100 years of blood gas and acid base analysis in clinical medicine

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The term acidosis was first mentioned in the medical literature in 1898 in connection with the description of diabetic ketoacidosis. Alkalosis was first used in human medicine in 1922 by the English physiologist J. S. Haldane; it had been used in veterinary medicine somewhat earlier.

In 1870, the Norwegians C. M. Guldberg and P. Waage formulated the law of mass action, which applies to chemical equilibria, and which the American L. J. Henderson used for carbonic acid/bicarbonate balance in 1907. He expressed the mathematical balance as:

$cH^+ = K(cH_2CO_3 / cNaHCO_3).$ K is the equilibrium constant.

As the concentration of free H+ ions in blood is as low as 40×10^{-9} mol/L, the Dane S. P. Sørensen suggested applying logarithms to such low numbers and even using the negative value so that the number would become positive. This value was called pH. For "normal" blood, the pH value is 7.40 for the concentration 40×10^{-9} mol/L or 40 nanomol/L of free H+ ions.

"Why not also a logarithm the low equilibrium constant values?" the Dane K. A. Hasselbalch suggested in 1917. This was done and Henderson's equation then became:

$$pH = pK + \log (cHCO_3^- / S \times pCO_2)$$

pK is the negative logarithm for the equilibrium constant described above. S is a transition factor for H_2CO_3 from mol/L to partial pressure of pCO_2.

The Dane E. Warburg was the first person to use the term "the Henderson-Hasselbalch equation". He got his doctorate in 1922 with the work "Carbonic Acid Compounds and Hydrogen Ion Activities in Blood and Salt Solutions".

Up to about 1923, there had been no uniform definition of what an acid or a base was. In that year, the Dane J. N. Brønsted formulated his new acid-base theory, which has dominated the field of acid-base since that time. An acid is a substance that forms H+ ions in solutions or, in other words, an acid is a proton donor. A base is a proton acceptor. Acid = Base + H+.

The Van Slyke technique

In 1917, Donald D. Van Slyke (from the Rockefeller Institute for Medical Research) introduced the gasometric method for determining the total CO_2 and the total O_2 of blood. He first worked out the method using the volumetric technique, and, 7 years later, the method was improved, and he then used the manometric measuring principle.

With this method, oxygen was released with ferricyanide, and carbon dioxide was released on adding an acid. Volume and pressure of the released gas were then measured and, using the general gas equation, the volume percentage of oxygen and carbon dioxide were calculated. As the dominant part of total CO_2 is made up of bicarbonate, and these ions "buffer" H^+ ions, the total CO_2 was later called the alkali reserve. Volumetric and manometric blood gasometry were routine procedures in many hospitals from the 1930s until well into the 1960s. Van Slyke also developed gasometric techniques for measuring total nitrogen, carbamide, glucose and lactic acid, etc.

In 1932, Van Slyke, together with internist John P. Peters from Yale University, published a two-volume edition of Quantitative Clinical Chemistry. The first volume was about interpreting laboratory answers, and the second volume dealt with methodology. This two-volume work continues to be one of the best-known reference works within clinical chemistry.

Astrup's equilibration technique

The Van Slyke instrument was gradually replaced by the Astrup equilibration technique regarding acid-base analysis. The polio epidemic that ravaged Northern Europe at the start of the 1950s gave Danish doctors the impetus to work out the genial technique, which the equilibration method turned out to be.

In Copenhagen, in 1952, the number of polio patients who suffered paralysis of the respiratory muscles was striking and these patients required artificial ventilation. The total CO_2 was determined and was found to be raised in the majority of the patients. This was interpreted as indicating that the patients had alkalosis. When pH was also determined, after a while, it turned out that the patients had low pH (acidosis).

The explanation was that respiratory acidosis with accumulation of CO_2 caused a low pH. During this period, it is true to say that acid-base analysis went from being a physiological laboratory practice to a clinical necessity. Measuring total CO_2 with the Van Slyke apparatus and pH with another instrument, however, gradually came to be considered cumbersome.

Professor Poul Astrup said that he volunteered to ventilate polio patients at the Blegdams Hospital in Copenhagen. The respirators were operated by hand, and while he sat and pumped he wondered: "Am I doing this too fast, too slow, or just right? Where can I get an easy answer to my question? I must know the patient's situation now, not in a few hours' time."

Astrup studied Van Slyke's work and used the knowledge that the titration line for CO_2 to pH was approximately a straight line in the physiological measurement area (pCO_2 from 1.5-15 kPa), presuming that pCO_2 was marked logarithmically.

Capillary blood is taken in three capillary tubes (approximately 50 µL in each tube). Two of these samples are transferred to the equilibration chambers and equilibrated with special gas mixtures with known high and relatively low pCO_2 for 3-4 minutes. For the third sample, there is no preprocessing of the equilibration stage. The pH should be measured for all three samples. The titration line can be drawn based on the pH measurements in the two equilibrated samples. After that, the pCO_2 is read from the titration line with the measured pH from the blood sample, which was not equilibrated.

