0060935	0.0001591
7	0.027675	0.0607897	0.0190249
8	0.000550	0.0263751	0.0028095
9	0.027675	0.0691393	0.0235189
10	0.059175	0.1051453	0.0520333
11	0.000025	0.0010564	0.0000594
12	0.012025	0.0376968	0.0073081
13	0.027675	0.0917640	0.0373784
14	0.012025	0.1239775	0.0563044
15	0.027675	0.0853067	0.0331860
16	0.218200	0.2179403	0.1790015
17	0.000125	0.0111021	0.0005066
18	0.000550	0.0056560	0.0001466
19	0.119050	0.1620405	0.1098244
20	0.004850	0.0466308	0.0094405
21	0.218200	0.2377149	0.1999186
22	0.001750	0.0116205	0.0006482
23	0.000550	0.0022444	0.0000243
24	0.004850	0.0147829	0.0011685
25	0.367025	0.3739152	0.3618602
26	0.027675	0.0529427	0.0151371
27	0.027675	0.1341217	0.0689888
28	0.004850	0.0098089	0.0005487
29	0.004850	0.0164825	0.0014270
30	0.001750	0.0158648	0.0011660

Table 3: Markers discovered in the simulated study by the selection criterion: biomarker *P*-value < threshold from a dataset with 3,000 uninformative (false) biomarkers and 30 true biomarkers when *P*-values are based on exact calculation or on normal approximation with or without logit transformation. The test statistic is the sensitivity at 90% specificity estimated with the empirical ROC[a]. Number of study subjects: 40 cases and 160 controls.

		Number of Markers		
Threshold for Sensitivity *P*-value		**Exact *P*-value**	**Normal *P*-value**	**Logit-Normal *P*-value**
0.0277	**Total Discoveries**	106	30	126
	False Discoveries			
	estimated[b]	84	84	84
	actual	82	15	106
	True Discoveries			
	estimated[d] (tdr[c])	22 (21%)	0 (0%)	42 (33%)
	actual(tdr)	24 (23%)	15 (50%)	20(16%)
0.0121	**Total Discoveries**	47	14	75
	False Discoveries			
	estimated	37	37	37
	actual	29	5	58
	True Discoveries			
	estimated(tdr)	10 (21%)	0 (0%)	38 (51%)
	actual(tdr)	18 (38%)	9 (64%)	17 (23%)

[a] equivalent to hypothesis testing with the positive predictive value

[b] estimated false discoveries = threshold-p × number of biomarkers

[c] tdr: True discovery rate = number of true discoveries/ number of discoveries

[d] estimated true discoveries = total discoveries – estimated false discoveries

Table 4: Markers discovered by the selection criterion: biomarker *P*-value < threshold from a dataset with 3,000 uninformative (false) biomarkers and 30 true biomarkers when *P*-values are based on exact calculation or on normal approximation with or without logit transformation. The test statistic is the empirical area under the ROC curve (AUC). True biomarkers have AUC = 0.758 (PPV = 0.30) while false (uninformative) biomarkers have AUC=0.50 (PPV = 0.10). Number of study subjects: 20 cases and 20 controls

		Number of Markers		
Threshold for AUC *P*-value		**Exact *P*-value**	**Normal *P*-value[1,2]**	**Logit-Normal *P*-value[1,2]**
0.0216	**Total Discoveries**	94	108	82
	False Discoveries			
	estimated[3]	65	65	65
	actual	68	80	56
	True Discoveries			
	estimated	29	43	17
	actual	26	28	26
0.01016	**Total Discoveries**	59	76	47
	False Discoveries			
	estimated[4]	31	31	31
	actual	37	51	26
	True Discoveries			
	estimated	28	45	16
	actual	22	25	21

1 standard error calculated using 500 bootstrapped samples of the data

2 similar results found with standard errors calculated using a large sample theory expression (11, 12)

3 estimated false discoveries = 3030 × 0.0216 = 65.45

4 estimated false discoveries = 3030 × 0.01016 = 30.78

Table 5. Candidate biomarkers for ER positive PR positive ductal breast cancer measured on preclinical plasma samples from 121 cases and 121 controls in the WHI observation study. The top 40 biomarkers ranked according to *P*-values for sensitivity at 90% specificity are shown. Rankings are with respect to exact *P*-values (top panel) and logit-normal *P*-values (bottom panel).

