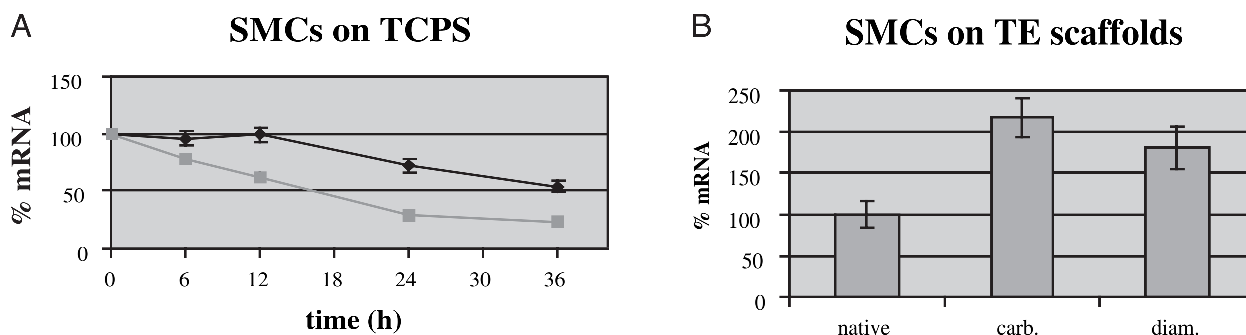

Figure 1. Ratios of cyclin E and tTG mRNA expression levels of umbilical vein SMCs cultured on g-TCPS (A) and on TE scaffolds (B). A: Cells were cultured in medium with 20% serum (–●–) and without serum (–■–) on gelatin coated TCPS for up to 36 h and mRNA expression levels were compared to time point zero. B: Cells were cultured in medium with 20% serum on native and crosslinked scaffolds and mRNA expression levels of cells cultured on crosslinked scaffolds (either with a carbodiimide (carb.) or with a diamine (diam) were compared to levels of cells cultured on native scaffolds.

can be used to compare growth behaviour of cells cultured on (and in) different scaffolds for TE applications. Crosslinking of TE scaffolds composed of collagen and elastin for small diameter blood vessel applications with a carbodiimide or with a diamine seems to be beneficial to SMC behaviour. We suggest that with this approach it will be possible to culture cells in a standardised way not only for obtaining an artificial media of a TE blood vessel using SMCs but also for all kinds of TE purposes using other cell types.

References

1. Ohtsubo M, Theodoras AM, Schumacher J, Roberts JM, Pagano M. Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. Mol Cell Biol 1995; 15: 2612-2624.
2. Volokhina EB, Hulshof R, Haanen C, Vermes I. Tissue transglutaminase mRNA expression in apoptotic cell death. Apoptosis 2003; 8: 673-679.
3. Heimli H, Kahler H, Endresen MJ, Henri ksen T, Lyberg T. A new method for isolation of smooth muscle cells from human umbilical cord arteries. Scand J Clin Lab Invest 1997; 57: 21-29.
4. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome 1996; 6: 986-994.

Ned Tijdschr Klin Chem Labgeneesk 2004; 29: 277-278

An ELISA for the determination of lipoprotein (a) with careful accuracy targeting

P.N.M. DEMACKER, E. BOLAT, H.I. TOENHAKE-DIJKSTRA and L.J.H. van TITS

Introduction

Recently, much interest has been focused on lipoprotein(a) (Lp(a)), since there is a lot of evidence that its circulating level represents an independent risk factor for ischemic heart disease even after correction for the contribution of the classical risk factors (1). Results that Lp(a) is a strong indicator for cerebrovascular disease have also been reported (2). Because of its independent athero-thrombotic risk, a rapid, accurate and simple method for its quantification is desired. Recently, the interlaboratory-CV for Lp(a) measurement was found to be large. It could be reduced to a few percent by the conversion of mg/l units to nmol/l units (3). Consequently, an ELISA in which the monoclonal antibody associates with an epitope on the unique fragment V is now in use as a reference method (3). The conversion to the nmol/l units has the drawback that most epidemiological studies in which mg/l units are used are no longer clinically interpretable. We here describe efforts to settle the accuracy of our Lp(a) method which may give insight into the relation of old and new standardization criteria.

Department of Medicine, Laboratory of General Internal Medicine, University Medical Center St Radboud, Nijmegen, The Netherlands

Materials and methods

Lp(a) (d: 1.045-1.063 g/ml) was isolated by density gradient ultracentrifugation and heparin-sepharose chromatography. By immunisation of a rabbit, specific antibodies to apo(a) were obtained not cross reacting with apolipoprotein B or plasminogen. With the IgG fraction a sandwich ELISA was developed

between 20 and 600 mg of Lp(a) per litre. We prepared a serum pool from 20 different sera with a gradual increase in concentration from 12 to 2300 U/l. The Lp(a) target value of the pool was approached by the Mercodia RIA method which gave a value of 390 U/l (= 273 mg/l, using the conversion 1U/l = 0.7 mg/l given by Mercodia). This working standard was used throughout the whole study as calibrator for the ELISA. Results were compared using the well known solid phase two-site immunoradiometric assay of Mercodia which uses two monoclonal antibodies directed against unique and separate antigenic determinants on the apolipoprotein(a) molecule. Results were also compared to a Delfia (4). In this method a polyclonal antibody is used. This assay was calibrated with the IFCC PRM-2 as standard (Prof SM Marcovina, Seattle). Mg/l units were calculated on the base of an average Mw for apo(a) of 317 kD. For Lp(a) samples, the concentration is inversely correlated with the size of apo(a) (5). Therefore, we assessed the isoprotein related bias of our ELISA by intentionally testing the accuracy of samples with a large range in Lp(a) concentrations.

