SUMMARY MINUTES

MEETING OF THE CLINICAL CHEMISTRY AND CLINICAL TOXICOLOGY DEVICES ADVISORY PANEL

MEETING

December 6, 2006

Holiday Inn Gaithersburg
Gaithersburg, Maryland

Clinical Chemistry and Clinical Toxicology Devices Advisory Panel Meeting

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Attendees

Chairperson
Bernard W. Steele, M.D.
Jackson Memorial Hospital
Miami, Florida

Voting Members
Ann M. Gronowski, Ph.D.
Washington University School of Medicine
St. Louis, Missouri

Alan T. Remaley, M.D., Ph.D.
National Institutes of Health
Bethesda, Maryland

Ruiwen Zhang, M.D., Ph.D.
The University of Alabama at Birmingham
Birmingham, Alabama

Consultants
Cindy L. Grines, M.D.
William Beaumont Hospital
Royal Oak, Mississippi

Stanley S. Levinson, Ph.D.
University of Louisville
Louisville, Kentucky

Santica M. Marcovina, Ph.D.
University of Washington
Seattle, Washington

Robert D. Shamburek, M.D.
NHLBI, National Institutes of Health
Bethesda, Maryland

Michael Y. Tsai, Ph.D.
University of Minnesota
Minneapolis, Minnesota

Karol E. Watson, M.D., Ph.D.

The David Geffen School of Medicine at UCLA
Los Angeles, California

William E. Winter, M.D.
Shands Hospital
Gainesville, Florida

Industry Representative
Thomas E. Worthy, Ph.D.
Worthy Consulting
Walnut Creek, California

Consumer Representative
Murray H. Loew, Ph.D.
George Washington University
Washington, D.C.

Executive Secretary
Veronica J. Calvin, M.A.
Food and Drug Administration
Rockville, Maryland

FDA Participants
Alberto Gutierrez, Ph.D.
Director
Division of Chemistry and Toxicology

Steve Gutman, M.D., M.B.A.
Director
Office of In Vitro Diagnostics

Carol C. Benson, MT(ASCP), M.A.
Associate Director for Chemistry

Courtney D. Harper, Ph.D.
Associate Director for Toxicology

Douglas Wood, MT(ASCP), MCSE
Division of Chemistry and Toxicology

Guest Presenter
Parvin P. Waymack, Ph.D.
Research Chemist
Centers for Disease Control and Prevention

CALL TO ORDER AND PRELIMINARY MATTERS

Panel Chairperson Bernard W. Steele, M.D., called the meeting to order at 8:09 a.m. He noted that the panel members present constitute a quorum and asked them to introduce themselves.

Panel Executive Secretary Veronica J. Calvin read the conflict of interest statement. No waivers have been issued with regard to any panel members or consultants. Dr. Parvin Waymack, a guest speaker, has acknowledged scientific collaboration with firms at issue.

Carol C. Benson, MT(ASCP), M.A., Associate Director for Chemistry, Chemistry and Toxicology Division, and **Courtney D. Harper, Ph.D., Associate Director for Toxicology, Office of In Vitro Diagnostics,** provided division updates.

FIRST OPEN PUBLIC HEARING

Russell G. Warnick, Berkeley HeartLab, Inc., spoke about improvements in lipid and lipoprotein testing. He said that the lipid panel which has dominated practice fails to identify half of patients at risk, and lipoprotein subclasses can better characterize risk.

Kenneth French, Atherotech, Inc., discussed the vertical auto-profile (VAP) cholesterol test, clinical relevance, and national guidelines.

Nehemias Muniz, Quantimetrix Corporation, discussed their work on measurement of high density lipoprotein (HDL) subfractions. They have found that not all subfractions are the same and they have different correlations with different risk factors, particularly comparing large and small HDL.

Samia Mora, M.D., M.H.S., Harvard Medical School and Brigham and Women's Hospital, discussed the question whether the increased CHD risk of patients with smaller LDL size is due to LDL particle size or particle number. Without adjusting for small LDL particle number, they found large LDL particle number is only weakly associated with IMT. But when both small and large particles were examined jointly, both were highly significantly associated with carotid intima-media thickness (IMT), even after adjusting for traditional risk factors. LDL particle size contributed little after accounting for particle number.

