clinical samples. The alternative of working with dilutions of cell lines suffers from a major disadvantage because circulating tumor cells vary widely in size, shape, weight, and antigen expression, whereas tumor cells from cultured lines are very homogeneous in these variables. Thus, the results from such a model system may not predict results with clinical specimens (6).

The lower detection rate and lower counts of cancer cells after ficoll centrifugation suggest a significant loss of cancer cells during processing of marrow samples. However, the possibility of antigen loss during ficoll centrifugation needs consideration in future studies. For leukapheresis samples, by contrast, there was no difference in the nominal detection rate (44%), and the yield of detected tumor cells was slightly, although not significantly, higher after ficoll separation. This could be attributable to the lower presence of erythrocytes in apheresis samples, specimens with extremely high white-cell counts, than in marrow or blood samples.

For PCR detection, the separation protocol had no influence on the detection rate. Culture experiments revealed that it is not always possible to culture viable cells from samples that are tumor cell-positive by other methods. One could hypothesize that RT-PCR also detected free mRNA liberated from apoptotic cancer cells, but this must be demonstrated.

We conclude that separation of nucleated cells by RCL from marrow or blood samples is superior to ficoll separation for the immunocytochemical detection of cancer cells, but that neither technique offers a clear advantage for leukapheresis samples or for RT-PCR analysis of any of these sample types.

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References

William Krüger*
Roman Jung2
Nicolaus Kröger3
Kai Gutensohn3
Walter Fiedler4
Michael Neumaier2
Fritz Jänicke5
Christoph Wagener2
Axel R. Zander1

1 Bone Marrow Transplantation Center,
2 Department of Clinical Chemistry,
3 Blood Transfusion Service,
4 Department of Oncology/Hematology, and
5 Department of Gynecology and Obstetrics,
University-Hospital Eppendorf
Martinistrasse 52
20246 Hamburg, Germany

*Address correspondence to this author at: Einrichtung für Knochenmarktransplantation, Abteilung Hämatologie/Onkologie, Universitäts-Krankenhaus-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. Fax 49-40-42803-3795; e-mail krueger@uke.uni-hamburg.de.

Is Lipoprotein(a) Cholesterol a Significant Indicator of Cardiovascular Risk?

To the Editor:

In the July 1999 issue of Clinical Chemistry, Seman et al. (1) present reference ranges and prevalence data for lipoprotein(a) cholesterol [Lp(a)-C], based on the examination of offspring and spouses of participants of the Framingham Heart study. These data will be potentially useful provided that the determination of Lp(a)-C is included in routine cardiovascular risk assessment in the future.

Seman et al. (1) measured Lp(a)-C by nonspecifically trapping Lp(a) with a lectin and subsequently determining cholesterol enzymatically. They claim that their method, in contrast to immunoassays for Lp(a), was not sensitive to the genetic size polymorphism of apolipoprotein(a) [apo(a)]. This, however, has not been demonstrated conclusively. Thus, to date, there are no data available as to whether the apo(a) size polymorphism affects the binding of Lp(a) to the lectin matrix.

In 1995, we published a method to measure Lp(a)-C using a two-step procedure: (a) we first removed lipoproteins with a density <1.006 kg/L by preparative ultracentrifugation; and (b) we then detected Lp(a) by electrophoresis of the infranate and subsequent cholesterol staining (2). In addition, we evaluated a commercially available agarose gel electrophoresis method that separated HDL, LDL, VLDL, and Lp(a) in one run (3, 4). Both of these methods are completely independent of the apo(a) size polymorphism, and they have the additional advantage that LDL-cholesterol (LDL-C) is already “corrected” for Lp(a)-C.

