Università degli Studi di Bari
Cattedra di Reumatologia

PROTEIN MEASUREMENT OF INFLAMMATION

(A statistical evaluation)

GIOVANNI LAPADULA

The existence of a quantitative variation in plasma proteins under different conditions, particularly during acute and chronic inflammatory diseases, is well known[1,4-7,11-13]. In fact, the quantitative determination of some serum proteins has today become a routine procedure for evaluating the intensity of the inflammatory process or for monitoring its course[2,3,10]. It is also possible that variations showing significant correlations during the inflammatory process completely lose their significance under normal conditions or during non-inflammatory diseases. Therefore, the evaluation of the changes in plasma protein pattern due to the inflammatory response should take into account not only the quantitative variations in the individual proteins but also the total behaviour of these inflammation indices. To obtain more accurate and useful data, a multivariate statistical analysis could be employed[8] as an appropriate tool for studying simultaneously the modifications in all the variables and their correlations.

The discriminant analysis that has often been suggested for diagnostic purposes is one of these multivariate techniques.

When different evaluation parameters of two groups of subjects are given (e.g. a group of normal subjects and another with some alterations, or two groups with different diseases), it is possible to use discriminant analysis to obtain a unique numerical value; this value is an overall measure (expressed on a linear scale) of the biological phenomenon to which these parameters are related. Furthermore, this technique makes it possible to find the most effective linear combination of these parameters; it is therefore possible to construct a linear scale on which any individual can be measured starting from the given multivariate data; this means that the measure obtained is the best for dividing the two original groups. In other words,

---

Key-words: Acute phase reactants; Discriminant analysis; Inflammation; Inflammation indices.

Accepted for publication on May 15, 1981.

La Ricerca Clin. Lab. *11*, 223, 1981.

once the best linear function (usually called 'Z') has been defined, its value for any subject can be determined starting from the initial values. It is easy to distinguish two groups: the former in which the 'Z' values of single members vary around a given mean value, the latter in which the 'Z' values vary around another mean.

If the considered parameters are related to the disease under examination, the two means should be appreciably different; it is nevertheless possible that there will not be a sharp distinction between the two groups so evaluated, but rather that they share some transvariant characteristics. Therefore, the determination of a threshold value of 'Z' (with 'Z_0' as an optimum discriminant threshold) is necessary to class the subjects examined into either one group or the other with an acceptable degree of reliability. The effectiveness of the discriminant function can be expressed in terms of probability of error. If this probability is satisfactory, the function can be used for diagnostic purposes to assign to different groups the subjects that exhibit the same given value of the parameters used to determine the function.

MATERIALS AND METHODS

A preliminary analysis (unpublished data) carried out on 450 cases of different inflammatory diseases was used to determine the most suitable parameters for calculating the discriminant function. That investigation was carried out on the following group of proteins: IgG, IgA, IgM, C3, C4, α_1-antitrypsin (AT), α_2-macroglobulin (M), α_1-acid glycoprotein (GA), fibrinogen (F), caeruloplasmin (Cae), C-reactive protein (CRP) and haptoglobin (Hp).

The quantitative evaluation of all plasma proteins was carried out by using the simple radial immunodiffusion technique[9]. Of the proteins considered, only the following six showed variations which could be related statistically to the inflammatory situation: AT, GA, F, Cae, Hp and CRP.

Furthermore, these proteins have always shown interrelated variations in the course of inflammatory diseases.

In order to calculate the value of the discriminant function, 60 subjects with rheumatoid arthritis (RA), free of treatment and free from extra-rheumatoid changes (especially from any liver disorders) and 40 young healthy subjects (aged 15 to 40 years) were selected.

The discriminant function was evaluated from the values of deviation and co-deviation of the parameters used; the significance of the above mentioned function was calculated using the method of the analysis of variance, applied to the groups discriminated by the function 'Z' (tab. 1). As for computing the optimum discriminating value ('Z_0'), the following rule was used: the value of 'Z' was selected which minimized the sum of the error probability in assigning different new cases to one of the two groups.

Since it might be assumed that the 'Z' function follows a normal distribution, the threshold value was computed from the following formula:

$$\frac{\overline{Z_A} - Z_0}{S_A} = \frac{Z_0 - \overline{Z_B}}{S_B} \quad \text{hence} \quad Z_0 = \frac{S_B\,\overline{Z_A} + S_A\,\overline{Z_B}}{S_A + S_B}$$

where S_A = the standard deviation for the discriminant values within the RA patient group; S_B = the standard deviation for the discriminant values within the healthy subject group; $\overline{Z_A}$ = mean of 'Z' for the RA patient group; $\overline{Z_B}$ = mean of 'Z' for the healthy subject group.

	degrees of freedom	sum of squares	variance	F	p
between groups	6	2.924 *	0.487 *	129.850 *	<0.001
within groups	93	0.349 *	0.004 *		
total	99	3.273 *			

* Calculations in this test were actually made to ten decimal places.

