CRYOGLOBULINAEMIA

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CRYOGLOBULINS

- Cryoglobulins are plasma proteins that precipitate at low temperature (0 to 4 degree Celsius) and dissolve at higher temperature
- They were first described in 1933 by wintrobe and Buell * (Bull John Hopkins Hosp 1933; 52:156-65)
- In 1962, Lospaluto et al. showed that cryoglobulins contains more than one immunoglobulin * (Am J Med 1962;32:142-7)

CLASSIFICATION*(Brouet et al.'s)*

- TYPE I:- Single Monoclonal Immunoglobulins
  - Type I CGB consists of a single monoclonal Ig, usually IgG or IgM, infrequently IgA, and very rarely monoclonal light chain protein
- TYPE II :- Mixed Monoclonal/ polyclonal IG
  - Type II CGB are mixed CGB consist of two or more IG of different classes. One component of the complex is a monoclonal protein(with a high proportion being IgM), that has rheumatology factor activity, in association with polyclonal IG component
- TYPE III :- Mixed polyclonal IG
  - Type III CGB are also mixed CGB, consisting of two or more IG of different classes, however, each component is a polyclonal IG

CRYOGLOBULIN TYPES & ASSOCIATED DISEASES

Types of CGB	Immunochemical composition	Associated diseases	Comments
Type I : **Single Monoclonal IG**	• IgG • IgA • Monoclonal L-Chain • IgM	B-cell dyscrasis: • Myeloma • W.Macroglobulinemia • CLL • Angioimmunoblastic • Lymphadenopathy • Hairy Cell Leukaemia	Cryoglobulin concentration is usually **HIGH** (> 5mg/ml)

CRYOGLOBULIN TYPES & ASSOCIATED DISEASES

Type of CGB	Immunochemical composition	Associated Diseases	Comments
Type II: **Mixed Monoclonal IG**	IgM-IgG IgG-IgG IgA-IgG	B- Cell dyscasias: ● Myeloma ● W. Macroglobulinaemia ● CLL ● Mixed essential CGB ● HCV	● Circulating immune complexes ● CGB con. Usually(5mg/ml) ● Mainly associated with monoclonal paraproteins

CRYOGLOBULIN TYPES & ASSOCIATED DISEASES

Type of CGB	Immunochemical composition	Associated Diseases	Comments
Type III : **Mixed Polyclonal IG**	• IgM-IgG • IgM-IgG-IgA	Autoimmune disease: • Rheumatoid arthritis • SLE • Sjogren's syndrome • hepatitis • Vasculitis	• Indicative of circulating immune complexes in response to Ig challenge in Rheumatoid disease and chronic infections • CGB conc. is usually < 1mg/ml (< 1%)

CLINICAL ASSOCIATION

- Type I cryoglobulinemia are more likely to be symtomatic and are usually associated with acrocyanosis, retinal haemorrhage, Raynaud's phenomenon, and arterial thrombosis
- High levels of type I cryoglobulins are associated with symptoms of hyperviscosity
- Mixed CG, are less commonly symptomatic and are usually associated with vascular purpura and Arthritis/ Arthalgia
- This is secondary to deposition within tissue (immune complexes) which activate complement and induce localized inflammation

CLINICAL ASSOCIATION

	TYPE I	TYPE II
Symptomatology	Common	Uncommon
Raynaud's	Common	Uncommon
Gangrene	Common	Uncommon
Purpura	Uncommon	Common
Arthralgia	Uncommon	Common
Nephritis	Uncommon	Common

CLINICAL PRESENTATION

- There is considerable overlap, the majority of type III CGB are present at low conc., with a cryocrit < 1%.
- Type I and type II CGB are often present at a higher conc., with a cryocrit usually > 1% , and occasionally up to 20- 40%
- The **commonest symptoms** of cryoglobulinemia are skin lesions, which are found upto 80% of patients
- Arthralgia and/ or arthritis (35 %)
- Glomerulonephritis producing nephrotic syndrome and / or hypertension
- Neurological symptoms (17%), including stroke, polyneuropathy, and mononeuritis multiplex