Rank by exact *P*-value	Marker	Estimated Sensitivity	*P*-value	False Leads Expected	Estimated True Markers	Estimated[a] False Discovery Rate
1	v2621	0.305	0.000050	0.12	0.88	8.9%
2	v689	0.339	0.000075	0.18	1.82	8.9%
3	v1830	0.281	0.000150	0.36	2.64	11.9%
4	V1619	0.287	0.000250	0.59	3.41	14.8%
5	V2261	0.264	0.000325	0.77	4.23	15.4%
6	V1954	0.261	0.000825	1.96	4.04	29.6%
7	V2407	0.248	0.000875	2.07	4.93	29.6%
8	V1873	0.259	0.001050	2.49	5.51	30.3%
9	V2542	0.264	0.001150	2.73	6.27	30.3%
10	V1851	0.246	0.001300	3.08	6.92	30.8%
11	V2512	0.248	0.001875	4.45	6.55	40.4%
12	V2193	0.237	0.002225	5.28	6.72	40.4%
13	V2706	0.244	0.002250	5.33	7.67	40.4%
14	V2765	0.242	0.002475	5.87	8.13	40.4%
15	V2622	0.244	0.002650	6.23	8.72	40.4%
16	V1693	0.239	0.002725	6.46	9.54	40.4%
17	V1969	0.239	0.003550	8.41	8.58	47.4%
18	V1745	0.239	0.003600	8.54	9.46	47.4%
19	V2424	0.225	0.003925	9.31	9.69	48.6%
20	V2123	0.235	0.004100	9.72	10.28	48.6%
21	V2302	0.236	0.004700	11.14	9.86	50.3%
22	V2321	0.233	0.004850	11.50	10.50	50.3%
23	V2548	0.233	0.005225	12.39	10.61	50.3%
24	V845	0.223	0.005500	13.04	10.96	50.3%
25	V1518	0.235	0.005700	13.51	11.49	50.3%
26	V2322	0.215	0.005725	13.57	13.43	50.3%
27	V2718	0.215	0.005725	13.57	12.43	50.3%
28	V2327	0.215	0.006450	15.29	12.71	51.9%
29	V2848	0.224	0.006550	15.53	13.47	51.9%
30	V2090	0.223	0.006875	16.30	14.70	51.9%
31	V2416	0.231	0.006875	16.30	13.70	51.9%
32	V2500	0.219	0.007000	16.60	15.40	51.9%
33	V2309	0.227	0.008400	19.92	13.08	59.8%
34	V2820	0.217	0.008700	20.63	13.37	59.8%
35	V2140	0.208	0.009075	21.52	14.48	59.8%
36	V2892	0.208	0.009075	21.52	13.48	59.8%
37	V2396	0.220	0.009325	22.11	14.89	59.8%
38	V2305	0.214	0.010700	25.37	12.63	62.5%
39	V1669	0.217	0.010800	25.61	13.39	62.5%
40	V1649	0.215	0.011075	26.26	15.74	62.5%

Table 5, continued

Rank by logit normal *P*-value	Marker	Estimated Sensitivity	*P*-value	False Leads Expected	Estimated True Markers	Estimated[a] False Discovery Rate
1	v1619	0.287	<.000001	<0.01	1.00	<0.1%
2	v1830	0.281	<.000001	<0.01	2.00	<0.1%
3	v1518	0.235	0.000009	0.02	2.98	0.7%
4	v2512	0.248	0.000045	0.11	3.89	2.7%
5	V2416	0.231	0.000087	0.21	4.79	4.1%
6	V2765	0.242	0.000104	0.25	5.75	4.1%
7	V2309	0.227	0.000195	0.46	6.54	6.4%
8	V2622	0.244	0.000255	0.60	7.40	6.4%
9	V1873	0.259	0.000263	0.62	8.38	6.4%
10	V2090	0.223	0.000285	0.68	9.32	6.4%
11	V1704	0.208	0.000298	0.71	10.29	6.4%
12	V1954	0.261	0.000324	0.77	11.23	6.4%
13	V2621	0.305	0.000454	1.08	11.92	7.6%
14	V2123	0.235	0.000496	1.18	12.82	7.6%
15	V2407	0.248	0.000505	1.20	13.80	7.6%
16	V1914	0.214	0.000529	1.25	14.75	7.6%
17	V2436	0.202	0.000543	1.29	15.71	7.6%
18	V2302	0.236	0.000617	1.46	16.54	7.8%
19	V1847	0.203	0.000643	1.52	17.48	7.8%
20	V1648	0.215	0.000686	1.63	18.37	7.8%
21	V1669	0.217	0.000701	1.66	19.34	7.8%
22	V2321	0.233	0.000725	1.72	20.28	7.8%
23	V2548	0.233	0.000756	1.79	21.21	7.8%
24	V1740	0.198	0.000851	2.02	21.98	8.4%
25	V2828	0.200	0.000899	2.13	22.87	8.5%
26	V2706	0.244	0.000994	2.36	23.64	8.6%
27	V2193	0.237	0.000996	2.36	24.64	8.6%
28	V2426	0.202	0.001012	2.40	25.60	8.6%
29	V291	0.198	0.001066	2.53	26.47	8.7%
30	V2892	0.208	0.001176	2.79	27.21	9.3%
31	V1969	0.239	0.001363	3.23	27.77	10.2%
32	V1851	0.246	0.001373	3.25	28.75	10.2%
33	V2417	0.198	0.001592	3.77	29.22	11.4%
34	V826	0.209	0.001655	3.92	30.08	11.5%
35	V2322	0.215	0.001777	4.21	30.79	12.0%
36	V2908	0.220	0.001991	4.72	31.28	12.9%
37	V2542	0.264	0.002021	4.79	32.21	12.9%
38	V689	0.339	0.002248	5.33	32.67	14.0%
39	V2814	0.198	0.002405	5.70	33.30	14.6%
40	V720	0.198	0.002612	6.19	33.81	14.9%