Immunoassays for Lp(a) differ widely from each other regarding their sensitivity to the apo(a) genetic polymorphism. Probably the least sensitive ones are enzyme-linked immunoabsorbent assays that use capture antibodies against apo(a) and detection antibodies against apoB (5, 6). To increase the accuracy and comparability of Lp(a) mass assays, an IFCC standard will soon be available in which the Lp(a) concentration is expressed in mmol/L. This standard will not, however, eliminate the problem that Lp(a) mass determinations are affected by the apo(a) polymorphism because it will be used in
connection with assays of varying architecture that are themselves influenced by the size of apo(a). In addition, the current cutoff values of the National Cholesterol Education Program (NCEP) guidelines already account for the bias of LDL-C by Lp(a)-C (7). The question, however, of whether quantification of Lp(a) in terms of cholesterol will be preferable over immunoassays will probably not be answered on the basis of methodological considerations only. Instead, this will depend on the ability of this marker to identify subjects at an increased risk for coronary artery disease (CAD) better than Lp(a) protein determination performed by methods independent of the apo(a) size polymorphism. From this point of view, we miss in the publication by Seman et al. (1) the discussion of our reports, in which we measured Lp(a)-C directly using electrophoretic techniques (2, 4).

Using our combined ultracentrifugation and electrophoresis assay, we performed a case-control study that included 399 female and male subjects. Lp(a) mass was determined on a Behring nephelometer, using a polyclonal antibody (Incstar). This method was standardized with a commercial calibrator (Immuno) (2). In that study, both Lp(a)-C and Lp(a) mass concentrations were significantly higher in patients with CAD demonstrated by coronary angiography than in healthy controls (P < 0.05). The odds ratios were 1.71 (95% confidence interval, 1.024–2.855) and 1.59 (95% confidence interval, 1.019–2.481) for Lp(a)-C and Lp(a) mass, respectively, suggesting that both methods have similar discrimination power. This was confirmed by virtually identical ROC curves. These data are very similar to the results of Seman et al. (1), who reported an adjusted odds ratio for Lp(a)-C of 2.293 in men.

Interestingly, in our study, the ratio of Lp(a)-C to Lp(a) mass tended to be lower in CAD patients than in controls (P = 0.097). The nephelometric assay used in this study was sensitive to the apo(a) size polymorphism, with larger apo(a) isoforms producing higher signals than smaller ones. Cross-sectional studies have shown that CAD patients possess smaller apo(a) isoforms than healthy individuals. Because our nephelometric Lp(a) immunoassay is influenced by the size of the apo(a) antigen, this would produce an increased Lp(a)-C/Lp(a) mass ratio in CAD patients if the amount of cholesterol per particle was not different between CAD and controls. However, the opposite was observed (2). This raises the question of whether Lp(a) particles from patients with CAD contain less cholesterol molecules, similar to atherogenic “small, dense” LDL particles (8). Regarding the average percentage of cholesterol content in Lp(a) particles, the averages (29.1% and 26.7%) we found in our studies (2, 4) are in good agreement with the average (27.5%) presented by Seman et al. (1). Seman et al. now have the data to answer the interesting question of whether the composition of Lp(a) is of predictive significance because they measured Lp(a) mass in addition to Lp(a)-C in a subset of 1000 samples, using an immunoassay that is not biased by the apo(a) polymorphism. If they are able to reproduce our finding suggesting the existence of small, dense Lp(a) particles, then the simultaneous assessment of Lp(a)-C and Lp(a) mass might represent a valuable tool to assess the contribution of Lp(a) to an individual’s risk of CAD.

References

Matthias Nauck*  
Winfried März  
Heinrich Wieland

University Hospital Freiburg  
Department of Clinical Chemistry  
Hugstetter Strasse 55  
D-79106 Freiburg i. Br., Germany

*Author for correspondence. Fax 49-761-270-3444; e-mail manauck@med1.ukl.uni-freiburg.de.

Correction
On page 2130 of the article by V. Ricchiuti and F. S. Apple, entitled “RNA Expression of Cardiac Troponin T Isoforms in Diseased Human Skeletal Muscle” (Clin Chem 1999;45:2129–35), the correct sequence for the human skeletal muscle troponin (sTnT) reverse primer should be: 5‘-GTT TCA GCT TCG CCA TCA GGT CGA ACT-3‘.

The authors apologize for the error.