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Molecular biology, measurement and clinical utility of the acute phase proteins

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Abstract - The acute phase reaction is a natural response to tissue injury and includes a range of metabolic activities which include alterations in the rate of synthesis of several proteins produced by the liver. It is recognised that the cytokines play a key role in mediating this response. Research is directed toward an understanding of this response from the stimulus through mediation to the role of the proteins produced. Measurement of the proteins in serum is of considerable value in the diagnosis, management and prognosis of many diseases that exhibit an acute phase response.

Inflammation, resulting from any form of tissue injury causes an increase in concentration of a number of liver-derived plasma proteins -the acute phase proteins (APP). This response is mediated by cytokines released from cells at the site of inflammation and is accompanied by several other systemic responses such as leucocytosis, fever and muscle proteolysis. The response is markedly conserved throughout evolution and may serve to replenish inflammatory mediators and inhibitors consumed during inflammation. The differential induction of these proteins by different cytokines does, however lead to speculation over whether the nature and chronicity of inflammation may be influenced by the pattern of acute phase proteins produced and whether the defects in the system may predispose to pathological consequences (ref.1).

MOLECULAR BASIS OF THE ACUTE PHASE REACTION

The bulk of acute phase protein synthesis occurs in the liver parenchymal cells with increasing numbers of hepatocytes being recruited during the first few hours of the inflammatory response (ref. 2). However synthesis of some acute phase proteins has been demonstrated in extra hepatic sites, in particular in cells of the monocyte-macrophage lineage (refs. 3, 4).An enormous amount of experimental work has been undertaken using whole animals and hepatocyte cultures to characterise the mediators of the acute phase protein response. It is now clear that the full spectrum of the response can probably be attributed to interleukin - 6 (IL6) acting in conjunction with interleukin-1 (IL1) and glucocorticoids. In addition, tumour necrosis factor can regulate a subset of acute phase proteins(refs. 5,6). In the rat, glucocorticoids and catecholamines may induce α_2 - macroglobulin synthesis without requirement of the cytokines (ref. 7). The cytokines responsible for the acute phase response are derived predominantly from activated macrophages at the site of injury though many other cell types may be capable of synthesizing them. When classified according to function it is clear that the acute phase proteins may all have roles to play in inflammation or the healing process which follows, (Table 1).

The rate of increase in their plasma concentration and the incremental change achieved following inflammatory stimulus in vivo, varies considerably and reflects their induction by different cytokines, their molecular size, volume of distribution and rate of metabolism both in the circulation and at the site of inflammation. Kushner (ref. 9) has defined acute phase proteins as those whose concentration increases by 25% or more following inflammation and has classified human acute phase proteins into three groups on the basis of the magnitude of their increase (Table 2) (ref. 10). During inflammation some proteins fall in concentration due to either a redistribution into the extravascular space (ref. 11) or cytokine mediated decreases in gene transcription (ref. 12). This group mainly contains transport proteins and includes, albumin, prealbumin, transferrin, retinol binding protein, alpha and beta lipoprotein and alpha - 2 HS glycoprotein.

KINETICS OF THE ACUTE PHASE RESPONSE

In man CRP and serum amyloid A (SAA) show the greatest response to injury although the rate of increase in plasma concentration and the incremental change achieved following the inflammatory stimulus varies considerably between the acute phase proteins. These variations possibly reflect the differential sensitivity to induction of synthesis although in a particular species the pattern of response to acute inflammation is constant. In chronic inflammation different patterns of response may be seen following different stimuli, in different inflammatory diseases, and following the same stimulus in different species or individuals of the same species. This complex situation may reflect alterations in the rate of synthesis of individual proteins in response to changes in regulating mediator patterns. Glibetic and Baumann (ref. 13) showed that during the progression from acute (less than 24 hours after injury) to

chronic inflammation in the mouse, there was a switch from a predominant expression of the gene for α_1 - acid glycoprotein 1 to the gene for α_1 - acid glycoprotein 2. In addition, the concentration of albumin mRNA returned to normal levels after an initial reduction of 50% whereas the mRNA for SAA, α_1 acid glycoprotein, haptoglobin and fibrinogen was maximal at 24 hours and decreased afterwards despite continuing inflammation. These findings afford some evidence that a change in regulation of protein synthesis for different proteins occurs during chronic inflammation.

