Value of two putative biomarkers for identification of HCC in the HALT-C Trial

Analysis Example

Selection of Cases and Controls

- Serum samples from cases selected closest to diagnosis and before any specific treatment of HCC.
- Controls matched 2:1 to cases based on treatment assignment and baseline fibrosis stage (fibrosis or cirrhosis).
- Controls had to be followed for at least a year after the matched case diagnosis.

Study Design for Stage 1 Testing

- Cases
  - Diagnosed with probable or definite HCC
  - Sample closest to the time of diagnosis but prior to treatment
- Controls
  - Did not have a diagnosis of HCC and had follow-up for at least 12 months after the case
  - Matched on treatment assignment and fibrosis stratum (Ishak fibrosis stage <5 or 5-6)

Stage 2 Study Design

- Serum samples from cases selected at time points prior to the diagnosis of HCC.
- In most cases 4-6 samples up to 2 years prior to diagnosis.
- From the same matched controls as in stage 1, similar selection of samples: 4-6 samples up to 2 years prior to the case diagnosis.

Samples

- Each sample is 0.25 ml with a numerical identifier that does not indicate whether a case or control.
- Coded masked samples are sent to the biomarker lab.
- Data analyst receives results and links to the HALT-C patient data.

To proceed with 2nd stage of testing

- Stage 1 results: For recognition of HCC at the time of diagnosis, do the proposed biomarkers perform as well as Alpha fetoprotein (AFP) or complement AFP?
- Is there sufficient evidence to decide whether to go to stage 2 of testing of samples prior to the diagnosis of HCC?

Sources of Samples

- 55 Cases and 110 matched controls
- 15 pts had measures from lead-in (5 HCC, 10 controls)
- 90 pts had measures from randomized phase (30 HCC, 60 controls)
- 60 pts had measures from observational phase (20 HCC, 40 controls)

1st Analysis Step: Quantitative/Ordinal Data

- Examine means and distribution of biomarker results
- Determine whether means differ between cases and controls (unmatched and matched)
- Is the difference in means as meaningful as for AFP?

2nd Step: Matched Odds Ratio Analysis of Quantitative Data

- Select a percentile cut-off for the biomarker data – cases and controls combined.
- In these examples, used cut-off of 67% because of ease of considering the results.
- Thus if there were complete separation of cases and controls, all cases would be classified in the upper 1/3rd and all controls in the lower 2/3rds.

Matched Odds Ratios (OR)

- Need to maintain the matching in the calculation of OR.
- Control for additional variables can be performed with conditional logistic regression analysis.

Analysis details: Breslow NE and Day NE. Statistical methods in cancer research. Volume 1- The analysis of case-control studies. IARC scientific publications No. 32. Chapter 5, Analysis of matched data, p 162-89.

Sample Means of Cases and Controls

Test	Mean cases	SEM	Mean controls	SEM	Mean difference (p-value)
Biomarker #1	5.16	0.28	4.59	0.21	0.08
Biomarker #2	4.77	0.24	3.97	0.18	0.004
AFP	196.5	30.8	16.2	21.9	<0.0001

Odds Ratio and Area Under ROC

Test (67% cut-off)	Matched OR	95% CI		AUROC (unmatched)
Biomarker 1 (5.53)	1.30	0.64	2.63	0.55
Biomarker 2 (4.83)	2.63	1.24	5.58	0.64
AFP (22.9)	8.88	3.39	23.21	0.79

AFP
Matched OR = 8.9 (3.4-3.4)

Cases	CONTROLS (AFP >=67% cutoff 22.9 ng/ml)			
	Both controls < 67% cutoff	One control < 67% cutoff	Both controls >=67% cutoff	Total
Case >=67% cutoff	21	10	3	34
	38.18	18.18	5.45	61.8
	61.76	29.41	8.82	
	56.76	71.43	75	
Case <67% cutoff	16	4	1	21
	29.09	7.27	1.82	38.2
	76.19	19.05	4.76	
	43.24	28.57	25	
Total	37	14	4	55
	67.3	25.5	7.2	100

Biomarker 1
Matched OR = 1.30 (0.64-2.63)

Cases	Biomarker 1 Controls >=67% cutoff (5.53)			
	Both controls < 67% cutoff	One control < 67% cutoff	Both controls >=67% cutoff	Total
Case >=67% cutoff	**7** 12.73 33.33 29.17	**10** 18.18 47.62 38.46	4 7.27 19.05 80	21 **38.2**
Case <67% cutoff	17 30.91 50 70.83	**16** 29.09 47.06 61.54	**1** 1.82 2.94 20	34 61.8
Total	24 **43.6**	26 **47.3**	5 9.1	55 100

Biomarker 2
Matched OR = 2.63 (1.24-5.58)

Cases	Biomarker 2 Controls >=67% cutoff (4.83)			
	Both controls < 67% cutoff	One control < 67% cutoff	Both controls >=67% cutoff	Total
Case >=67% cutoff	**11** 20.0 42.3 37.9	**11** 20.0 42.3 52.4	4 7.3 15.4 80	26 **47.3**
Case <67% cutoff	18 32.7 62.1 62.1	**10** 18.2 34.5 47.6	**1** 1.8 3.5 20.0	29 52.7
Total	29 **52.7**	21 **38.2**	5 9.1	55 100

Do the Two Biomarkers Complement AFP in LR Analysis?

	OR	95% CI	P-value	AUROC
AFP Alone	8.9	3.4-23.2	<0.0001	0.793
AFP plus	10.7	3.7-30.4	<0.0001	0.800
Biomarker 1	0.61	0.24-1.56	0.30	
AFP plus	7.7	2.9-20.5	<0.0001	0.794
Biomarker 2	1.8	0.7-4.2	0.21	

Neither biomarker was statistically significant when combined with AFP and neither provided meaningful improvement to AUROC.