[a]Benjamini-Hochberg estimates (13)

Legends for Tables and Figures

Table 1: Reference distribution for the sensitivity corresponding to 90% specificity estimated with the empirical ROC when calculated with data for 40 cases and 160 controls[a]. The reference distribution is used to determine exact *P*-values and was generated by 40,000 randomly chosen enumerations[b] of ranks for 200 subjects with the first 40 labelled as cases.

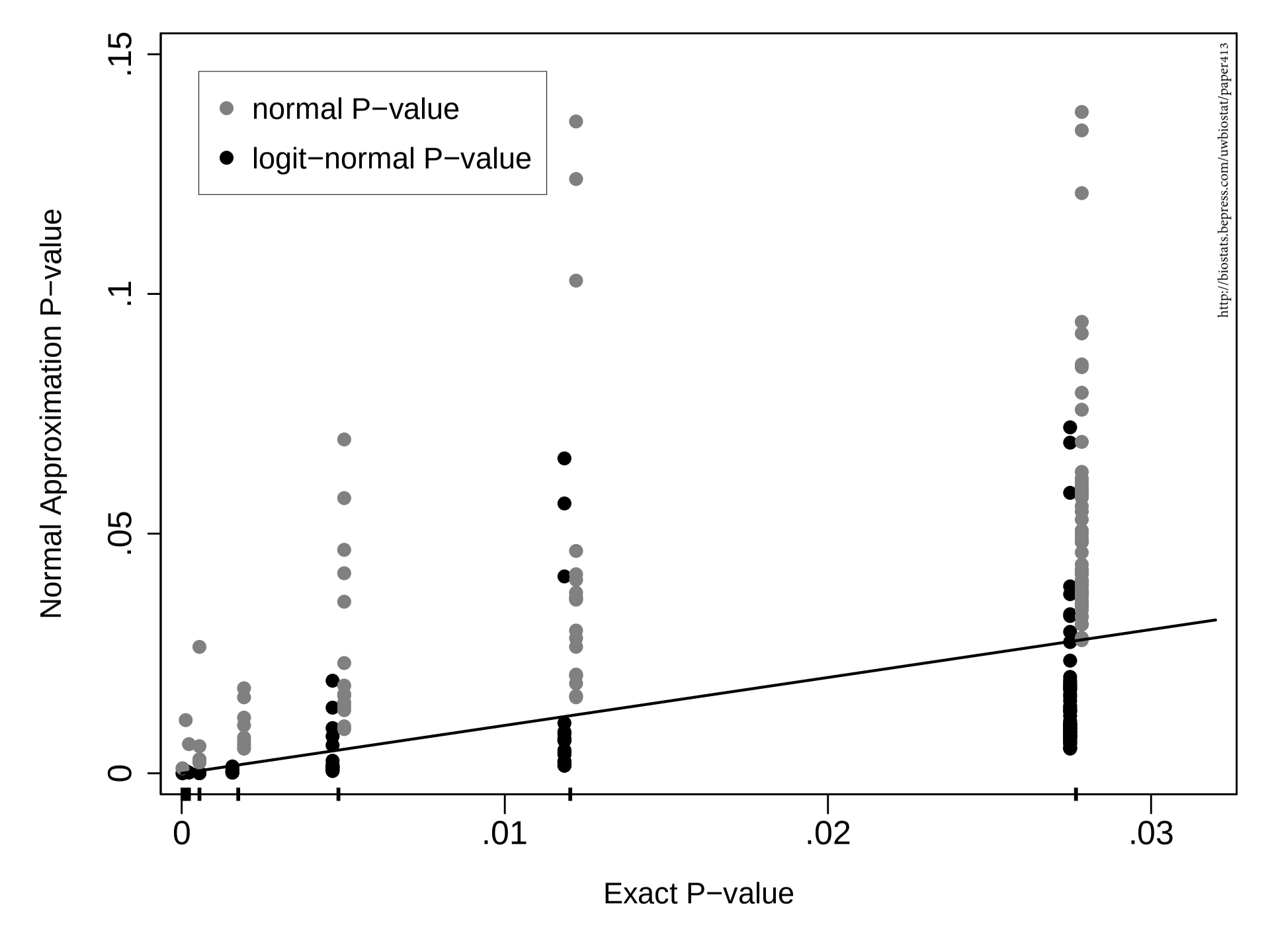
Table 2: P-values calculated for the 30 true biomarkers in the simulated biomarker study of 40 cases and 160 controls. Calculations use standard normal approximations to the distributions of the estimated sensitivity at 90% specificity with or without logit transformation or use exact methods. For at least 3 markers, *P*-values are substantially different.