CYTOKINES AND THE ACUTE PHASE RESPONSE *IN VIVO*

Evidence of correlations between plasma IL1 levels and acute phase reactants in man are not apparent despite the recent availability of sensitive immunoassay detection methods. Using bioassay methods serum levels have been detected in patients with burns (ref. 14) and following surgical procedures Nijsten et al also showed that IL6 detected in plasma within hours after burn injuries was 2 to 100 times the normal level while increases in CRP and α_1 - antitrypsin followed more gradually. In post-operative patients IL6 was detectable in serum within 3 hours, had reached a maximum at 24 hours and levelled off by 48 hours, whereas CRP could not be detected during the first 6 hours but gradually increased over the next 48 hours. The other acute phase proteins measured, haptoglobin, α_1 - antitrypsin and orosomucoid, increased gradually over a longer time course.

TABLE 1 ACUTE PHASE PROTEINS OF INFLAMMATION (ADAPTED FROM REF 8).

Protein	Postulated function
Inflammatory mediators	
Complement components	Opsonization, chemotaxis, mast cell
$C1_s$, C2, C3, C4	degradation
C5, C9, factor B	
Kininogenase (kallikrein)	Vascular permeability and dilation
Kininogen	
Factor VIII, fibrinogen	Clotting formation of fibrin matrix for repair
Prothrombin	
Plasminogen	Proteolytic activation of complement,clotting, fibrinolysis
C- reactive protein	Binding to phosphorylcholine in all membranes with complement activation and opsonization
Inhibitors	
Antithrombin III	Control of mediator pathways
C1 INH , factor I, factor H	
α_1 - Protease inhibitor (a_1 - antitrypsin)	Elastase, collagenase
α_1 - Antichymotrypsin	Cathepsin G
Thiol protease inhibitor	Cysteine proteases
Haptoglobin	Cathepsins B, H, L?
Scavengers of molecular products of inflammation	
Haptoglobin	Hemoglobin
Serum amyloid A protein	Cholesterol?
C-reactive protein	DNA?
Ceruloplasmin	$[0_2]$?
Immune regulation	
C-reactive protein	Interactions with T and B cells
α_1- Acid glycoprotein (orosomucoid)	Expressed on lymphocyte surface
Repair and resolution	
α_1- Acid glycoprotein	Promotes fibroblast growth, interacts with collagen
α_1 - Protease inhibitor,	Bound to surface of new elastic fibres
α_1 - antichymotrypsin , C1 INH	(?), inhibit remodelling by leukocytic protease

IL6 has also been reported in the serum and synovial fluid of patients with various rheumatic diseases; synovial fluid levels of IL6 were found to be a thousand-fold higher and to correlate positively with those in serum, suggesting localised production in the joint. Synovial fluid levels of IL6 could also be correlated with articular index, a clinical measure of local inflammation, whereas serum IL6 levels were positively correlated with CRP and negatively correlated with albumin in rheumatoid arthritis. Raised levels of IL6 in serum, urine and CSF have also been reported in various other inflammatory conditions involving acute phase responses.

MEASUREMENT

Whilst many of the acute phase proteins have a demonstrable biological function, immunoassay techniques are now preferred for the quantitation of these proteins. The first immunological methods included immunodiffusion and electroimmunoassay and were popular because of their economy and simplicity. In addition latex slide fixation techniques were used for the measurement of CRP to provide rapid semi-quantitative answers. However the advent of immunoprecipitation methods has revolutionized the measurement of the APPs. Immunoprecipitation methods depend on the polyvalency of the antigen and the bivalency of the antibody to produce an immune complex with an increase in size and refractive index and a concomitant increase in light scattering.

The principles of light scattering immunoprecipitation methods and strategies for method optimisation are described in several reviews (refs. 15,16). The choice of the antibody will depend on the required sensitivity, range and specificity of the method. In this respect it is particularly important to ensure that there are sufficient binding sites to achieve complex formation, avoiding the situation of antigen excess. The inclusion of polyethylene glycol (PEG) in the reaction mixture enhances the rate of immune complex formation and in some instances the calibration range of the assay. However it is important to avoid increasing the PEG level to the extent that nonspecific precipitation of proteins occurs; this may increase sample blanks and in the extreme, removal of the antigen of interest.

