Human Immunodeficiency Virus Infection and Anion Gap*†

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ABSTRACT

Although a normal or increased anion gap (AG) is commonly used to help assess acid-base balance, decreased AG has aided in the diagnosis of halogen ingestion and myeloma. Substantially increased levels of IgG cause a decrease in the AG. Patients with polyclonal increases in immunoglobulins, especially hepatic cirrhosis, also exhibit decreased anion gaps. Patients with human immunodeficiency virus (HIV) infection commonly show polyclonal increases in immunoglobulins. A case is reported of a patient with HIV infection who exhibited a decreased AG associated with increased polyclonal IgG (63 g per L). Unlike the electrophoretic profile of patients with hepatic cirrhosis, which commonly shows a β-γ-globulin bridge, reflecting a decreased immunoglobulin degradation, the profile of the patient with HIV infection was consistent with an increased immunoglobulin synthesis.

Examination of sera from 18 additional HIV positive patients indicated that, in general, the AG of HIV infected patients with normal renal function is significantly higher than in normal persons. The significance of this finding is as yet unclear. Nevertheless, decreased AG was associated with increased IgG. This may complicate the use of the AG in evaluating HIV infected patients because of frequent elevations in IgG. These relationships are now in the process of further investigation. Nevertheless, it is suggested that, with appropriate history and physical, identification of a decreased anion gap in conjunction with a polyclonal increase in γ-globulin may be reason to consider a work up for infection by HIV.

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Introduction

Investigations have shown that substantially increased levels of IgG cause a decrease in the anion gap (AG).\textsuperscript{2,5,16} Although this effect has most often been reported for patients with myeloma, patients with polyclonal increases in immunoglobulins, especially hepatic cirrhosis, exhibit decreased anion gaps.\textsuperscript{5,7} Patients with HIV infection consistently show increased levels of IgG and IgA.\textsuperscript{8,17} Here a case is reported of a patient with HIV infection who exhibited a decreased AG associated with increased IgG. To our knowledge, this effect has not previously been reported, and it may occasionally have diagnostic usefulness in identifying persons who are infected with HIV. Furthermore, since elevated AG may be associated with acid production as a result of infection, AG is used as a marker for evaluating the progression or establishment of opportunistic infections. Our data suggest that such evaluation may be obscured by the elevated levels of IgG, and that this factor should be considered when evaluating these patients.

Materials and Methods

Electrolytes and other chemistries were assayed with the Ektachem Analyzer.\textsuperscript{*} Immunoglobulins were measured by rate immunonephelometry using the Array.\textsuperscript{†} Protein electrophoresis was performed with universal II Agarose Film/12, with the Ciba Corning Protein system.\textsuperscript{‡} All other testing was performed in house by routine methodologies, except for T-cells, and AIDS tests which were performed at another Veterans Administration Hospital.

Additional sera from patients positive for HIV infection were obtained from the hospital clinics. These were assayed for BUN, creatinine, albumin, IgG, IgA, and AG. On the basis of these laboratory values, none of the patients appeared to have severe kidney disease, with the BUN, creatinine, and albumin values ranging between 2.5 to 8.9 mmol per L, 62 to 159 μmol per L, and 32 to 45 g per L, respectively (normal reference limits indicated below). Sera from apparently normal employees were obtained and assayed for AG.

Case Presentation and Results

The patient was a 44-year-old, black male who was admitted to the hospital because of a right swollen, tender, submandibular lymph node. Clinical examination indicated generalized lymphadenopathy and fever. Relevant routine chemistry, hematology, urinalysis, and coagulation laboratory tests, upon admission, with normal reference ranges in parentheses, were: albumin 35 g/L (35 to 48), AG ratio 0.5 (1 to 1.7), aspartate transaminase 153 U/L (0 to 50), alanine transaminase 84 U/L (0 to 45), creatinine 159.1 μmol per L (44 to 123). Urea nitrogen (3.2 to 7.5 mmol per L), alkaline phosphatase, and ketones were within normal limits. Complete blood count was unremarkable, except for a low hemoglobin of 100 g per L (130 to 180). Urinalysis showed trace positive for proteins, negative for bilirubin, and negative for ketones, but with many red blood cells. Coagulation studies indicated a normal prothrombin time.