INCIDENCE OF SIGN AND SYMPTOMS IN PATIENTS WITH CRYOGLOBULINEMIA(adapted from Brouet et al)

CLINICAL MANIFESTATIONS	INCIDENCE (%)
Cutaneous	80%
Vascular purpura	60%
Raynaud's phenomenon	50%
Arthritis/ Arthralgia	35%
Nephritis	17%
Neurologic	14%
Distal necrosis	10%
Urticaria/ livedo/ haemorrhage/ acrocyanosis	4%
Leg ulcers/ arterial thrombosis/ abdominal pain	

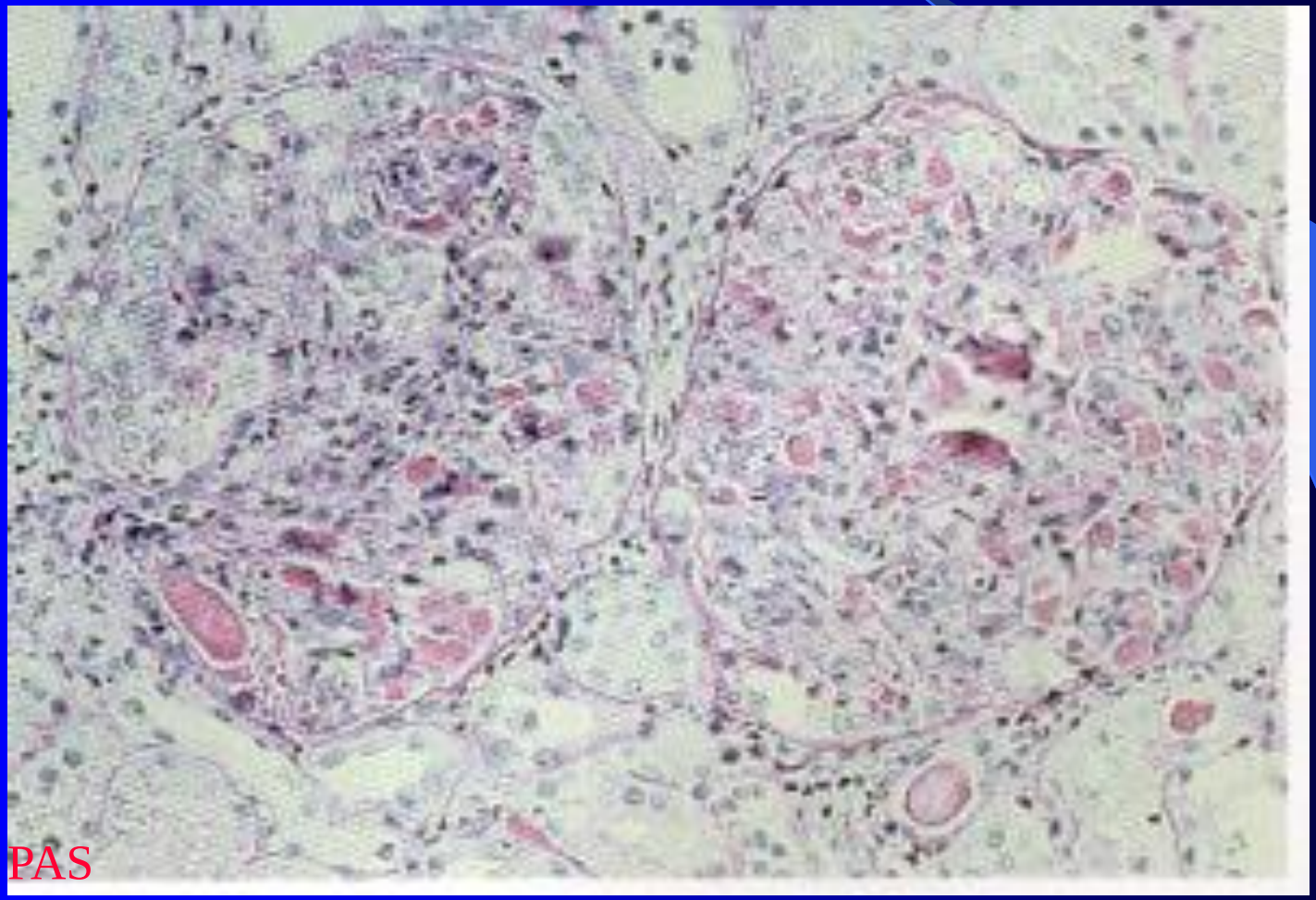
Classification of cryoglobulins

TYPE	FREQUENCY (%)	% AT CONC OF :		
		< 1 mg/ml	1-5 mg/ ml	>5 mg/ml
Simple (type1) • IgG, IgM, or IgA • Ig light chain	5-38	10	30	60
Mixed •Monoclonal (type11)	14-72	20	40	40
•Polyclonal (type 111)	23-54	80	20	0

DISEASE, CLINICAL, AND LABORATORY ASSOCIATION

TYPE	ASSOCIATIONS		
	DISEASE	CLINICAL	LABORATORY
1	Macroglobulinemia, myeloma	Necrosis, Raynaud' phenomenon, acrocyanosis	M-spike, increased viscosity, cryocrystals
11	HCV infection, CLL, Lymphoma, Sjogrens syndrome, macroblobulinemia	Purpura, neuropathy, keratoconjunctivitis	RF, nephritis, hepatitis, decrease C4
111	Chronic infections, autoimmune disease	Vasculitis	RF, nephritis