Light is scattered in all directions to differing degrees depending on the size of the scattering species and thus the apparent reaction kinetics. Hence method optimisation, will depend on the detector system. The detection of immune complex formation commonly involves nephelometry or turbidimetry. However the ideal nephelometer configuration involves a forward scatter angle for maximum sensitivity but this is only available with dedicated instrumentation. In addition a laser light source will also improve the sensitivity of detection. Despite this apparently superior sensitivity the advantages of nephelometry over turbidimetry are not born out in routine practice with the latter showing similar sensitivity with better precision (ref. 15). Improved precision is also achieved by employing a fixed time interval measurement or continuous reaction monitoring, both techniques also providing sample and reagent blank correction.

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If greater sensitivity is required the light scattering of the immune complex can be considerably enhanced by labelling the antibody with latex (ref. 17) or gold sol (ref. 18) particles. Latex particles are proving the more popular because of the ability to manipulate core refractive index, density, surface chemistry and size. Furthermore covalent coupling of antibody to particle provides better reagent stability and hence calibration curve stability. In an assay for CRP Price et al (ref. 19) showed a 100 fold increase in sensitivity , with calibration curve stability in excess of one month, for a latex enhanced, 30 sec reaction time, immunoturbidimetric assay.

It is now being claimed that biosensor technology will provide new diagnostic techniques that will enable rapid and accurate assays to be performed outside the laboratory. These techniques include electrochemical and optical immunosensors. One of the most exciting possibilities is that of surface plasmon resonance spectroscopy. The technique depends on changes in the nature of totally internally reflected light upon a change in refractive index at the surface of a prism like device. The change in refractive index could involve binding of antigen to immobilized antibody with additional sensitivity achieved using latex particles. One of the attractions of this technique is the rate of reaction.

Whatever methodology is used the desired accuracy will only be achieved with appropriate calibration. In several studies it has been shown that interlaboratory variation is not method but calibrator dependent, and that on a common calibrator can improve interlaboratory performance dramatically (ref. 20).

CLINICAL UTILITY

The measurement of APPs has become popular because (i) inflammatory diseases represent a common circumstance in clinical practice, (ii) the increase of APP constitutes one of the major characteristic changes which can objectively document the occurrence of an inflammatory process for the clinician, (iii) the relative merits of the different APP are now well documented, (iv) rapid and precise methods of measurement are available.

Does the ideal APP exist?

Some 30 proteins have been reported to increase in serum during the acute phase response (ref. 21). The most commonly measured proteins are CRP, haptoglobin α_1 - acid-glycoprotein or orosomucoid, α_1 - protease inhibitor (α_1-PI) previously termed α_1 - antitrypsin and α_1 - antichymotrypsin (Table 2).

An ideal APP should fulfil four criteria: (i) A strict dependence on the inflammatory process; additional factors may influence the serum level of an APP, for example oestrogens increase a_1 antitrypsin but diminish α_1 - acid glycoprotein values, haemolysis induces a dramatic consumption of haptoglobin. (ii) An independence of the clinical aetiology of the inflammatory process; CRP is an excellent marker of bacterial infections, whilst α_1 antitrypsin is better for viral infections of the liver. (iii) An ideal protein should exhibit rapid kinetics with a marked elevation after the inflammatory stimulus. A short half-life allows a quick return to normal values at the end of the acute phase period.(iv) Significant increase during a mild inflammatory process.

TABLE 2. MAJOR HUMAN ACUTE PHASE PROTEINS (ADAPTED FROM REF 9).

Protein		Normal plasma concentration (g/L)	Plasma Concentration in inflammation (g/L)	Response time (h)
Group III	up to 1,000 x increase			
	C-reactive protein	0.00007-0.008	0.4	6-10
	Serum amyloid A	0.001 - 0.030	2.5	6-10
Group II	2-4 x increase			
	α_1-Antichymotrypsin	0.3 - 0.6	3.0	10
	α_1 - Antitrypsin	1.0 - 2.0	7.0	
	α_1 - Acid glycoprotein	0.5 - 1.4	3.0	24
	Haptoglobin	1.0 - 3.0	6.0	
	Fibrinogen	2.0- 4.5	10	
Group I	about 50% increase			
	Ceruloplasmin	0.15 -0.6	2.0	
	C3	0.55 - 1.2	3.0	48-72
	C4	0.2 - 0.5	1.0	

