An International Quality-Assessment Program for Measurement of Aluminum in Human Plasma: A Progress Report

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In view of the increasing interest in measurement of aluminum in plasma of hemodialysis patients and the analytical difficulties, which may lead to false clinical interpretations, we organized an international interlaboratory quality-control program that has operated since 1983. The results obtained after four years (72 participants) demonstrate a lessened discrepancy of the results. This program allowed some laboratories to improve their results and even to solve some of their analytical problems. This surveillance will be continued and extended to include the analysis of dialysis fluids.

Aluminum (Al) presents a significant risk of toxicity among hemodialysis patients. This metal was shown to be one of the causes of chronic encephalitis as early as 1976 by Alfrey et al. (1), of senile dementia such as Alzheimer's disease (2), and of other tissue disorders (3).

Owing to the ubiquity of this element as compared with the small amounts in the organism, combined with a lack of analytical sensitivity, there is a great disparity of so-called normal values (4, 5). The improvement in the performance of the equipment used in electrothermal atomic absorption spectrometry, emission spectrometry, or inductively coupled plasma, together with a greater analytical strictness to avoid contamination, permitted calculation of a physiological concentration as <0.3 μmol/L, without completely resolving the great discrepancy of the results related to average or supranormal values. This inconsistency makes the dialysis treatment of patients more difficult and may even lead to diagnostic errors.

To minimize this discrepancy in results, it seemed desirable to test the specialized laboratories involved in supervision of hemodialysis patients. The European Economic Community, aware of these difficulties, adopted a resolution in June 1986 (6) requiring a quality-control scheme for all the laboratories in the member states. Preceding this decision, the Commission for Trace Elements of the Société Française de Biologie Clinique (SFBC) had in operation, since 1983 (7), an interlaboratory quality-control program, called "The Worldwide Interlaboratory Quality Control Aluminum" and including 72 participants from 17 different countries.

Henceforth, the latter will operate on an international level together with the two other major international quality-control programs—that of Dr. A. Taylor, of Surrey University (8) in Guildford, U.K., and that of Dr. J. Weber (Quebec University, Canada).

After the 12th control, we decided to evaluate the results to determine if the quality controls actually helped the laboratories improve the reliability, reproducibility, and accuracy of their results.

Materials and Methods

Plasma collection and sample preparation. Human plasma was chosen as the specimen in order to compare it with the plasma from hemodialyzed patients. The blood was collected in polypropylene bags (Prolabo, Rhone Poulenc, Paris, France) containing Al-free heparin and taken from volunteer donors who knew the purpose for which it was being used. Samples were screened for Australia antigen, HTLV III antibodies, and pathogens before any other treatment. The plasma was not eluted through Chelex ion-exchange resin so there would be no loss of any normal constituents. This ensured that control and test samples were analyzed under the same conditions. The specimens were stored at 4 °C and were never frozen.

For sample preparation and storage, we used polyethylene bottles and polystyrene tubes that had been previously decontaminated by washing and soaking overnight in Al-free HCl (E. Merck, Darmstadt, F.R.G.) and then rinsed five times with "ultrapure" water (>18 MΩcm) to avoid protein precipitation. The pipette tips were prepared in the same way. To check the effectiveness of this decontamination, we tested some of the tubes and bottles for remaining traces of Al, using the method described by Guillard et al. (9). After careful homogenization, the pool of plasma was divided into three 300-mL portions, in polyethylene bottles, and a constant volume of an aqueous solution of Al was added. The samples were mixed carefully for 1 h by constant rotating agitation. The method of standard additions and lyophilized human serum (Seronorm®, batch no. 105; Nycomed, Oslo, Norway) were used. Analytical recoveries ranged between 95% and 105%. Aliquots of 3 mL were dispensed into polystyrene tubes. The entire preparation time did not exceed 24 h.

Frequency of distribution. Since 1986, each participating laboratory has received three plasma samples (A, B, C), each with a different Al concentration, every two months on a predetermined schedule. For purposes of identification, each laboratory was assigned a code number. The analyses had to be completed by a certain time and the results were returned on a form, on which also was indicated information concerning the analytical method.

Treatment of raw data. For each sample, we first calculated the mean (X̄1) and standard deviation (SD1) for all values (n1, initial calculation). Then, after excluding results out of the 2 SD range, we determined with the remaining data (n2, final calculation) a new mean (X̄2), standard deviation (SD2), and coefficient of variation (CV2). The statistical analysis of these data, performed on a Macintosh Plus® (Apple Corp., Cupertino, CA 95014), resulted in histograms of average values. After each control, each participant

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received the results for the three samples in the form of histograms with the individual results as well as the number of participants retained (n), the mean value (X̄), and the coefficient of variation (CV). Furthermore, an annual report of the six controls, both in English and French, is sent to each participating laboratory.

Results

Table 1 summarizes the quality of the results for three different ranges of Al concentrations.

Table 2 compares results for the first and twelfth controls for the 16 laboratories participating since the first control, for two samples with similar concentrations (CL1 and CL12). For initial calculations, the mean values for X₁ were 1.44 and 1.20 μmol/L, and the CV₁ values were 47% and 17%, respectively, for CL1 and CL12. Final calculations (for the 13 remaining data) in the same way yield: 1.38 μmol/L and 1.19 μmol/L for X₂ and CV₂ 26.5% and 8.9% for CL1 and CL12, respectively.

Discussion

In Table 1, the percentage of "regular and good" laboratories is small (11%) for low concentrations (<1 μmol/L) as compared with the percentages obtained for all other concentrations—unsurprising in view of the difficulty in measuring physiological concentrations of Al in plasma. The "improving" group demonstrates the usefulness of the quality-assessment program: 39%, 32%, and 42% of the laboratories improved their results for the three ranges of concentration (<1, between 1 and 3, and ≥3 μmol/L, respectively).

Table 1. Progress in Quality of Results between the First and the Twelfth Control

<table>
<thead>
<tr>
<th>Concentration range of Al, μmol/L</th>
<th>Between 1 and 3</th>
<th>≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of labs. with results rated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular 29</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Regular and bad</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Regular and good 11</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Improving 39</td>
<td>32</td>
<td>42</td>
</tr>
</tbody>
</table>

Unfortunately, a very large proportion of the laboratories (36% to 50%) remained in the two groups characterized "irregular" and "regular and bad." This poor classification may be explained in two ways: either the laboratory has only recently become involved in the program or it may simply have had analytical problems.

An analysis of Table 2 substantiates this interpretation. In fact, for the 16 laboratories that have participated since the first control, we have compared the discrepancies of the CV, between two similar concentrations, the first (CL1) and the twelfth (CL12). We observe that for the final calculation, CV₂ evolved from 26.5% to 8.9%, a threefold improvement of the CV₂, thus substantiating the usefulness of the quality control.

Furthermore, the information we are seeking has a double objective. First, in collaboration with Surrey University, we try to select the best analytical conditions (10); and secondly, we seek to help and advise those laboratories who want it. This assistance played a positive role and allowed some laboratories classified "regular and bad" or "irregular" to improve their analytical performance within a year to become classified "good and regular."

For greater efficiency, we decided to increase the controls to seven per year. Since the sixth control, we have extended the quality control of Al to the analysis of dialysis liquid. These results will be reported after the sixteenth control.

References