The patient's sodium was 136 mmol per L (135 to 145), CO\textsubscript{2} was 31 mmol per L (24 to 31), chloride was 106 mmol per L (101 to 110), potassium was 4 mmol per L (3.6 to 5), and the AG without potassium was -1 mmol per L (5 to 14).\textsuperscript{3} Immunochromatographies showed an IgG of 63 g per L (7.2 to 16.8), IgM 3.8 g per L (0.63 to 2.77), IgA 4.7 g per L (0.69 to 3.8), and a normal k/A ratio. Protein electrophoresis showed a borderline decreased albumin fraction, and an increased γ-globulin fraction, with minor γ-bridging (figure 1, bottom). This is compared with the electrophoretic profile of a patient with a classical cirrhotic pattern showing γ-bridging (figure 1, top).

The patient was found to be HIV positive by enzyme linked immunosorbent assay (ELISA) and Western Blot. The CD 4 T-helper cells were 308 cells per mm (395 to 1601), CD 8 T-suppressor cells were 858 (161 to 807), and the CD 4/CD 8 ratio was 0.36 (0.6 to 3.4). He was classified, according to the Center for Disease Control schema for HIV infection (table I), as Group III (Persistent Generalized Lymphadenopathy).\textsuperscript{1}

Eighteen additional HIV infected patients and 18 normal sera were tested...
HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND ANION GAP

FIGURE 1. Electrophoretic densitometer profiles from patients with chronic liver disease (top) and human immunodeficiency virus (HIV) infected patient (bottom). The cirrhotic pattern was from a patient with chronic active hepatitis as a result of alcohol abuse and chronic hepatitis B infection, but negative for HIV antibody. This patient also showed a low anion gap (AG) (AG = 3 mmol per L). His immunoglobulin concentrations were: IgG = 61 g per L, IgM = 3.4 g per L, IgA = 8.5 g per L, and his γ/α ratio was normal. It demonstrates gamma bridging. From the area under the curve for each fraction: Top, albumin = 20 g per L (35 to 50), α1 = 2.0 g per L (2 to 4), α2 = 5.3 g per L (6 to 10), β = 6.0 g per L (8 to 12), and γ = 53 g per L (7 to 16). Bottom, albumin = 35 g per L, α1 = 1.0 g per L, α2 = 5.0 g per L, β = 7.0 g per L, and γ = 45 g per L (7 to 16). Parenthesis indicates normal reference ranges.

Anion Gaps from Patients Infected with Human Immunodeficiency Virus Compared with Normal Persons and Correlated with Immunoglobulins, and Albumin from Infected Patients

<table>
<thead>
<tr>
<th>Anion Gap (mmol/L)</th>
<th>Normal</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Median</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>8.8 (2.4)</td>
<td>8.0</td>
<td>12.8 (4.6)</td>
</tr>
</tbody>
</table>

Correlation Coefficients Between Anion Gap and Proteins for HIV Patients

<table>
<thead>
<tr>
<th>Rho</th>
<th>p</th>
<th>Range of Values (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>-0.52</td>
<td>0.035</td>
</tr>
<tr>
<td>IgA</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.60</td>
<td>0.014</td>
</tr>
</tbody>
</table>

*Mann Whitney test.
SD = standard deviation.
Rho = Spearman's nonparametric correlation coefficient.
p = significance level.
Figure 2. Distributions of anion gap (AG) for 18 human immunodeficiency virus (HIV) infected patients and 18 normal persons. (-----) Indicates the means of 12.8 (SD = 4.6) and 8.8 mmol per L (SD = 2.4), respectively. (......) Indicates two standard deviations (SD) above and below the means. See table II for significance levels.