Table 3: Markers discovered in the simulated study by the selection criterion: biomarker *P*-value < threshold from a dataset with 3,000 uninformative (false) biomarkers and 30 true biomarkers when *P*-values are based on exact calculation or on normal approximation with or without logit transformation. The test statistic is the sensitivity at 90% specificity estimated with the empirical ROC[a]. Number of study subjects: 40 cases and 160 controls.

Table 4: Markers discovered by the selection criterion: biomarker *P*-value < threshold from a dataset with 3,000 uninformative (false) biomarkers and 30 true biomarkers when *P*-values are based on exact calculation or on normal approximation with or without logit transformation. The test statistic is the empirical area under the ROC curve (AUC). True biomarkers have AUC = 0.758 (PPV = 0.30) while false (uninformative) biomarkers have AUC=0.50 (PPV = 0.10). Number of study subjects: 20 cases and 20 controls

Table 5. Candidate biomarkers for ER positive PR positive ductal breast cancer measured on preclinical plasma samples from 121 cases and 121 controls in the WHI observation study. The top 40 biomarkers ranked according to *P*-values for sensitivity at 90% specificity are shown. Rankings are with respect to exact *P*-values (top panel) and logit-normal *P*-values (bottom panel).

Figure 1: Correspondence between *P*-values for sensitivity at 90% specificity in the simulated data calculated using standard normal approximations and exact *P*-values. Values above the diagonal line are larger than exact *P*-values while values below the line are smaller than exact *P*-values. The display is restricted to the 106 biomarkers with exact *P*-values <0.05. Number of study subjects 140 cases and 160 controls.


normal P−value
logit−normal P−value
Normal Approximation P−value
0
.05
.1
.15
0
.01
.02
.03
Exact P−value
http://biostats.bepress.com/uwbiostat/paper413

Supplementary Table S.1: Ten independent replications of the simulation study reported in Table 3. Markers discovered by the selection criterion: biomarker *P*-value < 0.0277 from a dataset with 3,000 uninformative (false) biomarkers and 30 true biomarkers when *P*-values are based on exact calculation or on normal approximation with or without logit transformation. The test statistic is the sensitivity at 90% specificity estimated with the empirical ROC. Number of study subjects: 40 cases and 160 controls.

		False Discoveries Observed			True Discoveries Estimated[2]: Observed		
Replication	**Estimated[1]**	**Exact *P*-value**	**Normal *P*-value**	**Logit-Normal *P*-value**	**Exact *P*-value**	**Normal *P*-value**	**Logit-Normal *P*-value**
1	84	83	23	107	25 : 26	0[3]: 17	48 : 25
2	84	75	17	92	14 : 23	0 : 16	32 : 24
3	84	85	29	114	26 : 25	0 : 17	55 : 25
4	84	76	18	97	18 : 26	0 : 20	38 : 25
5	84	84	29	107	26 : 26	0 : 18	47 : 24
6	84	82	24	110	23 : 25	0 : 18	51 : 25
7	84	70	11	90	11 : 25	0 : 19	28 : 22
8	84	77	26	94	17 : 24	0 : 15	33 : 23
9	84	74	22	100	18 : 28	0 : 25	44 : 28
10	84	99	23	124	41 : 26	0 : 20	64 : 24

[1] estimated false discoveries = (threshold-p) × number of biomarkers. This is the same for all *P*-value methods

[2] total discoveries – estimated false discoveries = estimated true discoveries

[3] when the total number of markers discovered is less than the expected number of false discoveries we estimate that the number of true discoveries is 0

Supplementary Figure S.1: Rank orders of *P*-values versus corresponding estimated sensitivity at 90% specificity for the top 40 biomarkers according to exact *P*-values (top panel of Table 5) and for the top 40 biomarkers according to logit-normal *P*-values (lower panel of Table 5).