CRYOGLOBULINAEMIA GLOMERULONEPHRITIS

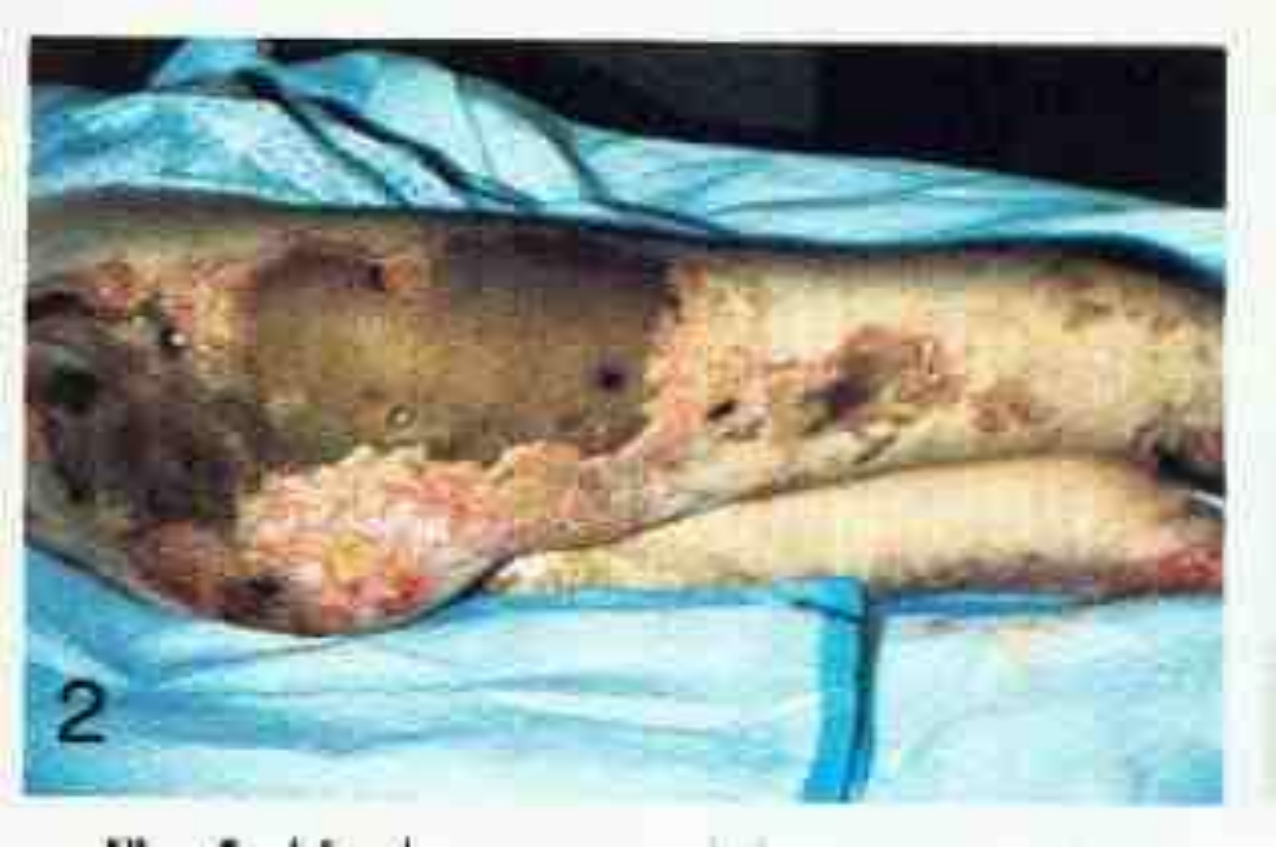


PAS

iple myeloma and type I cryo-