TABLE 3 DISEASES ASSOCIATED WITH A MAJOR ELEVATION OF ACUTE PHASE PROTEINS

Trauma	Surgery, burns
Infections	Bacterial Infections
Inflammatory disease	Rheumatoid arthritis
	Juvenile chronic arthritis
	Ankylosing spondylitis
	Polymyalgia rheumatica
	Behcet's syndrome
	Reiter's disease
	Systemic vasculitis
	Crohn's disease
Neoplasia	Lymphoma, Hodgkin's
	Carcinoma, Sarcoma
Ischeamic necrosis	Myocardial infarction

No single protein fulfills all these criteria, and a combination of two sets of proteins is to be recommended: (a) a protein with a short- half life and a large amplitude of variation. CRP constitutes the archetype of the marker of the acute phase period with a hundred fold increase during acute inflammatory processes. Serum amyloid A = was a possible candidate in this group of proteins but it appears to be too sensitive with rises occurring in very mild conditions without clear pathological significance; furthermore, due to its immunosuppressive effect it is difficult to get satisfactory antisera for nephelometric or turbidimetric measurements. (b) a protein with a slower response and a longer half-life such as haptoglobin α_1 antitrypsin or α_1 - acid glycoprotein which are good markers of chronic inflammatory diseases such as temporal arteritis (ref. 22).

These types of proteins are also very useful to monitor out-patients for whom samples are taken less frequently (ref. 23). In a study on 171 patients admitted to a gastroenterology unit, Calvin et al. (ref. 24) found that for assessment of inflammatory status α_1 anti chymotrypsin was the most sensitive test (95%) compared to CRP, haptoglobin, α_1 acid glycoprotein , α_1 antitrypsin and ESR, its specificity being 81%. They pointed out that a sample withdrawn eight days after an event inducing an acute phase response can give a normal CRP concentration. From the above considerations, the French Society for Clinical Chemistry (SFBC) has proposed the combined determination of CRP with haptoglobin and/or α_1 acid glycoprotein for the diagnosis and monitoring of inflammatory disorders.

What are the clinical indications of APP measurement?

Detection of organic disease. The elevation of serum acute phase proteins is a marker of tissue injury. Diseases associated with a major rise of APP (particularly CRP) are listed in Table 3. Alternatively systemic lupus erythematosus, scleroderma, ulcerative colitis, leukemia and viral infections are not characterized by an increase in CRP levels. Many clinicians have used APP measurement for screening purposes. As stressed by Kushner (ref. 25), the cost effectiveness of this strategy is questionable in asymptomatic patients or in people with trivial symptoms. On the contrary, with a clinical history of an evolving disease or when the indications are more focused (post surgical or neonatal period for infectious risk) APP measurements constitute a helpful tool for selecting who will need further investigations.

In these situations, the early detection of bacterial infections has been largely documented (ref. 26). Due to its rapid kinetics CRP is the most reliable APP for the follow-up of post-surgical patients and the rapid diagnosis of infectious complications. In the post operative period , as demonstrated by Fischer et al. (ref. 27), CRP levels which did not drop at day 3 or 4 are very suggestive of infection. However it is unhelpful and expensive to measure CRP at day 1 or 2 after the operation because of the "physiological" acute phase response induced by surgery. In neonatal sepsis Bienvenu reported an elevation of CRP and α_1 acid glycoprotein respectively in 73% and 85% of their cases (ref. 28). False negative results were essentially observed in B streptococcal infections. The particular interest of APP determination in this very early period of life essentially lies in the fact that in the neonates APP rises other than bacterial infections are very rare. In parasitic infections (ref. 29) APP elevations are only seen when the parasite leads to tissue destruction. Kostiala showed that in 25 out of 33 patients (76%) with deep seated fungal infections (essentially C. albicans) CRP increased up to 104-300 mg/L; this type of response resembles bacterial rather than viral infections.

Assessment of the extent of tissue injury and disease activity. After myocardial infarction the APP pattern is similar to the post surgical one (ref. 30). Some authors tried to correlate elevations of APP with the extent of tissue necrosis. The results are controversial (ref. 31) and have limited clinical value.

In cancer, raised APP concentrations are frequently observed (ref. 32) and a correlation with tumour metastasis is described. In the absence of other inflammatory disorders, CRP values at first presentation correlate with prognosis of some tumours such as prostate and bladder (ref. 33).