James Otvos, Ph.D., LipoScience, Inc., talked about nuclear magnetic resonance (NMR) spectroscopy, which has been used for almost ten years and is well validated analytically and clinically. Claims about clinical utility should be evidence-based, and broader utilization will now be enabled by decentralization of the assay.

Dr. Gronowski asked Dr. Otvos if the effects of freezing and storage on things like particle number and size have been studied. Dr. Otvos said the only issue is that freezing affects triglyceride rich samples and that freezing at minus twenty degrees for more than a couple of weeks may cause changes as well.

Dr. Watson asked how subclass distribution assays correlate with and add benefit to non-HDL cholesterol. Mr. French said VAP shows greater than 95 percent correlation in calculating Apo B while traditional total cholesterol minus HDL shows less correlation. Dr. Otvos suggested that non-HDL is more strongly associated with outcomes than LDL because it is a surrogate marker for LDL particle number. He referenced a recent paper in AJC that shows that in hyper-triglyceremic patients non-HDL cholesterol gets closer

than LDL cholesterol to LDL particle number but noted there are still discrepant situations.

H. Robert Superko, M.D., Fuqua Heart Center for Prevention, Piedmont Hospital, stated that many of the issues being discussed can be resolved using standard measures of triglycerides and HDL cholesterol and urged consideration of standardizing the field.

Dr. Marcovina asked Dr. Warnick if he had a correlation standard between determination of HDL 283 by differential precipitation technique in the gradient gel electrophoresis, and Dr. Warnick said no. Dr. Marcovina then asked Dr. Otvos for data on correlation between LDL particle number and total Apo B and about how LDL Apo B was measured in terms of its correlation with elevated particle number. Dr. Otvos said it was measured nephelometrically and the LDL was separated by ultracentrifugation but all that was done was one spin to remove very low density lipoproteins (VLDL). He also said the correlations are essentially equivalent between plasma Apo B and LDL particle number in the range of 0.9 to 0.95.

Dr. Levinson asked the speakers to comment on the conclusions of three papers, by Gardner et al., Dr. Campos, and Ernest Schaefer. Dr. Mora said the finding from MESA that small and large are negatively correlated may explain much of the confusion about LDL size. She said that particle number must be taken into account in addition to particle size. Mr. French was familiar with the papers and said they were looking at the overwhelming body of evidence and said a key issue is how the points are defined. Mr. Warnick said all early studies using absorbent dyes were compromised by the dyes being

non-stoichiometric, meaning they underestimate the dominant particles and that absolute quantification of particles can eliminate variability and some of the noise.

GUEST PRESENTATION

Parvin P. Waymack, Ph.D., Research Chemist, Centers for Disease Control and Prevention, presented his perspective on HDL-LDL subclass, subspecies, and subfraction analyses and challenges to standardization. His conclusions were that the method-dependent results make it challenging to compare studies, and each method is likely defining a different subpopulation of lipoproteins. It is not obvious which reference method is best for standardization; one based on density gradient would require arbitrary modifications to other methods which might not be appropriate. Direct comparison of these methods is needed, and commonly defined subfractions should be identified and characterized. Dr. Waymack acknowledged it may not be possible to harmonize all the methods, but the goal should be some standardization of defined subpopulations of atherogenic and anti-atherogenic lipoprotein particle concentrations.

Dr. Tsai asked how difficult it is to standardize them. Dr. Waymack said the problem is defining the analyte that will be standardized and that data associating risk with the specific particle does not exist. Dr. Tsai asked if they could just be standardized according to particle size. Dr. Waymack said it could be done by particle size or density but that other methods would have to be adjusted accordingly. He said the real issue is relating what you are measuring to risk. Dr. Tsai then asked if standardization is really necessary. Dr. Waymack said some sort of standardization is necessary and raised the possibility of standardizing each one separately.

Dr. Zhang asked Dr. Waymack for his understanding of the biology behind this method. Dr. Waymack said the source of the problem is the distribution of LDL particle concentration.

Dr. Remaley asked about the utility of proficiency tests and how such a program would be created for these assays. Dr. Waymack proposed a separate method for each peer group.