= 0.60). The positive relationship between IgA which tends to be negatively charged also supports these conclusions.

Discussion

Anion gap is most commonly used to assess the acid base balance of the serum. The finding of an elevated anion gap is virtually synonymous with the presence of a metabolic acidosis. In fact, the AG has been referred to as "undetermined acids". According to the law of electroneutrality, the sum of all of the anions in serum must be equal to the cations. The sum of the anions includes bicarbonate, chloride, phosphate, sulfate, protein, and organic anions, while the cations include sodium, potassium, calcium, and magnesium. The phosphate and protein have multiple charges. It is impractical to employ a formula which requires measurement of all constituents to access electrical neutrality. Sodium, potassium, bicarbonate, and chloride are the major ions in serum, accounting for 95 percent of the serum cations and the 85 percent of the anions and are easy to measure. Therefore, for practical purposes, these are used for assessing electroneutrality. Calcium and magnesium are considered unmeasured cations and phosphate, organic ions, and protein unmeasured anions. The unmeasured anions are present in somewhat greater concentration than the unmeasured cations, resulting in an anion gap.

The assessment of anion gap is useful clinically in differentiating between the two major types of acidosis: (1) acidosis with an increased anion gap, usually caused by organic acids, and (2) acidosis with a normal anion gap in which a concomitant increase in chloride occurs. Two formulas have been used:

\[ \text{Na}^+ + \text{K}^+ - \text{HCO}_3^- - \text{Cl}^- = \text{AG} \]

The appeal of the anion gap concept stems in part from the simplicity with which a complex, multivariable relationship can be simplified yet still reflect the electrical neutrality state. Because potassium is in relatively low concentration relative to the others, and because substantial changes in it are not compatible with life, it is usually not included in the calculation.

Although seen less frequently, decreases in the anion gap also have use for directing attention towards certain disorders. Theoretically, increased levels of calcium, magnesium, or potassium could cause a decreased anion gap, but ordinarily increases in these in the magnitude sufficient to decrease appreciably the gap is not compatible with life.
commonly a decreased anion gap occurs owing to halogen ingestion which causes an artifactualy decreased AG or owing to increased levels of IgG which cause a real decrease.\textsuperscript{6} Bromide has been the halogen most often responsible for causing a decreased anion gap. Because of its greater electronegativity, bromide reacts more strongly than chloride with many colorimetric reagents used to measure chloride,\textsuperscript{9} causing artificially elevated chloride levels. It also causes chloride to appear falsely elevated when ion-selective membrane techniques are used.\textsuperscript{15} A decreased anion gap has aided in the diagnosis of bromide intoxication.

A decreased AG is correlated with increasing IgG concentrations.\textsuperscript{2,7} This relationship has been shown for polyclonal and monoclonal gammopathies. Concentrations of monoclonal IgG greater than 50 g per L are associated with an AG below the normal reference range.\textsuperscript{5} This relationship has been useful in identifying patients with hitherto unknown plasma cell dyscrasia.\textsuperscript{2,5,16} This property is due to the cationic nature of the IgG.\textsuperscript{10} Characteristically, the isoelectric point of IgG paraproteins range between 7.5 to 9, while that of IgA is less than 7.5,\textsuperscript{2} accordingly, a decreased AG has not reported for IgA myeloma. Although albumin is the most abundant anionic serum protein, and has been estimated to be responsible for 75 percent of the AG,\textsuperscript{3} in practice, with concomitant hypergammaglobulinemia (IgG > 50 g per L), moderate hypoalbuminemia is weakly related to a decreased AG.\textsuperscript{5,7}

Polyclonal gammopathies occur as a result of an increased immunoglobulin synthesis or a decreased immunoglobulin degradation. The electrophoretic profile most commonly associated with an increased immunoglobulin production is the chronic inflammatory pattern in which elevated levels of immunoglobulins are observed in the gamma region, and albumin is moderately decreased. This pattern is often seen in chronic infections, and autoimmune disease.