In rheumatology, Hind & Pepys (ref. 34) reported that more than 90% of patients with rheumatoid arthritis (RA) had elevated CRP values. For these authors, CRP was the most precise objective laboratory test of disease activity: values exceeding 50mg/L are common in mild or moderate RA and frequent in severe RA. Furthermore, it has been shown that CRP concentrations are correlated with radiological changes (ref. 35). A correlation between disease activity and CRP levels has also been described in Crohn's disease (ref. 36).

Monitoring therapy in inflammatory and infectious diseases. In inflammatory disease, it is often difficult for the clinician to objectively assess the response to therapy. In this context, CRP provides a useful tool for the management of therapy in rheumatoid arthritis (ref. 37) and Crohn's disease (ref. 36) or ulcerative colitis (ref. 38).

The best indications of APP measurements are also valuable in the follow-up of antibiotic therapy during bacterial infections. Experience would suggest that a profile such as CRP and α_1 acid glycoprotein constitutes one of the most precise tools that the clinician possesses to control the treatment of neonatal infections: CRP with its short half-life (8 hours) indicates the efficiency of antibiotics while orosomcoid provides a guide for when to stop treatment.

These conclusions are also true for the monitoring of antibiotic therapy in adults; if a CRP is elevated when the diagnosis of bacterial infection is made and if this level does not decrease after 24 to 48 hours of antibiotics, it suggests an inadequate therapy and indicates the need for a change in antibacterial drugs.

Detection of Intercurrent infections. In diseases which do not usually induce an APP elevation a sudden and important rise in CRP levels is indicative of an intercurrent bacterial infection. This application of APP measurement has been well documented in systemic lupus erythematosus (SLE) and in acute leukemia.
Hind and Pepys (ref. 34) suggested that in SLE a serum CRP greater than 60 mg/L strongly suggests the existence of an infection a CRP level less than 30 mg/L makes a serious infectious disease unlikely.

In acute leukaemia, infections are a frequent cause of death, particularly in neutropenic patients. Among the rapid tests for the diagnosis of infections CRP is of special interest with serum concentrations above 100mg/L in bacterial infections (ref. 39); here again, the response to antibiotic therapy can be followed by CRP quantitation.

Differential diagnosis. The quantitative differences in CRP response during particular diseases that can present themselves under similar clinical pictures lead some authors to propose CRP as an aid to distinguish them. Hence RA, Crohn's disease, myocardial infarction will often demonstrate high CRP levels whilst SLE, ulcerative colitis (ref. 34) and atypical angina (ref. 25) show low CRP levels. CRP levels contribute to the distinction between bacterial (high CRP) and viral (low CRP) infections with clinical applications in pneumonia (ref. 40) and meningitis (ref. 41).

Reference for the interpretation of the variation of other proteins. The interpretation of an absolute protein serum concentration taken in isolation is very difficult particularly in the presence of inflammatory disease. Some authors introduced the concept of protein profiles (ref. 42) to compare the relative variations of the different plasma proteins between each other.

The transport proteins , albumin, transferrin, prealbumin, and retinol binding protein are considered as negative acute phase proteins. This has been clearly illustrated by clinical data in the post-surgical period where a maximum decrease of the "nutritional proteins" coincides with the acute phase proteins peaking (3-4 days after operation) and a return to normal values when the acute phase process has ceased (refs. 43- 44). Hence the diagnosis of malnutrition based on the concentration of transport proteins during the early post-surgical period is not possible. In general, when APP are elevated, the interpretation of "nutritional proteins" must be cautious, particularly during infection episodes which are frequent in malnourished people.

Apo A1 has also been known to be dramatically decreased during the acute phase response. In a study including 14 patients with sepsis admitted in an intensive care unit, Bienvenu et al reported a significant decrease of Apo A1 (0.50 $\pm$ 0.29 g/L) compared to a control group (1.01 $\pm$ 0.26 g/L) of patients undergoing elective surgery (ref. 45). SAA which circulates with HDL_3 could be implicated in these abnormalities because of its ability to displace Apo A1 from its binding to HDL. Another study designed to determine the changes in plasma apolipoproteins during inflammatory diseases, attempted to define the corresponding bias in estimation of cardiovascular risk on the basis of Apo A1 and Apo B measurements. On admission, Apo A1 levels in 133 hospitalized patients with inflammation was 1.04 $\pm$ 0.32 g/L(mean $\pm$ SD.) which was significantly lower than the level in 106 control patients (1.30 $\pm$ 0.30 g/L). Apo B concentrations were similar in the 2 groups. 60 days later, Apo A1 levels were not modified in 29 patients presenting a persistent inflammation, while in 31 patients in which inflammation had resolved a significant increase of Apo A1 was observed (1.43 $\pm$ 0.31 at day 60 versus 1.07 $\pm$ 0.32 g/L at first presentation). This variation in plasma apolipoproteins during the inflammatory process is comparable in magnitude with the difference reported between groups of patients with and without coronary - artery disease. Thus, the use of apolipoproteins for the screening of cardiovascular risk are inappropriate during the inflammatory response.