Dr. Levinson referenced other studies in which comparing NMR with gradient gel electrophoresis got less than fifty percent of people classified as pattern B were also pattern B on the other. Dr. Waymack responded by asking what is the real value of pattern A, pattern B, and the phenotype.

Referring to Dr. Waymack's statement that LDL cholesterol methods are well standardized, Dr. Marcovina asked him to define the limit of that standardization. Dr. Waymack said he does not think they are well standardized and that CDC does not standardize LDL cholesterol through the lipid standardization program. Dr. Marcovina referred to his statement that ultracentrifugation separation alters the lipoprotein composition, but Dr. Waymack said it is HDL cholesterol that shows the biggest changes with regard to that problem. He said routine methods of determining cholesterol are not as well standardized as they could be and said that different tests give different results, but there are a number that closely agree with the target value.

Dr. Winter inquired about data on head-to-head comparisons among the four methods. Dr. Waymack thought the only one was the one he already reviewed showing poor correlation between A and B phenotype in the four methods.

FDA PRESENTATION

Douglas Wood, Division of Chemistry and Toxicology, gave a presentation on identification of lipid fractions, the cholesterol pathway, effects of cholesterol, public health concerns, lipid subfractions, pertinent research, and NACB recommendations. He concluded that the proposed recommendations and published reports give insight on the current understanding of the clinical utility of such assays and the strengths and weaknesses of the potential biomarkers. However, FDA seeks the panel's advice on whether clinical use of these devices poses a public health risk and their effectiveness in measuring and diagnosing lipid disorders and atherosclerosis.

Dr. Grines asked for clarification of the NACB guidelines with regard to the statement that measurement of subfractions is not helpful and in some cases may be harmful. Mr. Wood said it had to do with the proposal to use subclasses in normal lipidemic patients and the possible side effects of using lipid lowering therapy in patients who may not need it. Dr. Grines asked if it had been proven to be harmful, and Mr. Wood said no and that he believes that is their definition.

Dr. Zhang asked if FDA had looked at actual studies as opposed to reviews and association recommendations. Mr. Wood said yes and that a number had been cited in the executive summary.

Dr. Marcovina asked about Mr. Wood's statement about consensus of experts in the field. Mr. Wood agreed that it was really a consensus of those experts participating on the NACB panel.

Dr. Tsai asked if there are specific papers documenting the harmful effects of performing these tests. **Alberto Gutierrez, Ph.D., Director, Division of Chemistry and Toxicology,** said that it was a general statement about potential harm and not specifically

addressed with respect to lipid subfractions. Dr. Grines said he had interpreted the statement as a warning, and Dr. Gutierrez reiterated that it may not be that the panel had specific reasons for saying they may be harmful.

Dr. Tsai suggested that the issue of harmfulness is somewhat irrelevant to the discussion since there is apparently no evidence for it and asked if the term could have been used loosely in terms of less than cost effective. Mr. Wood agreed and said he had merely stated the guideline rather than try to paraphrase it.

Dr. Remaley pointed out that the NACB guidelines are about five years old and many of the studies have been done since then. He also emphasized that they were draft recommendations which were not very well developed on the subfractions and the conclusions of which seem to lack any basis. Dr. Watson agreed and noted that the weight of evidence behind them is the weakest level there is.

Dr. Levinson added that the issue is whether the various methods agree with reference markers, and Mr. Wood agreed that is one of the questions the agency is asking the panel.

Dr. Winter asked about data on phenotypes in non-metabolic syndrome patients, normal triglyceridemic, normal HDL, normal LDL, and specifically for relative frequencies of the B phenotype in the control population versus the population that would have heart disease. Mr. Wood said there are papers available but did not have any data to present.

Dr. Zhang asked what kind of evidence there was for the statement that subclass has little to do with treatment over time. Mr. Wood said there had been studies which he said were not small.

Dr. Loew asked how judgments about normal versus dyslipidemic were made in regard to the HDL fractions comparison. Mr. Wood said they were made by the author but did not know how. He did not know of any similar comparison having been made for LDL fractions.

