Actual Ionized Calcium (at Actual pH) vs Adjusted Ionized Calcium (at pH 7.4) in Hemodialyzed Patients

To the Editor:

Ionized calcium is now the measurement of choice for evaluating calcium metabolic diseases (1, 2). Ionized calcium may be reported either as the actual ionized calcium, referred to the actual pH of the patients (arterial or capillary blood), or as adjusted ionized calcium, to a standard pH at 7.40. The latter has been shown to be as useful as actual ionized calcium in evaluating patients with chronic disorders of calcium metabolism (3). Although, theoretically, actual ionized calcium should be superior to measurement of adjusted ionized calcium in patients undergoing hemodialysis, because of the severe metabolic changes involved (3), studies showing this are lacking.

In a prospective study, we measured actual ionized calcium, adjusted ionized calcium, and parathyroid hormone (by Allegro Intact PTH; Nichols Diagnostic; San Juan Capistrano, CA) in 18 patients regularly undergoing hemodialysis. Blood samples were collected from the arteriovenous fistula in glass capillary tubes (Clinikubes™; Radiometer, Copenhagen, Denmark), before and after hemodialysis, for measurement of actual and adjusted ionized calcium at 37°C with a semiautomated ICA1™ analyzer (Radiometer) as previously described (4). The ICA1 calculated the concentration of adjusted ionized calcium from the measured ionized calcium and pH according to the slope for $\log \text{[Ca]}_{2+}/\Delta \text{pH}$, i.e., $-0.23$, which is the conversion factor used in all commercial analyzers and currently reflects the actual adjustment performed in the routine laboratory.

We observed a highly significant relationship between actual and adjusted ionized calcium ($r = 0.88$, $P < 0.0001$) but with a high residual variation across the regression line (Figure 1). The concentration of actual ionized calcium was increased in 27.8% and decreased in 16.6% of the samples, compared with 58.4% and 13.9%, respectively, for the concentrations of adjusted ionized calcium, corresponding to a diagnostic discrepancy between the two methods of 44.4%. In our study, the major factors responsible for the difference between actual and adjusted ionized calcium are probably the rapid changes in acid-base (from pH 7.37 to 7.46; mean values pre- and postdialysis), albumin (from 2.23 to 2.06 mmol/L), phosphate (from 2.06 to 1.04 mmol/L), and other anions such as lactate and free fatty acids (not measured). The adjusted ionized calcium, which is based on intraventricular changes (i.e., CO₂, loss), does not take the above-mentioned factors into consideration, which may therefore explain the high discrepancy between the two variables.

During dialysis, the actual ionized calcium increased in all patients (from mean 1.22 to 1.42 mmol/L) except one (pre- and postdialysis hyperparathyroid); postdialysis, 55% of the patients had values within the reference interval, compared with 6%, as judged by the value for adjusted ionized calcium. The patients showed an average decrease in parathyroid hormone of 53% (range 11%–79%); however, 52% of the patients remained hyperparathyroid postdialysis. This observation suggests that it might be clinically appropriate to increase the amount of actual ionized calcium in serum by using calcium supplement or calcitriol treatment, to achieve normal values for parathyroid hormone and thereby decrease the reabsorption of calcium from bones. However, this idea needs to be substantiated by a much larger clinical study.

We conclude that measurement of actual ionized calcium is preferable in patients undergoing hemodialysis, whereas adjusted ionized calcium is inappropriate because of the rapid changes in pH, albumin, phosphate, and other anions taking place in such patients.

References

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Atypical Electrophoretic and Isoelectrophoretic Patterns of Neutrophil Alkaline Phosphatase in a Case of Subacute Myelomonocytic Leukemia

To the Editor:

Reports of variations of alkaline phosphatase (AP; EC 3.1.3.1) isoenzymes in hematopoietic tumors and
leukemic cells are scarce (1–4). Electrophoretic changes of AP in leukemic leukocytes have been described only in patients in blast phase (3, 4).