The profile commonly associated with decreased immunoglobulin break down is the cirrhotic pattern in which levels of albumin and other proteins synthesized in the liver (most of the \(\alpha_1\), \(\alpha_2\), and \(\beta\) globulins) are severely decreased with a \(\gamma\)-bridge (figure 1, top). In chronic liver disorders, the increase in serum immunoglobulins is thought to be caused by reduced catabolism of immunoglobulins by the liver in the face of a normal or increased synthesis. The daily rate of metabolism of IgA is four times that of IgG.\textsuperscript{13} As a result, IgA is proportionally increased as compared to IgG. Because IgA is characteristically less cationic than IgG, upon electrophoresis, IgA largely migrates between the \(\beta\) and \(\gamma\) region, and is thought to give rise to the \(\gamma\)-bridge which is characteristic of cirrhosis.

Polyclonal elevations in IgG and IgA were noted as biochemical abnormalities characteristic of AIDS from earliest studies.\textsuperscript{8,17} Patients were found to have elevated numbers of B-cells spontaneously secreting immunoglobulins.\textsuperscript{8} This may seem surprising in a disease that causes the destruction of helper T-cells which normally facilitate antibody production. Although the exact reason for this apparent contradiction remains unknown, it is clear that it is a result of failure in immunoregulation.\textsuperscript{8,13} Furthermore, in agreement with what might be expected from an immunodeficiency disorder, the increase in immunoglobulins is largely a result of increased secretion by previously immunized B-cells (possibly memory cells), whereas antibody responses to new immunogens appear to be severely impaired.\textsuperscript{11,13} The electrophoretic profile (figure 1, bottom) is concordant with the mechanisms responsible for the protein alterations. The profile is consistent with a chronic inflammatory response, and an increased synthesis of immunoglobulins,
little γ-bridging is seen. This profile can be compared with a cirrhotic profile (decreased immunoglobulin degradation) showing gamma bridging (figure 1, top).

Decreased anion gap may be a useful clue for diagnosis of HIV infection in patients with a hitherto unknown diagnosis. Very elevated levels of immunoglobulins may precede advanced disease. Although our patient was suspected of HIV infection on the basis of history and physical, the decreased anion gap was identified as a part of the routine work up prior to definitive diagnosis on the basis of HIV testing. Classification in group III indicates that the patient had not yet progressed to AIDS. The inverse relationship between AG and IgG observed here for 18 HIV patients (table II) supports the contention that the very elevated level of IgG in our patient (63 g per L) was largely responsible for the low AG, although the borderline low albumin (35 g per L) may have also contributed. It is our conclusion that, along with myeloma, HIV infection be added to the list of diagnosis to be considered when a decreased anion gap is observed in relevant patients.

Also, it is our belief the finding that patients with HIV infection showed a much wider range of AG values with a significantly higher average value is of interest and possibly importance in evaluating these patients (figure 2, table II). Elevated values for AG may suggest to clinicians increasing acid levels owing to opportunistic infection. It is clear from our data that decreased albumin and elevated IgG concentrations, which are commonly seen in these patients owing to the HIV infection itself, may conceal low level increases in AG. Thus, the AG must be evaluated with these relationships in mind. Also, of interest is the finding that the mean AG value in these patients with relatively normal renal function and generally normal albumins was significantly increased above the mean normal AG value (figure 2, table II).

The present study used too few patients to determine if this difference was due to opportunistic infection, drug therapy (many of these patients were taking 3'-azido-3'-thymidine), or the HIV infection itself. If the latter reason was responsible, AG may be useful for following the extent of HIV infection. It appears that during the early period of HIV infection, prior to the reappearance of HIV antigen, there is substantial HIV replication in the lymphoid organs.12 Few markers are available for following the infectious load. Presently, the current authors are initiating a larger study to explore which of these alternatives is responsible for the increased AG in these patients.

Acknowledgment
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References
8. Lane, H. C., Masur, H., Edgar, L. C., et al: Abnormalities of B-cell activation and immuno-


