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M E M O R A N D U M

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FROM: Division of Biometrics, HFN-715

SUBJECT: Evaluation of Reactivity to HTLV-III Antibody Observed in Phase I of PHS Study.

Introduction

Under Phase I of the PHS study we consider the results obtained on about 15,000 plasma samples originating from plasmapheresis centers and the results of another 15,000 plasma samples originating from blood centers. The FDA received these samples from several blood suppliers through an agreement between the Office of the Assistant Secretary of Health, these blood suppliers, and the five manufacturers developing HTLV-III antibody kits. As batches of plasma samples from each source arrived at the FDA, they were distributed equally to the five manufacturers. Each manufacturer, using his own ELISA system, tested these samples and sent the results back to FDA for summary and statistical analysis. Each manufacturer had his own criteria for defining an initially reactive sample, but all initially reactive samples were retested by the manufacturer by ELISA and by Western Blot.

Data

For convenience I shall refer to plasma from plasmapheresis centers as Plasma, and to plasma from blood centers as Blood.

No attempt was made for this study to conduct a probability-based sample of the plasma supply at the time of collection. Also, due to the sensitivity of linking reactive samples with their donors, both Plasma and Blood samples were recoded several times. During this recoding the exact geographic origin of the samples was destroyed. For Plasma samples a rough geographic code (6 codes for the whole USA) survived for most samples and was used in our analyses. The lot numbers of the Blood samples referred also to some regionalization, but could not be retrieved for analysis due to time pressure. We were assured by the suppliers that the samples were collected in a short period of time to ensure that an individual could contribute only one sample. The data do not represent a random sample of the Nation's plasma.

Regional identification was not available for 458 samples. We allocated these samples to the manufacturers proportionally to the regional distribution of the 2644 samples with known regional codes. This distribution was as follows for each manufacturer:

Region	1	2	3	4	5	6
No. Samples	718	671	427	193	559	76

The great majority of samples tested were non-reactive by ELISA. For these the regional distribution was taken proportional to the one above, because confirmation of region would have been prohibitive. For the few reactive samples the regional code was confirmed and is, therefore, precise, although there were a few reactive samples with no code available.

Analyses - Comparison of Regions and Manufacturers in Data from Plasmapheresis Centers

Table 1 gives the number of Plasma samples analyzed by each manufacturer, the number found reactive on first ELISA, the number found reactive on second ELISA, and the number found positive by Western Blot. Table 2 expresses these results as percentages. It should be kept in mind, that a second ELISA and a Western Blot were performed only when the initial ELISA was reactive.

Each manufacturer received 2735 Plasma samples for analysis, but empty containers, illegible labels or poor material resulted in various losses. Manufacturer 1 returned results for the smallest numbers of samples (78.8 %). Various distributions of reactives by manufacturer and by geographical region are given in Tables 3 - 6.

To investigate differences between manufacturers and between regions we used a hierarchical model described by Bishop, Fienberg, and Holland?1 . The results can be summarized as follows:

Data	Results of hierarchical analysis
First ELISA: Number of Samples Analyzed, Number of Samples Reactive, by Manufacturer and by Region	For proportion reactive there is no significant interaction between manufacturers and regions ($p = 0.90$). There is no significant difference in average proportion reactive across regions ($p = 0.22$). There is, however, a significant difference in average proportion reactive among manufacturers ($p = 0.01$).

?1 Y.M.M. Bishop, S.E. Fienberg, P.W. Holland: Discrete Multivariate Analysis: Theory and Practice, The MIT Press, Cambridge, Mass. 1975.

Second ELISA: Number of Samples Analyzed, Number of Samples Reactive, by Manufacturer and by Region	Same findings as with first ELISA ($p = 0.83$, $p = 0.54$, $p = 0.01$, respectively)
Western Blot: Number of Samples Analyzed, Number of Samples Positive, by Manufacturer and by Region	For proportion positive, there is no significant interaction between manufacturers and regions ($p = 0.59$). There is no significant difference in average proportion positive across regions ($p = 0.64$). There is, however, a significant difference in average proportion positive among manufacturers ($p = 0.01$).
Western Blot: Number of First ELISA Reactives, Number of Western Blot Positives, by Manufacturer and by Region	There is no significant difference in the average proportion positive between manufacturers, between regions, nor is there a significant interaction (model with these factors missing has $p = 0.39$).

