<Research Article>

Development of a novel method to detect abnormal reaction patterns in plasma albumin measurements

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Summary The unexpected instrument failures and anomalous reactions during analyses can negatively impact the measurements and cause errors in the final reported results. In the present study, we examined methods to identify anomalous patterns in the absorbance values for various laboratory tests performed using biochemistry analyzers. First, we measured the variance as the difference in absorbance measurements between two adjacent points on the reaction monitor. Second, we measured the variance as the difference in absorbance measurements between two distant points. Third, we performed a principal component analysis using the machine learning technique to determine the difference in the absorbance measurements between two adjacent points. Based on the findings of these three techniques, we detected any anomalous patterns that can be expected in routine clinical use. Anomalous wave patterns in ALB measurements from 17,838 samples were identified in 13 cases (0.07%); notably, falsely high values of albumin (elevated by 1.4 g/L on average) were found in 10 of these cases. Our machine learning technique further identified anomalous wave patterns in 27 cases, with 18 cases being detected only by the machine learning technique. Our findings indicate that a system for real-time outlier detection may improve the overall reliability of laboratory tests in routine clinical practice as such a system would prompt re-run of the tests before the results are finalized and reported.

Key words: Biochemical analyzers, Time course, Machine learning, Anomalous patterns, Real-time

1. Introduction

The accuracy of automated biochemistry analyzers has improved in recent years, and the reagents used in these instruments have become more stable. These improvements have enabled the rapid delivery of laboratory test results with high accuracy. Many of the instruments also have the capability to detect errors resulting from unexpected events that occur during the analyses, including anomalous absorbance of fibrin in the samples, coloration of the samples that results from contamination or physical damage in the reaction cuvette, and anomalous absorbance due to insufficient reagents. Such safety features of the instruments certainly has an effect in preventing false reporting that results from analytical errors. However, it is

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difficult to validate the accuracy of the results each time by confirming that the volume of samples and reagents used were accurate and that there was no dripping or contamination of the reaction solution. It has also been reported that the presence of irregular proteins in the reagents can increase turbidity and interfere with the analyses [1-9].

There are limited number of studies that examined methods to prevent false reporting in cases of errors caused by unexpected instrument failures or anomalous reactions during the analyses; as such, it is highly likely that the influence of anomalous reactions on laboratory test results are disregarded in routine practice. In order to detect such anomalies, it would be most effective to analyze the time course of reactions using the time vs. absorbance curve for every test. However, it is practically impossible for an analyst to confirm the time course of the reaction for each test when there is a massive amount of data to process in routine clinical practice. Thus, errors that result from anomalous reactions may be preventable if the automated analyzers are equipped with a system that monitors the time course of reactions in real time and alerts when there are any outliers from the standard curve or absorbance range.

We previously demonstrated that anomalous reactions could be detected in routine clinical practice by performing statistical analyses of the absorbance measurements obtained from automated biochemistry analyzers and by using a system that indicates an error when there was a certain outlier within the data[8]. However, it was impossible to detect anomalies in absorbance, such as a gradual increase or decrease, using these anomalous dispersion-based anomaly-detecting systems.

Thus, we devised a turbidity anomaly-detecting method to detect anomalies in absorbance, such as gradual changes. Furthermore, we devised a different statistical approach as well as a machine learning technique (artificial intelligence (AI)-based method) to detect anomalous reactions.

ALB measurements were analyzed in this study because abnormal reaction patterns were relatively frequently observed in routine testing. In addition, falsely elevated ALB values may affect clinical assessment, particularly in patients with low ALB concentrations. In the present study, we analyzed abnormal reactions in ALB measurements.

2. Materials and Methods

Samples

A total of 17,838 lithium-heparin plasma samples collected from inpatients and outpatients were included in the study. Patients provided informed consent for biochemical testing of their samples, and the samples had already been tested for ALB in daily practice. The study was approved by the Research Ethics Boards of the New Tokyo Hospital (No. 0252) and the International University of Health and Welfare Graduate School (No.18-Io-186-3).