Results

The results we obtained for 11 sera by comparison with the Delfia are shown in figure 1. When the results in nmol/l using the PRM-2 IFCC calibrator were converted to mg/l units on the base of a MW of 317 kD, an excellent correlation was found in comparison with the results of our ELISA in mg/l. Note, none of the different sera behaved as an outlier underlining that specificity of both antibodies was similar. Results with our ELISA and the RIA method for 267 sera also agreed well: the correlation coefficient of 0.94 reflects the imprecision of about 10% in both assays. The between assay imprecision was of the same order as the between day CV, independent of the concentration ruling out any isoprotein dependency.

Conclusion

A polyclonal rather than a monoclonal antibody likely is less sensitive to the large molecular heterogeneity of the Lp(a) molecule. In addition, the use of a pooled serum, in which all isoproteins are equally present, warrants a further reducing of any sensitivity for isoprotein variation. Our results indeed prove the validity of this reasoning. By comparing our assay with that of a laboratory involved in classical epidemiological studies, good agreement was observed for the whole Lp(a) concentration range, suggesting isoprotein independent accuracy because of the inverse relationship between Lp(a) concentration and isoprotein size. Results could be expressed in U/l; mg/l or nmol/l on the base of the conversion factor 1U/l = 1 mg/l; for the conversion of mg/l to nmol/l an average MW of 317,000 seems to be the best approximation.

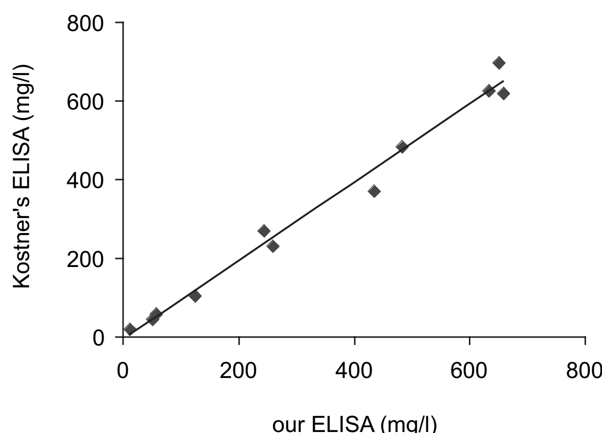

Figure 1. Comparison of results obtained with the developed ELISA (x) versus the Delfia (y). 11 sera were assayed with a gradual increase in the Lp(a) concentration; thus presumably containing isoproteins decreasing in the number of type IV type 2 kringles. The following linear regression equation was obtained: $y = 0.996\ x - 7.76$; the correlation coefficient: $r^2 = 0.982$; $n = 11$. Results of the Delfia originally in nmol/l were converted to mg/l using the average MW of 317 kD.

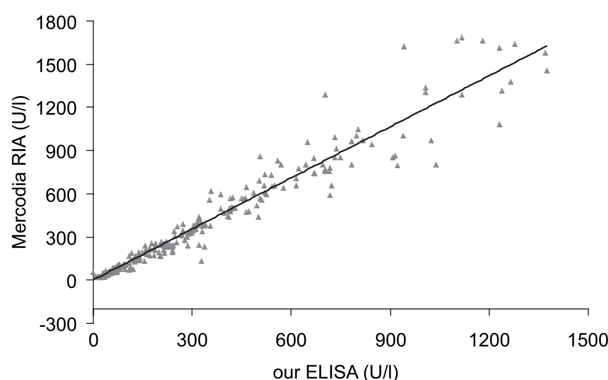

Figure 2. Results by the developed ELISA and by the RIA method of Mercodia in 267 freshly frozen serum samples with a large range in Lp(a) concentrations. Results were expressed in U/l, where 1 U = 0.7 mg. The regression equation was $y=1.18x - 6.5$; the correlation coefficient: 0.94.

References

1. Ariyo AA, Chan Tach MPH, Tracy R. Lipoprotein(a), vascular disease and mortality in the elderly. New Engl J Med 2003; 349: 8-15.
2. Zenker G, Koltringer P, Bone G. Niederkorn K, Pfeiffer K. Jürgens G. Lipoprotein(a) as a strong indicator for cerebrovascular disease. Stroke 1986; 17: 942-945.
3. Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, Couderc R, Dati F, Rifai N, Sakurabayashi I, Tate JR, Steinmetz A. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). Clin Chem 2000; 46: 1956-1967.
4. Kostner GM, Ibovnik A, Holzer H, Grillhofer H. Preparation of a stable fresh frozen primary lipoprotein(a), Lp(a) standard. J Lipid Res 1999; 40: 2255-2263.
5. Gavish D, Azrolan N, Breslow JL. Plasma Lp(a) concentration is inversely correlated with the ratio of kringle IV/ kringle V encoding domains in the apo(a) gene. J Clin Invest 1989; 84: 2021-2027.