Mr. Muniz said it was a very simple clinical distinction using the criteria of the NACB ATP-III guideline. Dr. Remaley said looking at the range can be misleading and that it would be more helpful to look at ROC area curves.

PANEL DISCUSSION

Dr. Grines highlighted the conflicting views on the importance of particle size. Dr. Watson said the field needs population-based studies prospectively following normal healthy people to see how well it predicts disease as well as studies showing that you can make a difference by treating people with a particular phenotype with a particular therapeutic intervention.

Dr. Zhang talked about the need for subclass, and he said that there is no well-designed study demonstrating that any one subclass or group of subclasses has better indication in clinical diagnosis or treatment. He also noted the limited number of studies and limited numbers of individuals studied. Dr. Levinson said the question seems to be whether these tests agree with total or HDL cholesterol and provide the same kind of information.

Dr. Shamburek agreed the studies are confusing and said they need to seriously consider whether the current tests are enough for clinicians at this point. Noting there are many factors which cause atherosclerosis, Dr. Winter did not imagine any one lipid test

would be 100 percent predictive and wondered if better markers are found who they should be applied to.

Dr. Remaley reiterated that current tests are not adequate since they do not identify half the patients at risk. He suggested that the panel is intended to advise FDA on whether these tests will be available rather than creating guidelines for their use and noted they may be very valuable in particular patient subsets, particularly for those of intermediate risk.

Dr. Marcovina said they should be open and evaluate each method independently and noted that fifty percent of those at risk are already being harmed by the lack of a test which identifies their risk. Dr. Levinson said that unless it can be shown that something is really a better predictor and can get a much better area under the ROC curve, even with other factors, he does not think they can add substantially to what is already done.

Dr. Grines said there is a need for more information which could be met by having these devices in clinical settings. She also noted that many patients presenting with infarcts have met cholesterol guidelines and said the guidelines may not be strict enough given the evidence on progression of atherosclerosis in patients who meet the guidelines. She hoped that additional knowledge from these subfractions may allow us to predict in whom it will progress.

Dr. Watson agreed with the points about the unmet need but believes strongly there could be harm both from using the data to overtreat and as an excuse to undertreat. She agreed the tests should be available but said it should be made clear they are to be used only for something like additional risk assessment.

Dr. Tsai said that after balancing the current risk with the risk of having too many tests that are not standardized that there is some usefulness to these tests. Dr. Shamburek said there is a distinction between research and clinical practice and that they need to look at the clinical utility of the individual tests.

Dr. Levinson talked about the atherogenic phenotype and how it can be environmentally produced in people who are insulin resistant and overweight and proposed looking at those issues rather than looking at particular subtypes.

Dr. Winter asked about reproducibility, long term stability, and reference materials of the various assays. Mr. French replied said the VAP test has less than three percent variation in reference to a CDC reference laboratory and their proficiency testing program. Dr. Winter asked if that is in reference to subfractions or concentrations of cholesterol in HDL and LDL. Mr. French did not of any data on the subclasses but said it was in reference to total cholesterol, HDL, LDL, VLDL, Lp(a), and intermediate density lipoproteins. Dr. Gronowski asked if it was less than three percent total CV, and Mr. French said yes. Dr. Winter pressed for data on the subfractions, and Mr. French, with help from a colleague in the audience, said it is three percent or less.

Dr. Zhang asked what the three percent CV means. Dr. Otvos said for the NMR assay the coefficients of variation are better for the pooled subfractions at less than five percent for total LDL particle number than the individual subfractions which are greater than that but generally less than ten percent. With regard to standardization, he said frozen pools of serum are used as day-to-day standardization or for quality control material. The NMR data uses a chemical reference standard that is measured every day,

and quarterly proficiency testing comparing data from all the machines shows very good agreement.

Dr. Levinson asked about the effect on reproducibility if the assays were available in multiple labs all over the country. Dr. Gutierrez said reproducibility would be looked into but did not think going into specifics would be helpful for this meeting. Dr. Winter commented on the fact of patients without lipid abnormalities getting heart disease because of other risk factors.