Instruments and reagents

The automated biochemical analyzers (BioMajesty™ Series JCA-BM6010, JEOL Ltd.) was used for the analyses. The BCP method (L-type Wako ALB-BCP, FUJIFILM Wako Pure Chemical Corporation) was used as the reagent for measurement of the plasma ALB concentration. The analytical parameters were established as designated by the manufacturer. Briefly, the sample volume was 2.6 μL, the first reagent volume was 140 μL, and the second reagent volume was 70 μL. The dominant wavelength on measurement was 596 nm, and the complementary wavelength was 658 nm. The ALB level was calculated from a difference in absorbance between 5 minutes after mixing the first reagent with the sample and 5 minutes after second-reagent addition.

Detection of anomalous variance (conventional method)

Based on the previous study[8], we calculated the difference (variance) in the absorbance measurements for the dominant and complementary wavelengths between adjacent photometric points for all ALB analysis. Measurements were considered anomalous if they exceeded 10 times the mean value of variance for routine tests in a 7-day period.

Detection of anomalous turbidity

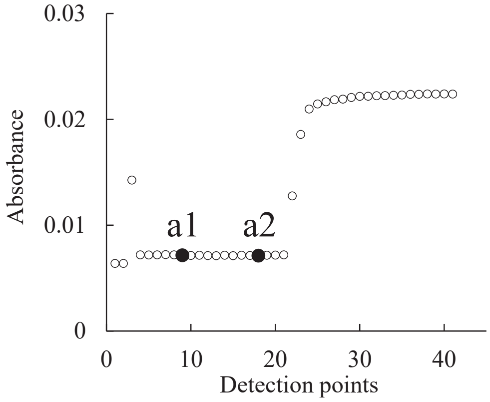

Fig. 1. The variance of difference in the absorbance measurements in the R1 interval (dominant wavelengths). The variance of differences were calculated from the difference in absorbance between the 9th (a1) and 18th (a2) points.

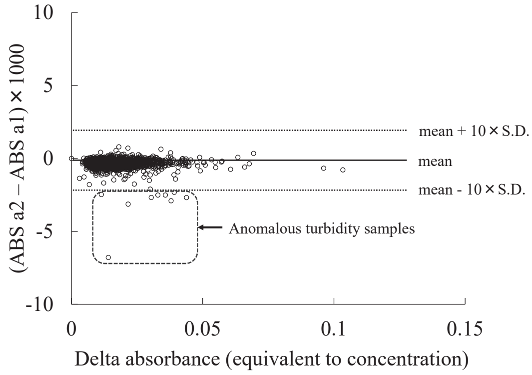

Fig. 2. The threshold calculation method for determining abnormal reactions in the R1 interval (dominant wavelength). The horizontal axis shows Delta absorbance (corresponds to the concentration of the component)

For each test, we measured the variance of difference in the absorbance measurements for the dominant and complementary wavelengths in the R1 interval (between point 9 and 18, 2.5 and 5 minutes after the addition of the first reagent to the sample; photometric measurements were obtained at approximately 14-second intervals [42 points over 10 minutes]) (Fig. 1).

Similarly, we measured the variance of difference in the absorbance measurements for the complementary wavelength in the R2 interval (between point 19 and 42, 0.1 and 5 minutes after the addition of the second reagent). Measurements were considered anomalous (i.e., turbid) if they exceeded 10 times the mean value of variance for routine tests in a 7-day period (Fig. 2).

AI-based (deep learning) detection of anomalous wave patterns

For the AI-based analysis, the 13 outlier cases detected by variance/turbidity analysis and 1,600 randomly selected routine ALB measurements were analyzed (total n = 1,613). For each test, we measured the absorbance of the dominant wavelength after the addition of the second reagent and determined the difference in the measurement between two adjacent points. The data were reduced to 3D using principal component analysis. The three axes (PC1, PC2, and PC3) represent the first, second, and third principal components, respectively. These components correspond to orthogonal directions that explain the largest variance in the absorbance-curve dataset and were used solely for dimensionality reduction and visualization of waveform characteristics. The calculation flow consisted of data standardization, calculation of the covariance matrix, calculation of the eigenvalue and eigenvector for the covariance matrix, selection of principal components, and dimensionality reduction. Outliers were detected using the Local Outlier Factor method[10], which is one of the machine learning methods based on Python language. Lastly, the data were visualized as a 3D image.