On a nomogram, which was later worked out by Professor Ole Siggaard-Andersen, it was possible to read base excess (BE), buffer base (BB) and standard bicarbonate parameters, which express the metabolic (non-respiratory) part of acid-base disturbance.

Interpretation of the Henderson-Hasselbalch equation

In 1964, Lorentz Eldjarn from Norway created a box with a three-dimensional coordinate system. The pH was marked on the X axis. The $p\mathrm{CO_2}$ was logged on the Y axis and the relevant bicarbonate level was marked on the Z axis. The graphic presentation was a double curved plane where the titration line for blood and spinal fluid was plotted.

It was possible to project this complicated plane down or into one of the three main symmetrical planes. Projection down or into the plane with the X and Y coordinates was Siggaard-Andersen's curve nomogram where isobicarbonate lines were straight lines, projection into the plane with X and Z as coordinates became Davenport's nomogram with plotted isobars for $p\mathrm{CO_2}$.

The last projection with Y and Z as coordinates was called the Cohen and Kassirer nomogram with plotted iso-pH lines. A fourth nomogram with log $p\mathrm{CO_2}$ on the X axis and BE on the Y axis was called the Grogono nomogram and was also intended to simplify the interpretation of acid-base disturbances. In recent years I have thought a lot about the extent to which these nomograms have contributed to the understanding of acid-base disturbances, but there is no doubt that they did help quite a bit.

Direct measurement of electrodes

Throughout the 1950s and the 1960s there was a lot of international focus on creating electrodes that could directly measure $p\mathrm{O_2}$ and $p\mathrm{CO_2}$ in blood samples. It was successful, eventually, and the most practically usable electrodes were subsequently called Clarke for the $p\mathrm{O_2}$ electrode and Stow/Severinghaus for the $p\mathrm{CO_2}$ electrode.

The three electrodes, pH, $p\mathrm{CO_2}$ and $p\mathrm{O_2}$, were then put together in one instrument, and analyzers for direct measurement of blood gases were available. Radiometer called its first analyzer of this type the

Acid Base Laboratory 1 (ABL 1). This was the first fully microprocessor-controlled, fully-automated blood gas analyzer to become available commercially.

The big transatlantic acid-base debate

At the start of the 1960s, it could be said that Astrup and Siggaard-Andersen's major input regarding acid-base put Copenhagen on the world map, and after a while the term "The Copenhagen School" began to be used. When the acid-base status of a patient was required, an "Astrup" was simply requested. I have heard such an acid-base status being requested as recently as 2011 at a hospital in Norway!

With the introduction of the metabolic parameters such as base excess (BE) and standard bicarbonate from "The Copenhagen School", articles soon started to appear in leading journals such as The Lancet and the New England Medical Journal, etc., criticizing these new parameters! Two internists in Boston, William Schwartz and Arnold Relman, were particularly critical of the two parameters mentioned and claimed that BE, in particular, was not independent of $p\mathrm{CO_2}$, especially in the case of hypercapnia. They pointed out that actual bicarbonate could well be used as the metabolic parameter. We call actual bicarbonate a mixed indicator that is present in both metabolic and respiratory acid-base disturbances. The presence of actual bicarbonate is most pronounced in metabolic acid-base disturbances.

A few years later, BE was modified and called "base excess extracellular fluid" (BEecF). The basis for this was that in vivo $\mathrm{O_2}$ and $\mathrm{CO_2}$ do not just equilibrate with blood but with all the extracellular fluid space. The buffer capacity is much higher in blood with hemoglobin present compared to the interstitial space, which does not contain hemoglobin and subsequently has a much lower buffer capacity.

It was then thought that the blood was diluted with extracellular fluid (fluid in the interstitial space). The concentration of hemoglobin in this diluted fluid would then be approximately one third of that of blood. Siggaard-Andersen had now worked out an

"alignment" nomogram, where BE could be easily read. This meant, for example, that with a hemoglobin concentration of 15 g/dL or 9 mmol/L, you would use a hemoglobin concentration of 5 g/dL or 3 mmol/L when making your calculation and thus obtain the value of the BEecF.

Another element that led the Boston School, in particular, to not recognize BE, was that BE was a mystical, artificial parameter and was calculated from complicated algorithms. The various manufacturers of blood gas analyzers worked out their own algorithms for calculating BE. These algorithms contained pH, pCO_2 and hemoglobin. Some also included pO_2.

I was able to demonstrate, in 1980, that the calculation of BE varied quite a bit when analyzers from different manufacturers were compared, and this could have clinical consequences in some instances, particularly in metabolic alkalosis. Throughout the 1980s and the 1990s the calculation was standardized so that today Van Slyke's algorithm or modifications of it are generally used. pO_2 is not included in this calculation.

