TWO PATIENTS PRESENTING WITH RAYNAUD'S PHENOMENON & THE DEMONSTRATION OF CRYOGLOBULINAEMIA

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Various tests in pathological conditions make use of an abnormal precipitation of proteins. Hence Bence Jones proteins which precipitate from heated urine are relevant in multiple myeloma. Precipitation of monoclonal macroglobulin forms the basis of the SIA Test. However most of these techniques have been replaced by electrophoretic and immunological methods. There remains a heterogenous group of conditions characterized by the presence of cryoglobulin where the demonstration of limited solubility remains a cornerstone in diagnosis.

The cryoglobulin precipitation tests was first described over 50 years ago[1]; it remains an essential investigation in the recognition of cryoglobulinaemia and is also useful in the detection of immune complexes in general.

Two patients both presenting with Raynaud's phenomenon in whom pathological quantities of cyroglobulin were present will be briefly discussed.

Case I

A 54 year old gentleman presented with a two month history of severe acrocyanosis and gangrene of four fingertips (three on right hand, one on left hand). He also complained of severe pain and tenderness over the middle and terminal phalanges. He was a Type I diabetic since 14 years of age. Apart from his presenting complaint, physical examination was unremarkable and peripheral pulses were normal. Relevant investigations showed:

Blood count and differential	— *Normal*
ESR	— *15 mm/hr*
Serum Creatinine	— *166 umol/l (80-124)*
Creatinine Clearance	— *25 ml/min*
ANF (latex aggl.)	— *Negative*
RA test	— *Positive 1:320 (N 1:20)*
Bence Jones Proteins	— *Negative*
S.P.E.: Alb	— *40 g/l (30-45)*
Gamma globulin	— *26.1 g/l (7-13)*
Immunoelectrophoresis	
	IgG 2800 mg/dl (710-1540)
	IgA 681 mg/dl (60-490)
	IgM 270 mg/dl (37-204)
B.M. aspirate	— *Normal findings*
CXR	— *Normal*
Abdominal C/T Scan	— *Normal*
Cryoglobulin	— *96 mg/dl*
Immunoelectrophoresis of purified cryoglobulins	— *IgM & IgG*

Case II

A 21 year old lady presented with a 6 month history of acrocyanosis involving the digits of both hands and feet. The only other relevant point on examination was the presence of livido reticularis of both lower limbs. There was nothing else of relevance in her history.

Investigations:

Blood count and differential	— *Normal*
Serum creatinine, U&E	— *Normal*
ESR	— *5 mm/hr*
ANF (latex aggl.)	— *Negative*
RA	— *Negative*
Cryoglobulin	— *36 mg/dl*

Detection and Analysis of Cryoglobulin

It is important to adhere strictly to an optimal technique in detection of cryoglobulins. This is because the temperature at which cryoglobulins begin to precipitate from serum varies considerably and may be as high as 35°C in some patients.

The syringe and blood container should be preheated to 37°C. Blood is collected and kept at 37°C for 4-6 hours, allowing a clot to form and retract. The serum is then collected in a graduated pipette and stored at 4°C for a period of seven days. If cryoglobulins are present, floccula are seen to develop and a deposit may form at the bottom. The next steps involve quantitative and qualitative analysis of the cryoglobulins. The pipette is centrifuged and the precipitate washed, redissolved and reprecipitated four times in saline at 37°C and 4°C respectively. The precipitate is then finally dissolved in acetate buffer at pH 4 at room temperature. Failure to detect a cryoglobulin (false negative result) may be due to loss of the protein due to adsorption on erythrocytes membranes if clotting of blood occurs below 37°C. Cryoglobulin may also become adsorbed to serum lipids and remain suspended as floccules. (Normal subjects are occasionally observed to have cryoglobulins in the range of 80-100 ug/ml, but such trace amounts are not apparent on gross inspection).

The extent to which a purified cryoglobulin is further analysed immunochemically depends on what information is sought and on what facilities are available. For routine characterization, electrophoresis and immunoelectrophoresis using antisera to whole human serum, to γ, μ and α chains and to κ and λ chains will suffice. This will enable cryoglobulins to be classified into single components or mixed types.

Classification of Cryoglobulins

Brouet et al, have developed a practical scheme for classifying cryoglobulins. This is based on immunochemical characterization of immunoglobulins as monoclonal or polyclonal.

TYPE I: Type I cryoglobulins are composed of monoclonal immunoglobulin usually IGM or IgG, rarely IgA. Usually the immunoglobulin is present in the serum in high concentrations (more than 5 mg/ml). These patients often have

multiple myeloma, Waldenstrom's macroglobulinaemia and other lymphoproliferative diseases.

TYPE II: This type of cyroglobulinaemia consists of a monoclonal component which has Rheumatoid Factor Activity (usually IgM) and a polyclonal IgG which behaves as an antigen for the IgM rheumatoid factor. This type also occurs in lymphoproliferative and autoimmune diseases but is characteristic of Essential Mixed Cryoglobulinaemia.

TYPE III: This is the most common type and consists of a polyclonal immunoglobulin (usually IgM) with rheumatoid factor activity and a polyclonal immunoglobulin (usually IgG) which again behaves as the antigen for the rheumatoid factor. Type III cryoglobulinaemia is associated with autoimmune disorders and persistent infection e.g. bacterial endocarditis. Unlike Type I and Type II, the amount of cryoglobulin is usually low (less than 1 mg/ml).

Clinical Correlations of Cryoglobulinaemia

The most common clinical features are related to cutaneous manifestations, usually vascular purpura and Raynaud's phenomenon. Acrocyanosis occurs only in a minority of patients.

In a review of available literature the following manifestations were found in decreasing frequency: arthritic pains, evidence of glomerulonephritis, neurological disorders (usually peripheral neuropathy), coagulation abnormalities (predominantly haemorrhage) and unexplained abdominal pain (Ref. Table 1).

Comments

Digital gangrene and cold or exercise-induced purpura have been closely associated with cold insoluble immunoglobulin, especially mixed cryoglobulins with antiglobulin activity. However, the 'in vivo' role of cold agglutination and precipitation as regards pathogenesis of the illness has not been confirmed. It is also possible that vascular injury and cryoprecipitation are both manifestations of circulating immune complexes and are coincidentally found together.

The management of cryoglobulinaemia obviously lies with this cause. In mixed essential cryoglobulinaemia (which is a classical example of an immune complex disease), plasmapheresis is the treatment of choice.

Concluding, it is worth stressing that demonstration of proteins with inherently limited solubility remains a valuable diagnostic measure in these syndromes as well as a useful guide to prognosis and therapy.

TABLE 1.
Review of literature (296 patients) with the incidence of signs and symptoms and indicating symptomatology of the two patients presented.

Manifestations	*Incidence*	*Patient 1*	*Patient 2*
Cutaneous	86%	x	x
Arthritis	34%		
Nephritis	36%	x	
Neurological	20%	x	
Haemorrhage/Thrombosis	5%		
Abdominal pain	2%		

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