3. Results

Accuracy of the ALB measurements

Prior to the experiments, we examined the intra-assay precision and intermediate precision of the ALB measurements (n=20). The average measurements were 27.5 and 30.9 g/L, with a coefficient of variation (CV) of 0.28% and 0.56%, respectively.

Detection of anomalous variance and turbidity

Outliers were detected in 0.07% of cases (13/17,838). One of the cases was an outlier in the measurement of both variance and turbidity, while

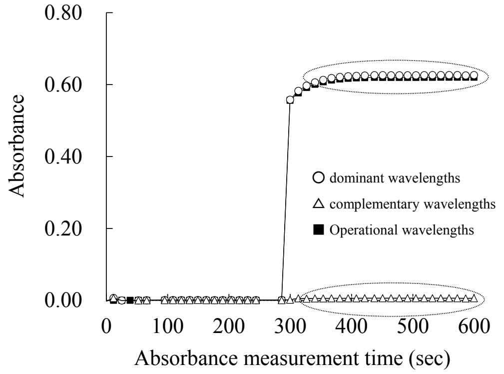

Fig. 3. Example of normal case. After addition of the second reagent, the absorbance (dotted circle area) approximately plateaued at both the dominant and complementary wavelengths. The absorbance at the operational wavelength used was the difference between the absorbance at the dominant wavelength and the absorbance at the complementary wavelength.

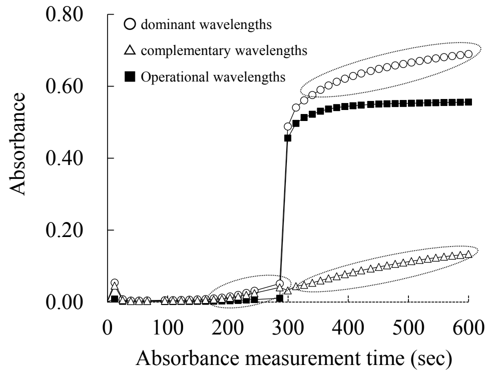

Fig. 4. Example of an abnormal case. After addition of the first and second reagent, the absorbance (dotted circle area) continued to increase at both the dominant and complementary wavelengths.

the remaining 12 cases were outliers in the measurement of turbidity. In all of the turbidity outlier cases, the reaction remained active compared to the standard time course which showed a plateau (Fig. 3, 4). The range of abnormal absorbance increases over 10 minutes was 0.06 to 0.16 at the dominant wavelength and 0.04 to 0.12 at the complementary wavelength. The reaction plateaued in these outliers when the samples were diluted two-fold. We diluted the specimens 2-fold and analyzed all abnormally responding cases. As a result, these abnormal curves were no longer observed. In addition, the ALB values obtained from undiluted specimens were significantly higher than those obtained after two-fold dilution (paired t-test, $p < 0.001$). The mean difference between the two measurements was 1.4 g/L (Table 1). Falsely elevated ALB values were identified in 10 of the 13 cases, whereas the ALB values

Table 1 The Albumin measurements in undiluted and 2-fold diluted specimens

Sample No.	Undiluted measurements (g/L)	2-fold dilution measurements (g/L)
1	26	24
2	33	31
3	30	29
4	22	19
5	25	24
6	23	21
7	23	20
8	30	29
9	31	30
10	36	35
11	19	19
12	27	27