Tab. 1 - Significance of discriminant function; analysis of variance.

The estimation of error probability for the optimum discriminating threshold requires the postulation of a hypothesis regarding the nature of the distribution of 'Z'. A reasonable hypothesis is that 'Z' follows a normal distribution. This makes it possible to compute the mean deviation in terms of 'standard deviation' and to compute for each group the expected frequency for values of 'Z' higher than 'Z_0' (in RA patients) or, alternatively, lower than 'Z_0' (in healthy subjects). The evaluation of this frequency was carried out by computing the 'probability density function':

$$\varphi(c) = \left(1 \Big/ \sqrt{2\pi}\right) e^{-\frac{c^2}{2}}$$

where $c = \dfrac{x - \mu}{\sigma}$ and then the probability:

$$Q(c) = \int_{-\infty}^{c} \varphi(c)\, d(c)$$

This probability has been found equal to 0.026.

RESULTS AND DISCUSSION

The first noteworthy result obtained by using multivariate analysis in this investigation is the demonstration of the possibility of calculating the partial correlation coefficients separately for the individual proteins considered, as shown in the following formula, where the discriminant function was calculated from 60 RA patients and 40 healthy volunteers:

$$'Z' = 4.63\ AT + 10.45\ GA + 2.09\ F + 24.92\ Cae + 2.87\ Hp + 112.90\ CRP$$

The function 'Z' is, for practical purposes, a measurement of the inflammatory state.

As mentioned above, the discriminant analysis provides a value on a linear scale for every subject. In the present case, it is a measure of inflammatory activity determined from the levels reached by the selected serum proteins. Partial correlation coefficients thus indicate the value of variation of single serum proteins (mg%) in relation to variations in the inflammatory state.

In other words, since inflammatory activity is the only dependent variable, partial correlation coefficients do express the value of variation in the inflammatory situation that is required to cause an increase of one measurement unit in the protein considered. Therefore, an increase of 4.63 arbitrary measurement units (AU) in

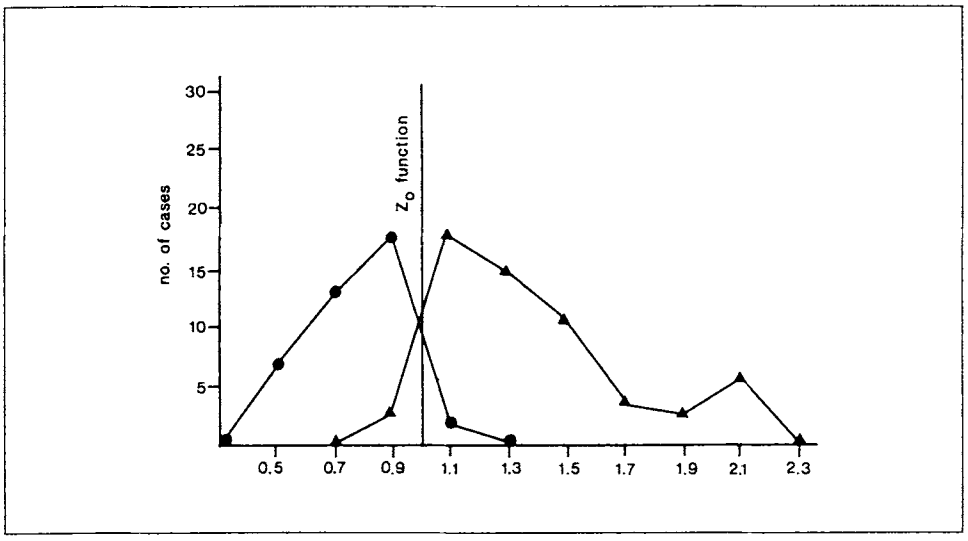

Fig. 1 - Curves of distribution of inflammation index in RA patients (▲—▲—▲) and in healthy volunteers (●—●—●). Note the transvariation pattern of this function and the dispersion of values wider in RA patients than in healthy subjects.

the inflammatory activity is required to obtain an increase of 1 mg% in AT, whereas an increase of 24.92 AU is necessary to obtain an increase of 1 mg% in Cae, etc.

Thus, it is possible to construct a sensitivity scale of the individual proteins investigated: F, Hp and AT show the highest sensitivity as related to the inflammatory state; GA and Cae show intermediate sensitivity; CRP shows low sensitivity, effectively increasing only during a severe inflammatory situation.