These analyses confirm that the distribution of Plasma samples to each manufacturer was successful and that no manufacturer inadvertently received substantially more samples from one region than did the other manufacturers. On the other hand, differences between manufacturers were consistently observed in the proportions of first ELISA reactives, second ELISA reactives, and Western Blot positives. Such differences in proportion reactive may arise from differences inherent in the set of samples received by each manufacturer, or from differences in the individual ELISA systems. The lack of observed significant differences in the average reactivity rates of the regions supports the notion that the sets of samples received by each manufacturer were comparable. Therefore, the observed significant differences may reasonably be attributed to basic differences in the performance of the various ELISA systems.

There were no significant differences observed when using the Western Blot as confirmatory test of the first ELISA. This lack of significance should not be attributed to consistency of results. On the contrary, wide variability in rates and small cell frequencies render any interpretation of the results imprecise.

Analysis - Data from Blood Centers Compared to Data from Plasmapheresis Centers

As mentioned above, it was not feasible to retrieve some geographic identification for any Blood samples and an analysis by geographic code was not possible. We could compare, however, the reactivity rates of the Plasma samples with those of the Blood samples for the five manufacturers. Table 7 gives the number of Blood samples analyzed by each manufacturer, the number found reactive on first ELISA, the number found reactive on second ELISA, and the number found positive by Western Blot. Table 8 expresses these numbers in various percentages. Using the same hierarchical model as in the analyses of the Plasma samples, we found the following:

Data	Results of hierarchical analysis
First ELISA: Number of Samples Analyzed, Number of Samples Reactive, by Manufacturer	For proportion reactive, there is a significant interaction between manufacturers and types of sample ($p = 0.01$). Therefore, the average effect due to manufacturer and the average effect due to sample type are meaningless.
Second ELISA: Number of Samples Analyzed, Number of Samples Reactive, by Manufacturer	Same as for first ELISA ($p = 0.01$)
Western Blot: Number of Samples Analyzed, Number of Samples Positive, by Manufacturer	Same as for first ELISA ($p = 0.01$)
Western Blot: Number of of first ELISA reactives, Number of Western Blot Positives, by Manufacturer	Same as for first ELISA ($p = 0.01$)

The observed significance of the interaction term in each of the data sets analyzed makes an interpretation of the main effects difficult. Comparing the columns of Table 2 with those of Table 8, we see that the percent reactive for manufacturer 1 in Table 8 is nearly twice that seen for this manufacturer in Table 2. For the other manufacturers this doubling was not seen, explaining the significant interaction. Nevertheless, the distinctions between manufacturers remain.

Summary

The data collected under Phase I of the PHS study were not a random sample of the Nation's blood plasma supply and, therefore, it is not possible to derive a statistically supportable estimate of a national prevalence rate for HTLV III antibody.

In the distribution of Plasma samples to the five manufacturers, each region appears to have been proportionally represented, despite the many logistic difficulties and extraordinary time pressure encountered. We can therefore, state with confidence, that the observed differences in reactivity rates are greater than would be expected by chance alone and are attributable to differences in the ELISA systems of the various manufacturers. The lack of significant differences in the confirmatory rates of the Western Blot are a function of the small numbers involved in these comparisons and of the large observed variability of the rates.

Plasma samples from Blood centers could not be analyzed with respect to their regional distribution. When comparing reactivity rates between Plasma and Blood samples, reactivity rates among manufacturers for Plasma were found to be significantly different from those for Blood.

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cc.
HFN-837/L. Smallwood
HFN-710/Chron.
HFN-715/BLG 2.2 HTLV-III, PHS Study, Phase I
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