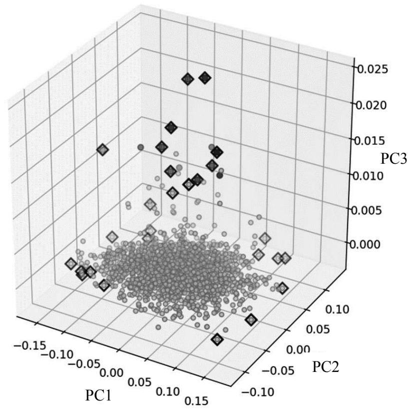

Fig. 5. The 3D image created by Python. Outliers were detected using the Local Outlier Factor method, which is one of the machine learning methods based on Python language. (n=1613) PC1-3: first, second and third principal components. Cases indicated by the black diamond shapes correspond to the outliers for variance and turbidity that were detected in the previous experiment. These outliers were detected in the third principal component. Cases indicated by the grey diamond shapes correspond to the cases with anomalous wave patterns. These cases were detected in both the first and second principal components.

obtained before and after two-fold dilution were comparable in the remaining three cases.

AI-based detection of anomalous wave patterns

The 13 outlier data and a total of 1,600 cases were randomly extracted to examine the data on the time course of the ALB reaction in routine practice by AI. Figure 5 shows the 3D image created by Python. Cases indicated by the black diamond shapes correspond to the outliers for variance and turbidity that were detected in the previous experiment. These outliers were detected in the third principal component. Cases indicated by the grey diamond shapes correspond to the cases with anomalous wave patterns. These cases were detected in both the first and second principal components. Anomaly detection based on machine learning identified 27 outliers (1.67%) in a total of 1,613 cases.

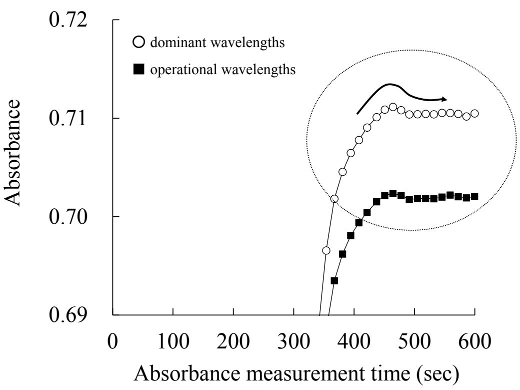

Fig. 6. Example of an abnormal case detected only by AI method (y-axis enlarged). After addition of the second reagent, a local distortion in the absorbance curve was observed.

Comparing AI anomaly detection with dispersion and turbidity detection, 9 cases were identified by both methods and 18 cases were identified by machine learning but not by dispersion or turbidity detection. These 18 cases showed either gradual or slight localized changes in the absorbance curve (Fig. 6). Four cases were identified by dispersion and turbidity detection but not by machine learning. The remaining 1,582 cases were not detected by either method.

4. Discussion

We previously reported that detecting reaction curve errors in automated analyzers is useful for detecting abnormal reactions in bilirubin measurements. While this technique has the potential to be universally applied to many measurement parameters, the threshold for detecting abnormalities needs to be set for each parameter. In this study, the results shown in Figure 2 suggest that abnormal reactions can be efficiently detected by defining abnormalities as values more than 10 times the average variance. As a result, anomalous reaction wave patterns were detected in 13 of 17,838 samples (0.07%), and all detected wave patterns met the criteria for anomalous variance or turbidity. In the present study, we further analyzed cases of abnormal ALB reactions, and detected in 1 of the 17,838 cases by the detection of anomalous dispersion on the absorption curves of automated biochemistry analyzers. In addition, 12 cases were detected on analysis using the anomalous turbidity-detecting function. We confirmed reaction curves in these 13 cases. As a result, there were abnormalities in reaction wave patterns in all cases. In addition, falsely elevated ALB values were identified in 10 cases, as the initial measurements were higher than the values obtained after two-fold dilution, which eliminated the abnormal reaction patterns (mean difference: 1.4 g/L). In the remaining three cases, the ALB values obtained before and after two-fold dilution were comparable despite the presence of abnormal reaction patterns. In these cases, increases in absorbance were observed at both the primary and secondary wavelengths to a similar extent, resulting in minimal changes in the absorbance difference used for ALB calculation.