The optimum discriminant threshold ('Z_0'), which was calculated from the discriminant function, provides an effective and reliable ($p = 0.026$) tool not only for ascertaining the presence of inflammatory state in subjects showing inconclusive symptoms of inflammation based on some laboratory findings, but also for quantifying the extent of inflammation in every subject investigated. In fact, the discriminant 'Z' will assume progressively increasing values (progressively higher than the discriminant threshold) as the inflammatory state in subjects with RA worsens. This finding, particularly the constant proportionality between the inflammatory state and the function 'Z', permits the actual measurement of the inflammatory state in the individual subject under examination. In fact, the ratio between the value of the function in a given case and the numerical value of the optimum discriminant threshold will be lower than 1 in subjects with minimal or no inflammatory phenomena, equal to 1 in subjects in a borderline situation and higher than 1 in subjects with inflammation in progress (fig. 1). The values which the inflammatory activity can bring about are obviously extremely wide; they monitor in a highly precise manner the actual instantaneous inflammatory state in every individual subject through the changes of the six proteins considered.

The score of inflammatory activity obtained would be highly useful for diagnostic purposes, particularly for those subjects whose symptoms cannot be assigned reliably to an inflammatory disease because of weak and not clear-cut clinical manifestation and poor or conflicting laboratory results.

Moreover, the use of a single score synthesizing the quantitative determination of six serum proteins simplifies greatly the interpretation of the laboratory results; finally, it provides a method for evaluating the efficacy of any anti-inflammatory treatment in the patients, on the basis of the fact that the mean average turnover of acute phase reactants is four days.

SUMMARY

Statistical analysis allows a better evaluation of plasma protein patterns during the inflammatory state, in which there is an increase in the levels of acute phase reactants. An index is proposed for an overall evaluation of the modifications in plasma proteins induced by inflammation.

REFERENCES

1. AMBANELLI U., TROISE W., FADDA G., NERVETTI A.: Serum and synovial fluid concentration of α_2-macroglobulin in rheumatoid arthritis - Z. Rheumaforsch. *34*, 408, 1975.
2. AMOS R. S., CROCKSON A. P., WALSH C., MCCONKEY B.: Rheumatoid arthritis C-reactive protein and erythrocyte sedimentation rate during initial treatment - Brit. med. J. *i*, 195, 1977.
3. AYLWARD M., MADDOCK J., WHEELDON R., PARKER R. J.: A study of the influence of various antirheumatic drug regimens on serum acute phase proteins, plasma tryptophan and ESR in rheumatoid arthritis - Rheum. and Rehabil. *14*, 101, 1975.
4. BACH ANDERSEN R., FRIIS TH.: Metabolism of fibrinogen in rheumatoid arthritis and in a control group - Acta rheum. scand. *17*, 94, 1971.
5. CROCKSON A. P., CROCKSON R. A., MCCONKEY B.: C-reactive protein in rheumatoid arthritis - Arthr. and Rheum. *21*, 491, 1978.
6. DECKER B., MCGUCKIN W. F., MCKENZIE B. F., SLOCUMB C. H.: A study of some 'acute phase reactants' in rheumatoid disease - Arthr. and Rheum. *3*, 49, 1960.
7. JAYLE M. F., ENGLER R.: Les différents profils des variations des protéines plasmatiques dans les états inflammatoires - Path. et Biol. *22*, 645, 1974.
8. KOMATSUBARA Y., HIRAMATSU S., HONGO I., MAEDA A., SODA T., BOTAN Y.: Multivariate analysis of serum proteins in rheumatoid arthritis - Scand. J. Rheum. *5*, 97, 1976.
9. MANCINI G., CARBONARA A. O., HEREMANS J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion - Immunochemistry *2*, 235, 1965.
10. MCCONKEY B., DAVIES P., CROCKSON R. A., CROCKSON A. P., BUTLER M., CONSTABLE T. J., AMOS R. S.: Effects of gold, dapsone and prednisone on serum C-reactive protein and haptoglobin and the ESR in rheumatoid arthritis - Ann. rheum. Dis. *38*, 141, 1979.
11. ROUX H., AQUARON R., BERGEAUD F., GRANGIER J., RECORDIER A. M.: Le dosage de la céruloplasmine sérique en rhumatologie - Rev. Rhum. *38*, 99, 1971.
12. SWEDLUND H. A., HUNDER G. G., GLEIG G. J.: α_1-antitrypsin in serum and synovial fluid in rheumatoid arthritis - Ann. rheum. Dis. *33*, 162, 1974.
13. WALSH L., MCCONKEY B.: α_1-antitrypsin and rheumatoid arthritis - Lancet *ii*, 564, 1977.

Requests for reprints should be addressed to:

GIOVANNI LAPADULA
Cattedra di Reumatologia
Università degli Studi di Bari
70124 Bari - Italia