Perspectives: will acute phase cytokines replace APP measurement?

It has been proposed that the measurement of the "acute phase cytokines" could be a more precise and more sensitive tool in the exploration of inflammatory and infectious diseases.

Increased concentrations of TNFα , IL 1β and IL - 6 in body fluids have already been reported in many clinical

conditions . These 3 cytokines are elevated in synovial fluid during RA (refs. 46-48) with a correlation with disease activity for IL - 1 and IL - 6 ; but, this rise is not specific for RA as it is also found in other inflammatory rheumatoid diseases. In septic shock. TNFα levels are remarkably high and this cytokine has been identified as having a pivotal role in the pathophysiology of the disease (ref. 49). In the diagnosis of graft rejection, the increase of TNFα in serum (ref. 50) and of IL - 6 in serum and urine (ref. 51) should be of some help in the early diagnosis of renal rejection with a rise occurring two to three days before clinical evidence.

In our opinion, the measurement of acute phase cytokines should be limited to a restricted number of clinical situations such as for example sepsis /septic shock for TNFα and IL-6. Furthermore, the introduction of these new parameters will largely benefit from the development of low cost and rapid methods for their determination, in accordance with the rapid turn-over of these molecules. Hence, the quantitation of APP and particularly CRP will stillremain for the next few years the major tool in the exploration of inflammatory disease.