Some new data were obtained from neutrophil alkaline phosphatase (NAP) in a patient with a chronic myelomonocytic leukemia corresponding to M₄ type of the French–American–British classification. This 75-year-old Caucasian woman, examined in our hospital unit, was found to be in a stable phase of the disease. Hematological results were as follows: erythrocytes, 4250 000/µL; hemoglobin, 159 g/L; mean cell volume, 87 fL; platelets, 458 000/µL; leukocytes, 19 000/µL, with 75% polymophonuclear cells, 15% monocytes, 2% immature granulocytic cells, and 8% lymphocytes. The bone marrow granulocytic series was increased, as were the monocytes (13%). Bone marrow biopsy confirmed cellular hyperplasia and showed slight densification of the reticular network. Serum lysozyme was then 60 mg/L. Functional study of polymophonuclear cells enabled us to demonstrate normal production of superoxide anion (3.88 nmol/min per 10⁸ cells; control 3.76), normal spontaneous migration on agarose (0.86 vs 0.94 in control), and a slightly reduced chemotactic response towards autologous activated serum (patient’s index 1.36; control 1.47). The myeloperoxidase score was within normal rate (300). NAP score was increased (208; controls 42.3 ± 13). NAP assay (5) showed a high amount of enzyme activity: 14.2 nmol/min per 10⁶ cells (normal controls: 6.5 ± 1.0 units). All these results were unchanged over the two-year period of study.

We separated neutrophils from venous blood, then extracted and solubilized NAP as previously reported (6). Contrary to other cases described (1–4), this patient, studied in a remission phase of subacute myelomonocytic leukemia, had an abnormally high NAP activity.

An agarose gel electrophoretic study performed according to Schreiber and Sadro (7) disclosed a faster migrating NAP isoenzyme. Miller et al. (3, 4) found a supplementary fast isozyme (LAP f) only in patients in blast phase. By means of various analytical methods they suggested it to be a different isoenzyme, with a distinct molecular mass, heat inactivation response, and isoelectric point. However, they presented neither isoelectric focusing pattern nor pl value (4).

We also used isoelectric focusing typing, both as previously described (6) and in the presence of separators to obtain a flattened pH profile (8).

Figure 1 illustrates patterns of NAP isoenzymes obtained for the two types of isoelectric-focusing agarose gels. In the broad pH gradient range 3.5–9.0 (Figure 1a), enzymatic activity was generally resolved into two isozyme groups: a major group from pl 6.1 to 7.0 and a minor group from pl 4.4 to 4.9 (6). But NAPs from the patient with a chronic myelomonocytic leukemia (lane 3) systematically disclose a pattern distinct from that of normal preparations run in parallel (lanes 1 and 2). Almost the whole enzymatic activity focuses nearer the cathode and spreads out between pl 6.0 and 7.2. In contrast, the anodic bands are very weak. In the narrow pH gradient range 3.5–7.0 (Figure 1b), several separators were added to carry ampholytes in isoelectric-focusing gel to improve the separation of clustered cathodic isozymes, thus allowing their resolution into subsets of AP bands. Moreover, an important fraction of enzymatic activity (65%) remained on the Whatman filter paper deposit, instead of 25% in the case of the first gel. The NAP diagram of the patient studied (lane 3) again differs from the others: the faint anodic bands are focused nearer the anode and a great proportion of enzymatic activity is shown in the more cathodic zone (flattened part of pH gradient).

Indeed, the isoelectricfocusing diagram of the NAP isoenzymes was markedly distinct from all other cases studied so far, by the very unequal distribution of NAP activity between the two main isozyme groups and also their shift in pH gradient. In particular, the weakness of anodic isozyme bands could be related to a greater thermolability of NAP isoenzymes. Thus 90% of enzymatic activity was lost at 56 °C after 10 min vs 75–80% lost in normal controls. NAP from our patient was more heat labile than that of the atypical form described by Damle et al. (2) in Hodgkin’s nodes, non-Hodgkin’s nodes, and chronic myeloid leukemic cells—despite the age of our patient. Indeed, Vergnes et al. (9) demonstrated that thermostability of NAP isoenzymes actually increased with aging: 35% of total better enzymatic activity was thermostable in a group of 75-year-old subjects in good health, vs 20–25% in younger controls.