SECOND OPEN PUBLIC HEARING

William Cromwell, M.D., Medical Director, Division of Lipoprotein Disorders, Presbyterian Center for Preventive Cardiology and Wake Forest University School of Medicine, discussed the clinical utilization of lipoprotein subfractions.

Paul Ziajka, M.D., Ph.D., Director, The Florida Lipid Institute and Chief Medical Officer, Atherotech, stated that the traditional lipid profile does not work very well in screening for risk and using advanced methods can almost double the ability to detect risk. Noting that these advanced lipid parameters are already widely used, he said the issue is not whether to allow their use but how to regulate and standardize their use.

Herbert K. Naito, Ph.D., M.B.A., NorthStar Consulting Service, advocated selective use of the emerging risk factors and the development of standardization programs.

Dr. Muniz addressed earlier questions related to a paper by Ensign. He said many of the criteria used were created by the author and are not the recommendations for the two gel electrophoresis method.

Dr. Tsai asked for clarification of Dr. Cromwell's comment that use of these lipid profiles can lead to differential therapy and whether sometimes he would preferentially use fenofibrate. Dr. Cromwell said he does believe lipoprotein can help change individual patient management. Dr. Tsai inquired further about diet and use of fibrate to lower triglycerides. Dr. Cromwell characterized it as the effect on composite dyslipoproteinemia and said the effects of diet and medications should be directed not only towards the lipid disorder but also towards LDL particle excess.

Dr. Gronowski asked if he had evidence that lowering small LDL and increasing larger LDL changes a patient's outcome. Dr. Cromwell said he would be more concerned with the total number of LDL particles. He referred to a study showing that only LDL particle number by NMR was significantly associated with prospective risk, and the same was true of HDL particle number.

Dr. Watson said that she would use statin and combination therapy on Dr. Cromwell's case CG and was not sure that advanced lipoprotein testing would change that. Dr. Cromwell agreed that the patient could benefit from combination therapy but said the question is what sources of risk are present but said that even after lowering LDL the patient could still have high numbers of LDL particles.

Dr. Gronowski asked if there had been any interventional clinical trials with prespecified outcomes showing improved outcomes based on particle number. Dr. Cromwell said there was one old one and added a caveat about statin trials.

Dr. Grines asked why Dr. Watson would treat patient CG. Dr. Watson said the guidelines say to not necessarily treat individuals with a predominant striking risk factor

strictly according to the guidelines and to use clinical judgment for such patients. She noted that the patient had two really strong risk factors.

Dr. Shamburek asked if Dr. Cromwell had measured only Apo B. Dr. Cromwell said Apo B and NMR are two ways one could assess LDL particle number. Dr. Marcovina pointed out there could be direct measurement of LDL particles with Apo B, and Dr. Cromwell agreed. Dr. Watson agreed that Apo B is commonly done and is a good marker for particle number. Dr. Grines inquired whether the results can be trusted. Dr. Watson said it is a very good test in some ways more reliable than lipoprotein measures of LDL.

Dr. Levinson said that statistically one can't really tell a difference between non-HDL cholesterol and Apo B. Dr. Marcovina suggested if that is true then it would make the case for determining LDL particle number by any other method.

Elizabeth Schilling, CRNP, University of Maryland Medical Center, discussed the benefits of using lipoprotein subfractionation in clinical settings.

Dr. Winter asked if she had said that 68 percent of patients had the metabolic syndrome and if she would have only recognized it because of the Atherotech. Ms. Schilling said she was able to look at particle density with the small dense LDL and that she did not have information on patients' weight and blood pressure. Dr. Winter asked if that validates the test since the clinician sees the patient and would know the BMI. Ms. Schilling said she was simply stating it for information since public averages of metabolic syndrome are usually in the range of thirty to forty percent. Dr. Winter asked if all of those patients with metabolic syndrome had elevated BMI. Ms. Schilling said no and that there were plenty normal patients, but she did not have a percentage.

Dr. Tsai asked for clarification of her point about metabolic syndrome. Ms. Schilling said her point is that there is a correlation, not that she is redefining metabolic syndrome.