REFERENCES

1. J.T. Whicher, C.I. Westcott, P.A. Dieppe "Protides of the Biological Fluids. (Proceedings of the 34th Colloquim)" H. Peeters (ed): pp 605 - 609 New York : Pergamon Press, (1986).
2. I. Kushner , G. Feldman. J Exp Med 148: 466-477 (1976).
3. H.R. Colten. Mol Immunol 19: 1279-1285. (1982):
4. A E. Kuta, L. L.Baum. J Exp Med 164: 321-326. (1986).
5. H. Baumann, C. Richards, J. Gauldie. J Immunol 139: 4122-4128. (1987):
6. J, Gauldie, C. Richards, D. Harnish et al. Proc Natl Acad Sci USA 84: 7251-7255. (1987).
7. J. Van Gool, W,Boers, M. Sala et al. Biochem J 220: 125-132. (1984).
8. J.T. Whicher, P A.Dieppe. Clin Immunol Allergy 5: 425-446. (1985):
9. I.Kushner Ann NY Acad Sci 389: (1982):
10. I. Kushner, A. Mackiewicz. Dis Markers 5: 1-11. (1987):
11. F. C. Ballantyne, W. J. Tilstone, A. Fleck. Brit J Exp Pathol 54: 409-415. (1973).
12. G. Ramadori, J. D. Sipe, C .A . Dinarello et al . J Exp Med. 162: 930-942. (1985).
13. M.D. Glibetic, H. Baumann . J Immunol 137: 1616-1622, (1986).
14. M.W.N. Nijsten, E. R. De Groot, H .J. Ten Duis et al . Lancet ii, 921. (1987).
15. C .P. Price, K. Spencer, J. T. Whicher . Ann Clin Biochem 20 1-14 (2983).
16. J. T. Whicher, C .P. Price. In Principles of Clinical Biochemistry (D L Williams, V Marks, Editors) 2nd edition, Heinemann, p 472-483 (1988).
17. J .P. Galvin. In Diagnostic Immunology: Technology Assessment and Quality Assurance (J . H. Rippey , R. M. Nakamura , Editors), College of American Pathologists p 19-30 (1983).
18. J .W .H. Leuvering, P. H. J .M. Thal , M. Vander Waart et al. J Immunoassay 1, 77-80 (1980).
19. C. P. Price, A. K. Trull, D. Berry et al. J Immunol. Meth 99 205-11 (1987).
20. D. G. Bullock, G. Dumont, A. Vassault et al. Clin Chim Acta 187 21-36 (1990).
21. J. Bienvenu. Ann. Biol. Clin. 42 47-52. (1984).
22. J .B. Paolaggi, D. Chaouat, D. Barres et al . Rev. Rhum 49. 413-419 (1982).
23. B. S. Bull , W .C. Levy , J .C . Westergrad et al Lancet 377-380. (1986).
24. J. Calvin, G. Neale, K J. Fotherby et al . Ann. Clin. Biochem. 25. 60-66. (1988).
25. I. Kushner. Hospital Practice March 30. 13-28. (1990).
26. J. Gauldie, L. Lamontagne , A. Stadnik Surv. Synth. Path. Res 4. 126-151. (1985).
27. C .L. Fischer, C. Gill , M G. Forrester et al . Am. J. Clin. Path. 66. 840-846. (1976).
28. J. Bienvenu, F. Bienvenu , L. Sann. H. Peeters. Ed. Pergamon Press. 34. 573-579. (1986).
29. I. Kostiala. J. of Infection. 8. 212-220. (1984).
30. I. Kushner, M. L. Broder , D. Karp. J. Clin. Invest. 61. 235-242. (1978).
31. L .M. Killingsworth. Plasma proteins implicated in the inflammatory response in "Marker Proteins in Inflammation" (R C Allen , J. Bienvenu, P. Laurent, R. M .Suskind Editors), pp 21-32 Walter de Gruyter (1982):
32. E. H. Cooper , J. Stone. Advances in Cancer Research 30. 1-43. (1979).
33. M .B. Pepys , M.L. Baltz. Adv. in Immunology 34. 141-212 (1983)
34. C. R .K. Hind, M. B. Pepys. Internal Medicine for the Specialist 5. 112-151. (1984).
35. R .S. Amos, T J. Constable, et al : Br. Med J. 1. 195-197. (1977).
36. E .A.,Fagan, R .F. Dyck, P. H. Maton et al B: J. Clin. Invest. 12. 351-359. (1982).
37. R .K. Mallya , F C. de Beer , H. Berry , et al : J. Rheumatol 9. 224-228. (1982).
38. N. A. Buckell , J E. Lennard-Jones, M. A. Hernandez. Gut. 20. 22-27. (1979).
39. K. P. Schofield , F.Voulgari, D. I. Gozzard . J. Clin Path 35. 866-869. (1982).
40. P .L. Mac Carthy, A. L. Franck, R. C. Ablow et al : J. Pediatr. 92. 454-456. (1978).
41. D. Clarke, K. Cost. J. Pediatr. 102. 718-720. (1983).
42. J. C. Frot, P. Giraudet, H. Hofmann, et al : Plasma protein profile: A model of data for interpretation with special reference to inflammatory conditions. In: Marker Proteins in Inflammation. (R C Allen J. Bienvenu, P. Laurent, R M Suskind Editors), Vol. 1, p253-273. Walter de Gruyter. (1982).
43. K. F. Aronsen , G. Ekelund, C .O. Kindmark , et al : Scand. J. Clin. Lab. Invest. 29. Suppl. 124, 127-136. (1972).
44. A. Lepape, J. P. Perdrix , B. Rondelet , V. Banssillon, J. Bienvenu, H. Bernon , A. Roulet , P. Laurent . Surgery induced modification of plasma proteins level. In: Marker Proteins in Inflammation. (P Arnaud, J. Bienvenu, P. Laurent . Editors), Vol 2,497-501. Walter de Gruyter. (1984).
45. J. Bienvenu , P. Deshaires, H. Bernon, et al : Ann. Biol. Clin. 46. 343-346. (1988).
46. S. J. Hopkins , A. Meager. Clin. Exp Immunol. 73. 88-92. (1988).
47. J. A. Eastgate , N G.,Wood, F. S. Gigiovine, et al : Lancet. ii. 706-709. (1988).
48. A .J. G. Swaak, A. Van Rooyen, E. Nieuweenhuis, et al : Scand. J. Rheumatol 17. 469. (1988)
49. P. Damas, A. Reuter, P. Gysen, et al : Crit. Care Med. 17. 975-978. (1989).
50. C. P. J. Maury, A. M. Teppo. J Exp. Med. 166. 1132-1137. (1987).
51. M. H .J. Van OersVan Der, A . Heyden , L. A. Aarden. Clin Exp. Immunol. 71. 314-319. (1988).