The patient’s NAP was also sensitive to the tissue-nonspecific AP isozyme inhibitor L-homoarginine (residual activity, 23%; normal control liver/bone AP, 20.8% ± 1.5%) and was resistant to L-phenylalanine, the inhibitor of placental AP and Regan isozyme (residual activity, 84%; normal control liver/bone AP, 90% ± 2%).

These data suggest that the patient’s NAP is the tissue-nonspecific or liver/bone type, and rule out its relation with placental and Regan isozymes. However, in this case of subacute myelomonocytic leukemia, the isoenzyme expressed by granulocytic cell lines does not share the electrophoretic and isoelectricfocusing properties usually recognized in normal neutrophils (Figure 1). It therefore appears to be a variant isozyme. Our results indicate the expression of an atypical form in this patient. On the basis of these findings, we conclude that a combination of agarose gel electrophoresis and isoelectric focusing is of particular interest in clinical chemistry. A systematic implementation of these methods should provide better discrimination between AP variants in myeloproliferative and myelodysplastic disorders.

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Age-Related Correlations between Weight and Lipoprotein(a) Concentrations

To the Editor:

Corsetti et al. (1) recently reported that serum concentrations of lipoprotein(a) (Lp(a)) in obese subjects were virtually identical to those found in healthy subjects: the weight-reduction program had no effect on Lp(a) concentrations. Some recent experiences of our research group are consistent with the conclusions drawn by these authors and enlarge their message.

A screening program for the risk of developing atherosclerosis or diabetes was carried out in the village of Arolo in northeastern Italy. Almost one-fourth (1014) of the 4677 inhabitants participated, giving clinical, dietary, and pharmacological histories and undergoing a panel of biochemical tests.

Weights and heights were measured and the subjects were asked about their weight at 20 years. For the present study we selected without conscious bias 120 subjects (61 women and 59 men), ages 21-53 years (mean 32.5, SD 14.68, median 53 years). The mean weight was 69.4 kg (SD 13.33, median 69, range 44-112 kg). The body mass index (BMI), kg/m² was 26.0 (SD 3.78, median 25.7, range 15.7-35.3). The reported weight and BMI at 20 years were, respectively, 60.2 kg (SD 10.37, median 60, range 39-92 kg) and 22.5 (SD 2.74, median 22.5, range 16.6-32.2); both were significantly (P <.001) lower than the values determined at the time of the screening.

The mean Lp(a) concentration in 118 subjects (2 subjects showed undetectable Lp(a) concentrations and were not included), measured with a commercially available enzyme immunoassay (TintEli; Biopool, Sweden), was 0.252 (SD 0.274) g/L; the median was 0.132 g/L and the range 0.116-1.182 g/L. The distribution was highly skewed, as previously reported (2).

We compared the weight gain and loss of subjects of very different ages by dividing the weight change by the number of years of age exceeding 20 and multiplying by 100, as expressed in the following formula:

Weight change ratio =

\[
\frac{\text{BMI} - \text{BMI at 20 years}}{\text{present age} - 20} \times 100
\]

Using simple linear regression between Lp(a) concentrations and weight change ratios, we determined that the correlation was not statistically significant (r = -0.097, t = -1.36, P = 0.291).

Of the 116 subjects, 48 showed a weight gain >10 kg. As Figure 1 shows, the distribution of BMI as a function of Lp(a) concentration in the subjects who gained weight also was not statistically different from that in the subjects who did not gain substantial weight. These results are in agreement with Rosengren et al. (2), who reported no influence of BMI on Lp(a) concentrations.

References


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Caffeine Concentrations in Adult Patients Chronically Taking Theophylline

To the Editor:

Theophylline (1,3-dimethylxanthine) is widely used to treat acute and chronic asthma and chronic obstructive pulmonary disease (COPD) because of its ability to relax bronchial smooth muscle (1).

Caffeine (1,3,7-trimethylxanthine) is a xanthine that occurs naturally in coffee, tea, cocoa, chocolate, cola beverages, and mate. Because of its stimulant effect, caffeine is consumed in...