Dr. Zhang asked Ms. Schilling if she thinks the lipid subclasses will bring changes to diagnostic practice. Ms. Schilling said it changes the aggressiveness of therapy and that patients and clinicians are more likely to be compliant when they have the data and they see a change with simple interventions. She said such an assay will impact clinical practice. Dr. Zhang asked for her opinion about how to standardize and improve clinical practice.

Ms. Schilling said she would begin by doing more assessment of cardiovascular risk by looking at the whole patient and that medical providers must be educated regarding more appropriate use of these tests.

Dr. Remaley asked how clinicians use these tests for each risk category and whether they advocate using them as screening or simply ancillary tests. Ms. Schilling said that all of her patients get the test since she does preventive cardiology. She advocated that primary care providers use it for anyone with a strong family history and anyone who has had an event with normal cholesterol. She does not think it is the right test for routine screening in primary care settings.

Dr. Winter asked if clinicians had used HDL subfractions and found it to be of clinical value. Ms. Schilling said it has been very valuable. Dr. Cromwell said the data on HDL subclasses were very confounding and confusing and predicted that the value will vary based on patient characteristics.

Dr. Tsai asked Ms. Schilling why she would not have found metabolic syndrome without Atherotech and if she would not have specifically measured for metabolic syndrome. She agreed she would look for it in the absence of the technology but said she uses the test to measure the success of treatment.

Dr. Levinson said a determination of clinical usefulness can only be made if there is a study with a very high predictive value or a prospective study showing clinical benefit and did not think Ms. Schilling's determination had met either of those criteria. Ms. Schilling said that is true but that intuitively based on the data it makes sense and that just treating LDL is not working.

Dr. Shamburek cautioned about ethnic differences with regard to small dense LDL and said it is possible many patients would be overtreated if one depends solely on small dense LDL. Ms. Schilling concurred and stated that among sub-Saharan Africans Lp(a) is not indicative of risk, but she could not explain the population differences.

Dr. Superko said there have been a number of studies from reputable investigators demonstrating the clinical utility of the HDL subclasses and said it is only useful if it changes the patient's treatment. He said he had participated in a meeting with the CDC as well as scientists and well known investigators in the field and suggested that a similar group of people be convened to ensure that the panel hears the entire scientific story rather than industry biases.

Dr. Winter asked what data the panel is missing that such experts could share. Dr. Superko said they need to determine in what patient subsets the information is clinically useful and provided examples from various studies including HATS, the National Asian Indian Heart Disease Study, and a Quebec study. Dr. Winter asked if the first studies

cited favored measuring Apo A-1 or fractionating to HDL-3. Dr. Superko said he is talking about LpA-I using an affinity chromatography method developed in France to look at lipoprotein particles with only A-1 and that it is a very good method of determining risk in some studies.

Dr. Marcovina pointed out that the method used in the Greg Brown study was actually developed at the University of Washington not in France by Fruchart. Dr. Zhang asked for a summary of what was missing from the FDA's presentation. Dr. Superko talked about the difficulty in standardizing laboratory methodology. He said one thing missing is the history of lipoprotein subfractionation and its relation to coronary disease. Issues remaining are whether this testing is useful for everyone, determining what the testing is useful for, and the lack of a primary outcome study.

Dr. Tsai disagreed that the panel has not read or done part of the work in this area. Dr. Superko apologized and said he was referring mainly to the information presented during the course of the meeting.

Dr. Levinson talked about odds and risk ratios and said to really discriminate well one needs a ratio of at least one to 200. Dr. Superko agreed that relative risk increase does not necessarily correlate with discrimination in terms of prediction of individuals. He said the field is moving towards treating disease rather than treating a number derived in a laboratory and that what is needed is some measure of disease and disease chance.

Dr. Watson said focusing solely on insulin as the predominant risk factor would be a mistake since in clinical trials the best insulin synthesizers available show an excess of cardiovascular events and not a decrease. Dr. Superko noted there are also studies using weight loss in terms of diabetes prevention and which show dramatic reduction in

development of Type-2 diabetes, the assumption being that the reduction is related to treating insulin resistance. Dr. Watson noted the most recent study of rosiglitazine that showed not only improvement in progression to Type-2 diabetes but also a statistically significant increase in cardiovascular events. Dr. Superko said diet, exercise, and weight loss would be the most often used therapy.