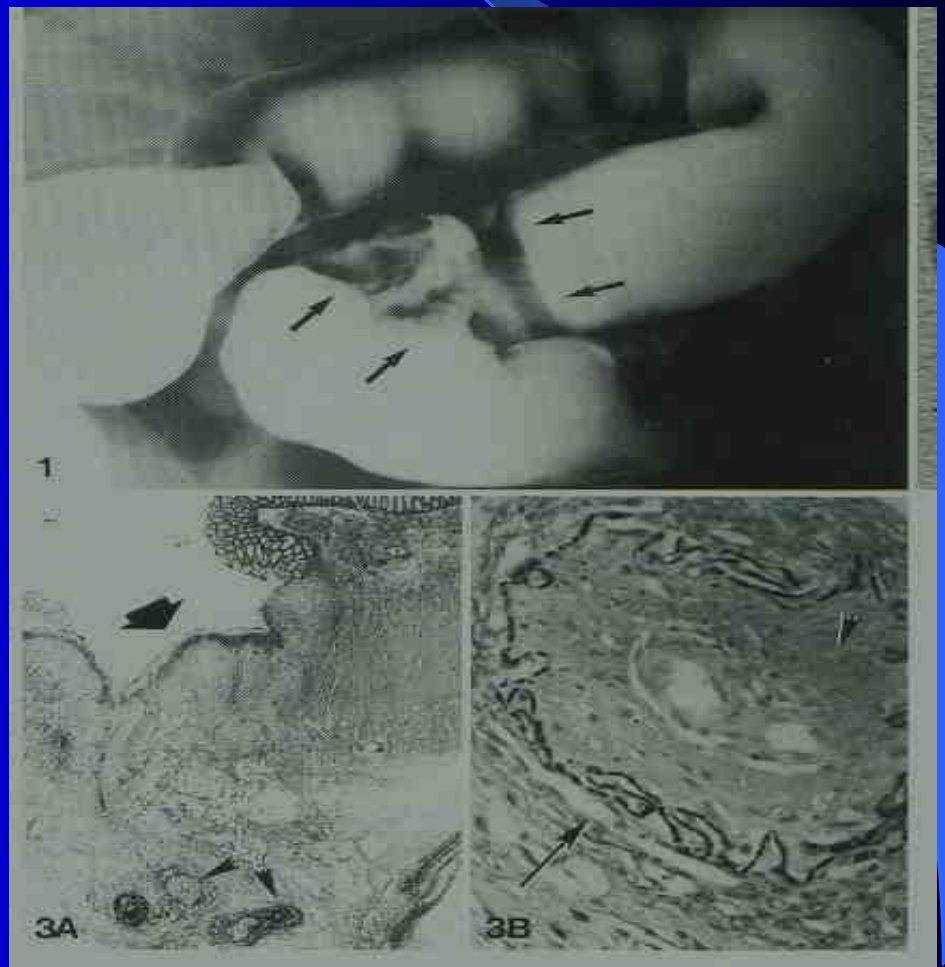
Reticulated purpuric to bronze pigmentry change in patient with type I CG