The Copenhagen School have subsequently admitted that it would have been better to call the BE parameter "titratable acid" but with the sign reversed. This was certainly a familiar term from the titration of urine for measuring the excretion of acids or bases.

Stewart's approach to the interpretation of acid-base disturbances

In 1981, the Canadian Peter Stewart introduced a new calculation method that he felt would improve our understanding of acid-base disturbances, particularly metabolic disturbances. The traditional definition of acids and bases was abandoned, and two new parameters were introduced: SID = strong ion difference, which in simplified form is $Na^+ + K^+ - Cl^-$, and Atot, which is the total amount of weak acids present.

Using these two parameters and pCO_2, he formulated six equations and created a "summation equation" that gave detailed information about the acid-base status.

Furthermore, it may be mentioned that the SID was identical to Buffer Base that was introduced by Singer and Hastings in 1948.

Another feature, which should be mentioned with regard to Stewart's approach, was that there was greater focus on the role of albumin and chloride in the understanding of acid-base disturbances. I include an example, which has led to discussion in international media: According to Stewart's theory an acute isolated hypoalbuminemia would be regarded as a mild metabolic alkalosis.

The negative albumin charges will, due to a reduction of the albumin concentration, mean that these will have to be replaced to maintain electrical neutrality. This is primarily achieved by increasing the bicarbonate concentration, which is then interpreted as metabolic alkalosis. This is not an acid-base disturbance and should not be treated with acid therapies!

Stewart's method became popular among researchers, but was not very user-friendly in the busy everyday clinical setting due to complicated equations and the need for programmable calculators.

New electrodes for measuring electrolytes introduced in blood gas analyzers

From the end of the 1960s, ion-selective electrodes were developed for the macro electrolytes in blood plasma. It was particularly electrodes for sodium, potassium, chloride and calcium that were used and that were found to be reliable. These electrodes were built into the blood gas analyzer.

Simon's discovery in 1969 that valinomycin was a particularly suitable carrier substance in a potassium electrode was of major importance. With regard to calcium, two answers had to be reported as ionized calcium varied depending on the pH.

When the pH was between 7.60 and 7.20, the "adjusted" calcium value was calculated to pH = 7.40. In this way, it was possible to distinguish between, for

example, hypercalcemia, which was just secondary to acidosis, and actual hypercalcemia! After a while, several types of directly measuring electrodes were built into the blood gas analyzers, such as glucose and lactate electrodes.

Spectrophotometry and oximetry

Spectrophotometric determination of oxygen saturation was introduced at the start of the 1930s. During the Second World War the need to measure oxygen saturation increased due to the military needs of flying at high altitudes without stabilization of the hypobaric conditions.

During the 1970s and 1980s, there was a vast development in multiwavelength oximetry. Absorption spectra for hemoglobin derivatives, particularly within the wavelength area of 500-700 nm, were recorded. Measurements at over 100 different wavelengths were recorded to calculate the four common hemoglobin derivatives: oxyhemoglobin, deoxyhemoglobin, CO-hemoglobin and methemoglobin. It was also possible to measure sulfhemoglobin if this was present in the case of sulfa therapy.

Simultaneous measurement of oxygen saturation and partial pressure of oxygen soon became a clinical necessity for plotting the position of the oxyhemoglobin dissociation curve (ODC) and also the pO_2 50 %, which could tell about the hemoglobin's affinity for oxygen.

With the introduction of oximetry to the blood gas analyzers, the blood sample had to be split; one part went to the electrometry measurements and one part went to the oximetry measurements so that the modern blood gas analyzer now gives at least 11 directly measured parameters, four from the oximeter and seven from the electrometer.

In addition, glucose and lactate measurements became possible, so that on the printout there are 13 directly measured values as well as a number of calculated parameters.

Quality control

Many different test solutions have been prepared to control the quality of the measurements in the blood gas analyzers. What is common to them all is that the gases are very difficult to control, particularly low pO_2 (hypoxemia). The reason is that oxygen is not particularly water-soluble, and when the test ampoule is opened, oxygen in the air quickly diffuses into the solution. When using hemoglobin-containing solutions, it is difficult to produce these with deoxyhemoglobin, because this is immediately converted into oxyhemoglobin when it comes into contact with air.

In major international quality control tests, where many types of instruments are tested, the total coefficient of variation can be up to 15 % for low pO_2 values of 7-8 kPa. Ranges for these results can be 6.0-9.5 kPa! The large variation is caused by both preanalytical and analytical factors! Tonometry has also proved to be a good method for carrying out quality tests on pCO_2 and pO_2.

Conclusion

When you read the two-volume work by Peters and Van Slyke from 1934, it is surprising how much biochemical and physiological knowledge they had then! There has, however, particularly following the Second World War, been an incredible technological development resulting in today's blood gas analyzers playing a very important role, particularly within intensive care medicine.

Otherwise it may be pedagogically correct in the future to rename BE and call it hydrogen ion excess with the opposite sign!

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