Dr. Zhang also disagreed with Dr. Superko's claim that the panel is missing data.

PANEL RESPONSE TO FDA QUESTIONS

1. Is there sufficient information available to conclude that HDL and/or LDL subfractions can be used:

a. to assess a patient's risk of developing CVD?

The panel generally felt that small dense LDL can add useful information for assessing a patient's risk of developing CVD but that these tests should only be used in certain populations at this time. Better data is needed on which specific subfractions should be used.

b. to diagnose dyslipidemia?

The panel generally felt that LDL, but not HDL, subfractions can be useful in diagnosis of dyslipidemia. There were concerns that this information may cause confusion among clinicians. A large portion of the panel had trouble with the use of the word "diagnosis" and did not think the subfractions meet the criteria for diagnosis.

c. to monitor treatment of dyslipidemic patients?

The panel was in near universal agreement that there is not enough evidence to use LDL and HDL subfractions for monitoring treatment of dyslipidemic patients.

d. for any other use?

The panel in general said no. Comments were made regarding continuing research in this area and for use in research applications looking at various aspects of metabolism pathology.

2. If sufficient information is available for clinical use, should HDL and/or LDL subfractions be used:

a. as a stand-alone test?

b. as an adjunctive test to be used with other traditional risk assessment tools (e.g., Total, HDL, and LDL cholesterol) and clinical judgment?

The panel agreed that HDL and LDL subfractions should never be used as stand-alone tests. There was general agreement that LDL subfractions should be used as an adjunct to other traditional risk assessment tools, but several panel members noted that it should only be used in select populations and never in a patient with an abnormal lipid profile. Concerns were expressed regarding HDL subfractions.

Dr. Levinson said no with regard to use as an adjunct because he said it could only be used in very selective cases. Dr. Shamburek also said no because there is not enough data.

Dr. Gutierrez asked for more information on what selected populations should be looked at and how to look at things like accuracy in a specific population. He also asked the panel whether submissions should be required to have data only for the particular population targeted.

Dr. Winter said as an adjunct for those individuals who have normal lipid profiles but still have other risk factors. Dr. Watson said individuals with a personal or family history of atherosclerosis not in proportion to traditional risk factors. Dr. Levinson said those who have had an event but do not have any known risk factors. Dr. Remaley said patients with intermediate risk with whom there is a dilemma regarding how aggressively to treat them but only as a positive risk factor. Dr. Tsai agreed with Dr. Remaley and Dr. Watson. Dr. Marcovina also agreed with Dr. Remaley.

Dr. Shamburek said those with a personal history would be treated anyway and did not think existing data is clear regarding intermediate risk. Dr. Zhang thought they

needed strong outcome data to make any determination. Dr. Gronowski agreed and suggested that research begin with the populations mentioned. Dr. Grines suggested also looking at those with mild coronary disease but no clinical symptoms or abnormal stress test. Dr. Loew also thought more data was needed. Dr. Winter noted that saying it might be used in particular populations is not an endorsement of its use.

3. When used either as a stand-alone test or in conjunction with other lipid measurements (with values defined as non- cardiac risk by the NCEP ATPIII guidelines), will changes in treatment based upon the abnormal lipid subfractions pose an acceptable level of benefits compared to risk to the patient?

Some panel members felt there is no data showing the value of this testing and therefore there could be harm. Others felt the subfractions could be used as a positive risk factor to get people into treatment.

Dr. Tsai noted the results are fairly complicated and thus may or may not be useful for primary practitioners. Dr. Shamburek was troubled by lumping all of the tests together and was unsure these methods would be able to identify patients who are being undertreated. Dr. Winter said the benefits would likely be greater than the risks if LDL fractionation is used predominantly to decide if a patient should move from intermediate to more intensive therapy.

4. How would the accuracy of these subfractions be established? What is an appropriate reference method? What are appropriate acceptance criteria when comparing to the reference method?