Massive cutaneous infarction in patient with M. Myeloma (type I)



The diagnosis of CG is often first suggested by the cutaneous manifestation

Involvement of GIT (up to 20%) with EMC



Gastrointest radiol 1988;13:160-2

40 year- old Italian lady present with palpable purpura on her leg

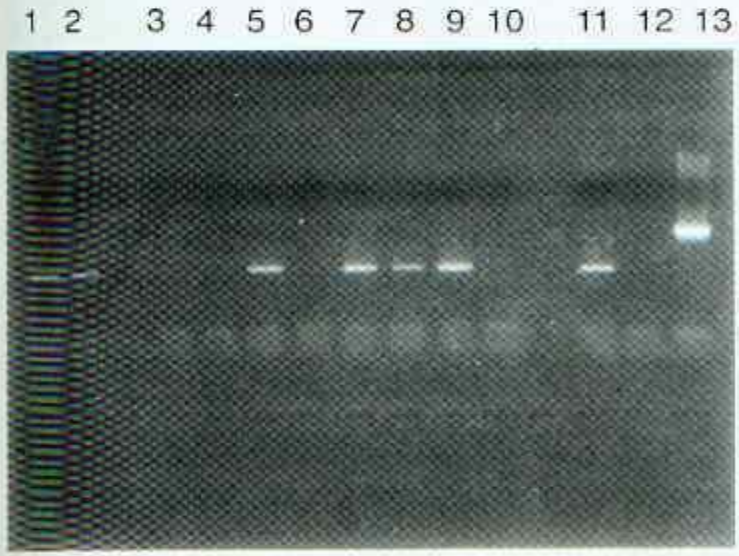
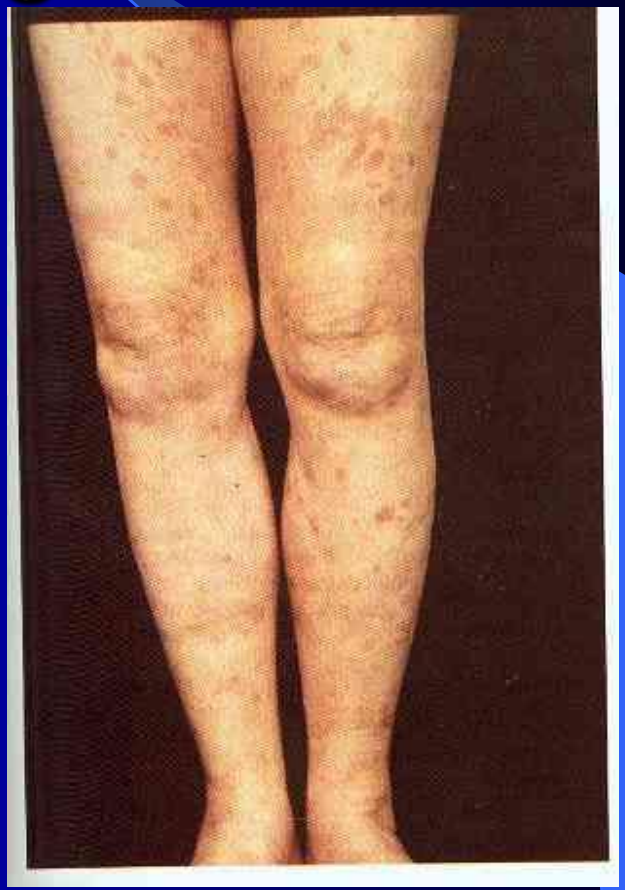


Figure 2. The results of RT–PCR for HCV-RNA in the serum and in the cryoprecipitate. No 1: serum of our patient; no. 2: cryoprecipitate of our patient; nos 3–10: serum of other hepatitis patients; no 11: serum from a patient known to have HCV-RNA; no 12: serum from a patient known not to have HCV-RNA; no 13: marker. HCV-RNA was detected in both the serum and the cryoprecipitate in our patient.