Therefore, concerning the ALB concentration, the turbidity-detecting function facilitated the detection of anomalous wave patterns that could not be detected using a conventional anomalous dispersion-detecting method, suggesting the usefulness of this function.

We demonstrated that these anomalous wave patterns showed a gradual increase in the absorbance of both dominant and complementary wavelengths after the addition of the reagents. Some wave patterns showed an increase in absorbance after the addition of the first reagent. These gradual patterns that were identified by detecting anomalous turbidity could not be identified as anomalous variance using the method that we previously reported. These anomalous wave patterns were identified in a repeat experiment, and normalized when the samples were diluted two-fold. This finding suggests that these anomalous wave patterns were not generated due to an error caused by the instrument; instead, this indicates that the components in the samples affected the measurements. In the present study, the abnormal reaction-related falsely high values of ALB ranged from 0 to 3 g/L (mean: 1.4 g/L). In 5 of these, there were 2 g/L or greater increases. The coefficient of variation (CV) for the accuracy of our ALB analysis (intermediate precision) was 0.56% at a mean value of 30.9 g/L; therefore, the observed increase in ALB values was considered greater than the expected analytical variation. Additional studies are needed to determine factors, such as comorbidities and patient characteristics, that may be common among these outliers.

We demonstrated that falsely elevated ALB values caused by anomalous reactions ranged from 1 to 3 g/L (mean: 1.4 g/L). ALB is widely used as an indicator of nutritional status[11-17,] as well as in the assessment of nephrotic syndrome[18,19] and the Child-Pugh classification of liver cirrhosis[20]. Therefore, falsely elevated ALB values may influence the interpretation of laboratory findings, particularly when ALB concentrations are close to clinically important decision thresholds. Using our method, we were able to detect outliers in this low concentration range of ALB. Furthermore, we demonstrated that the reporting of falsely high values can be prevented by diluting the samples. Collectively, these findings indicate that our method may be highly applicable in routine use. In our analyses, 9 cases were identified as outliers by both machine learning (principal component analysis) and the detection of anomalous variance and turbidity.

In addition, 18 cases were identified as outliers by machine learning. These additional cases showed either gradual or slight localized changes in the absorbance curve that could not be detected by the measurement of turbidity. The application of principal component analysis to multi-dimensional data allows for reduction of such dimensionality while minimizing the loss of information from the data[21,22]. In our analysis, we were able to detect wave patterns that deviated from the standard pattern by visualizing the reduced data as a 3D image. The merit of performing the local outlier factor (LOF) method with respect to such three-dimensional data after principal component analysis is that a phenomenon of approximation of the distance between high-dimensional data points may be suppressed. In addition, the use of Python language facilitated displaying abnormal values with different markers. Additional analyses of such anomalous wave patterns may be effective in defining the criteria for outlier detection, which would further enable the detection of a subtle anomaly. Although machine learning detected additional subtle waveform abnormalities, some of these findings may represent over-detection rather than clinically significant analytical errors. Therefore, prospective studies are required to determine the optimal balance between sensitivity and specificity and to clarify the clinical significance of these additional outliers. For implementation in routine clinical practice, thresholds should be optimized to balance the detection of clinically relevant abnormal reactions and excessive repeat testing due to over-detection. Because analytical imprecision may vary across concentration ranges, concentration-dependent thresholds based on analytical performance may be useful. However, since this was a retrospective study, we were unable to perform additional measurements on the specific cases to validate our ALB measurements. Future studies should be performed with real-time machine learning in order to determine the validity of ALB measurements for outliers.

5. Conclusion

In the present study, we detected anomalous wave patterns based on variance, turbidity, and AI-based machine learning technique. These techniques were effective in identifying several cases of anomalous reactions that would be considered problematic in routine clinical practice. Thus, these techniques of detecting outliers are useful in providing accurate results for clinical laboratory tests. Future studies are needed to determine whether these methods are also effective in the measurements of factors other than ALB.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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