Dr. Winter said the electrophoresis and VAP should be compared to traditional ultracentrifugation and that, absent a predicate for NMR, independent labs should be used to look at its robustness. Dr. Remaley thought it may be a mistake to try to get the methods to agree since they separate the subfractions based on physical properties. He thought existing test programs for other electrophoretic techniques could be used for electrophoresis and suggested separate accuracy based assessments for NMR and density

gradient ultracentrifugation. Dr. Levinson proposed comparing with Apo B and for LDL subtypes also with non-HDL cholesterol.

Dr. Zhang suggested looking product or assay-specific and that the sponsors should emphasize standardization of their own products. Dr. Winter proposed sharing sera among sites and comparing results. Dr. Worthy thought it would be valuable to have some sort of reference preparation and reference method and said that though it might be difficult to develop a reference material it would open up the entire field to standardization and compared it to what happened with hemoglobin A1C. He proposed shared responsibility since it may be difficult for industry to shoulder the entire burden of conducting the necessary studies.

Dr. Levinson was not sure the comparison with A1C was valid given that the methods are different and do not agree very well with one another. Dr. Winter encouraged an exchange of samples between the different technologies to see how the same sera are characterized by each assay.

5. How should expected values be determined for lipid subfraction assays? Is it possible to make meaningful test interpretations in cases where reference ranges for normal and "diseased" patients overlap?

The panel generally felt the expected values should be derived from healthy people rather than population studies and that more data is needed to identify where to locate cutoff points.

Mr. Wood clarified that "expected values" refers to reference ranges. Dr. Marcovina stated that even if each method had data from a large healthy population they would still not have a reference range because a good percentage of that population will be at risk. Dr. Levinson acknowledged that doing it after the fact is not ideal but suggested that each manufacturer could devise reference ranges based on their own

experience. Dr. Shamburek said each method would have to be dealt with separately since they are so different. Dr. Winter said that with a sensitive test and safe medications one can argue for sensitivity over specificity.

Dr. Gronowski said the cutoff would be set based on what value is associated with unacceptable risk. Dr. Levinson agreed and said that would require a vast amount of data from multiple studies. Dr. Watson said large scale prospective population-based studies are needed. Dr. Remaley said it may go back to the practice of medicine because we may not be able to have a single cutoff depending on risk, and Dr. Steele agreed. Dr. Loew said they need a clear definition for what constitutes disease.

Dr. Gutierrez asked if ROC studies should be conducted in the specified populations or in the normal population. Dr. Levinson thought it might be better to just look at disease groups and see how extreme their values are. Dr. Remaley noted that the control population must be carefully defined or else the apparent utility may be diminished by including people with disease. Dr. Worthy suggested that manufacturers should be involved before decisions are made about how to conduct studies.

6. If used (either as an adjunctive test to traditional lipid measurements or as a stand alone diagnostic) to diagnose or predict risk for dyslipidemia or atherosclerosis, does the lack of standardized nomenclature or differences in assay performance (e.g., reference ranges, precision, fractions analyzed, etc.) pose an unreasonable risk to the patient?

The panel agreed there is a lot of room for confusion with people using different assays and supported something along the lines of a workshop including all the stakeholders to try to come up with standard nomenclature.

Dr. Winter highlighted the importance of treating samples appropriately. Dr. Tsai did not think the lack of standard nomenclature is serious enough to preclude use of these

tests. Dr. Gronowski said standardization would be optimal and otherwise significant physician education will be needed to address the impact of changing methods.

7. Is there a difference in the assessment of lipid subfractions based upon particle size versus particle number? If so, what are the strengths and weaknesses of each method? Please discuss.

The panel felt that they do not have the data to make a decision. A head-to-head study comparing the methods in the same population is needed.

Dr. Tsai said that currently only NMR gives particle numbers and the other methods provide particle size but noted it may be possible to derive particle number from particle size and density. Dr. Remaley noted there may be differences related to the underlying physical structure of the particles.

ADJOURNMENT

Dr. Steele thanked the participants and adjourned the meeting at 4:21 p.m.

I certify that I attended this meeting of the Clinical Chemistry and Clinical Toxicology Devices Advisory Panel on December 6, 2006, and that these minutes accurately reflect what transpired.

Veronica J. Calvin
Executive Secretary

I approve the minutes of the December 6, 2006, meeting as recorded in this summary.

Bernard W. Steele, M.D.
Chairperson

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