The results of RT-PCR for HCV-RNA in serum and cryoprecipitate

HEPATITIS C VIRUS-ASSOCIATED CRYOGLOBULINEMIA

- When no underlying disease is present, this is referred to as essential mixed cryoglobulinaemia (EMC)
- In EMC, the possible role of hepatotropic viruses has been suggested by the high frequency of co-existing liver abnormalities
- The prevalence of anti-hepatitis C virus (HCV) antibodies and the correlation with clinical and serological parameters of EMC have been investigated

HEPATITIS – ASSOCIATED CRYOGLOBULINEMAI

- Chronic hepatitis C virus (HCV) infection is frequently associated with a variety of autoimmune phenomenon
- Mixed cryoglobulinemia (MC) appears in up to 50% of chronic HCV- infected patients
- This vasculities (*caused by the deposition immunocomplexes in small vessels*) is thought to cause clinical symptoms called Meltzer's triad (purpura, arthralgia and weakness)

MECHANISM OF VASCULITIS

- The striking association between HCV infection and MC has conducted to the hypothesis that HCV is of major importance in the production of MC with followed vasculitis
- Both hepatrophism and lymphotrophism have been reported for the hepatitis C virus

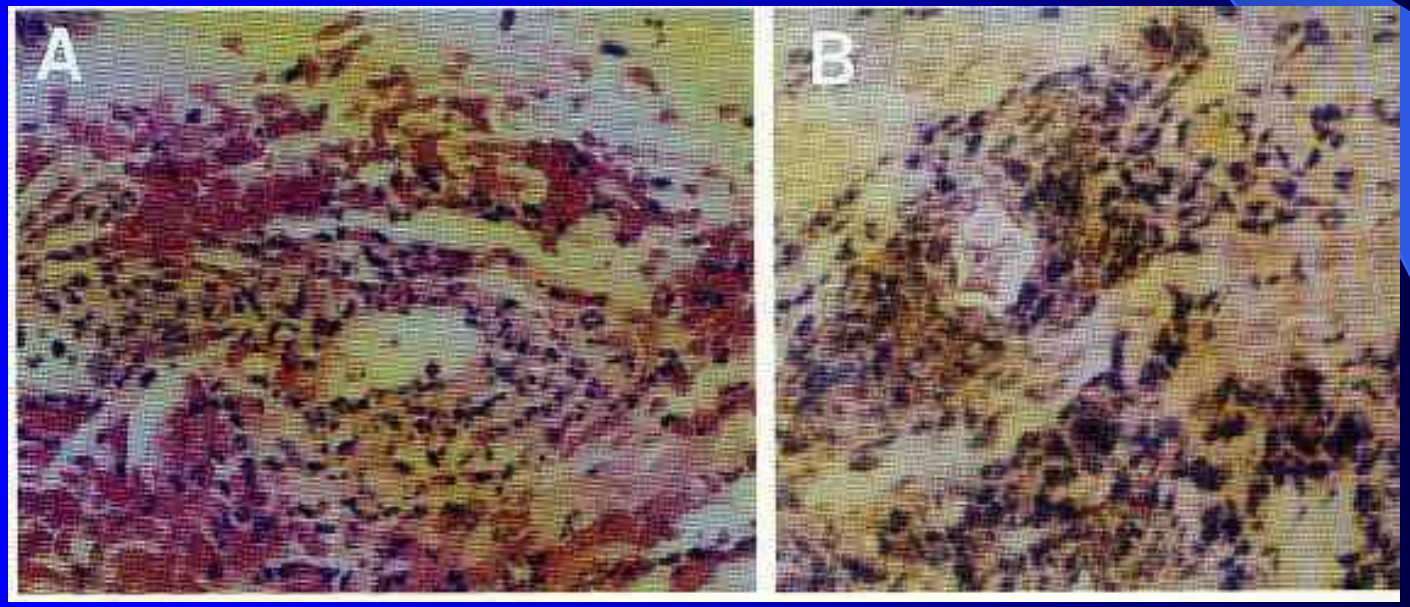
MECHANISM OF VASCULITIS

- Infection of B- cells by HCV could probably lead to a bcl-2 translocation and immunoglobulin gene rearrangement
- This could result in clonal lymphoproliferation and in synthesis of monoclonal IgM with rheumatoid factor activity
- These IgM form immunocomplexes with IgG in the clod, which are finally responsible for the vasculitis

Localization of HCV in cutaneous vasculitis lesion, in patient with Type II CG

Several distinct patterns of **HCV** staining were present

Serial sectioning of skin Bx specimen from a HCV-positive patient with MCG



A: Shows the classical leukocytoclastic vasculitis (H& E)

B:Virion form of HCV (brown staining)using the antisense riboprobe

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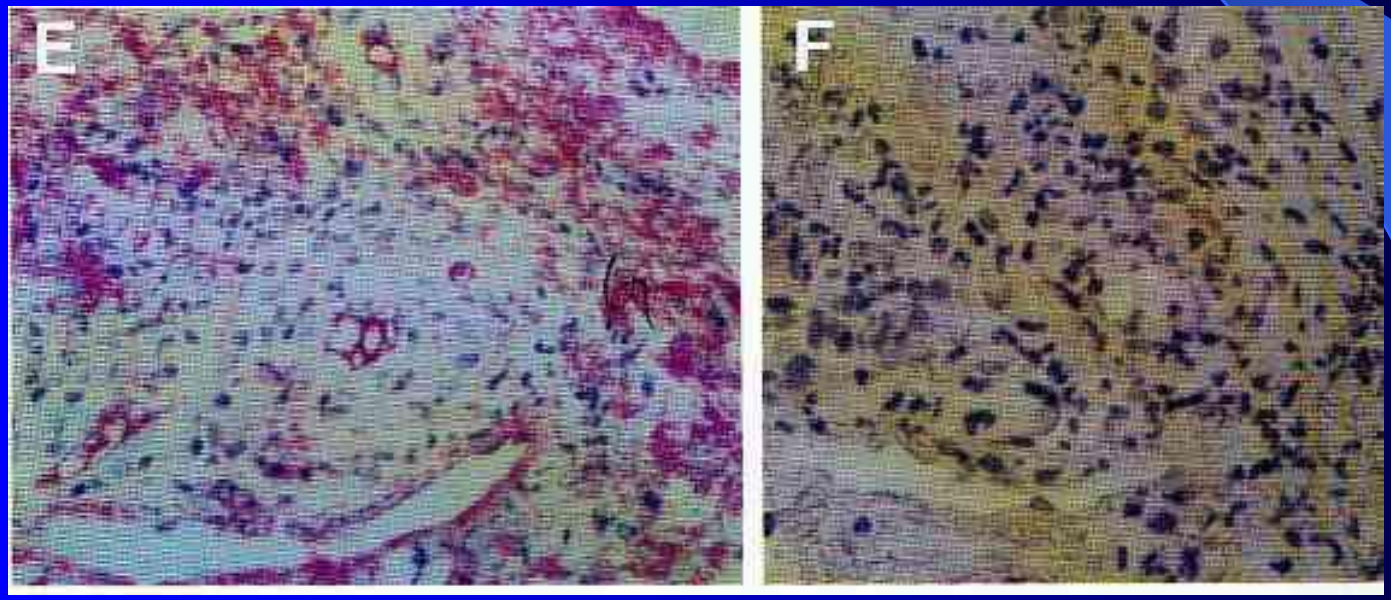


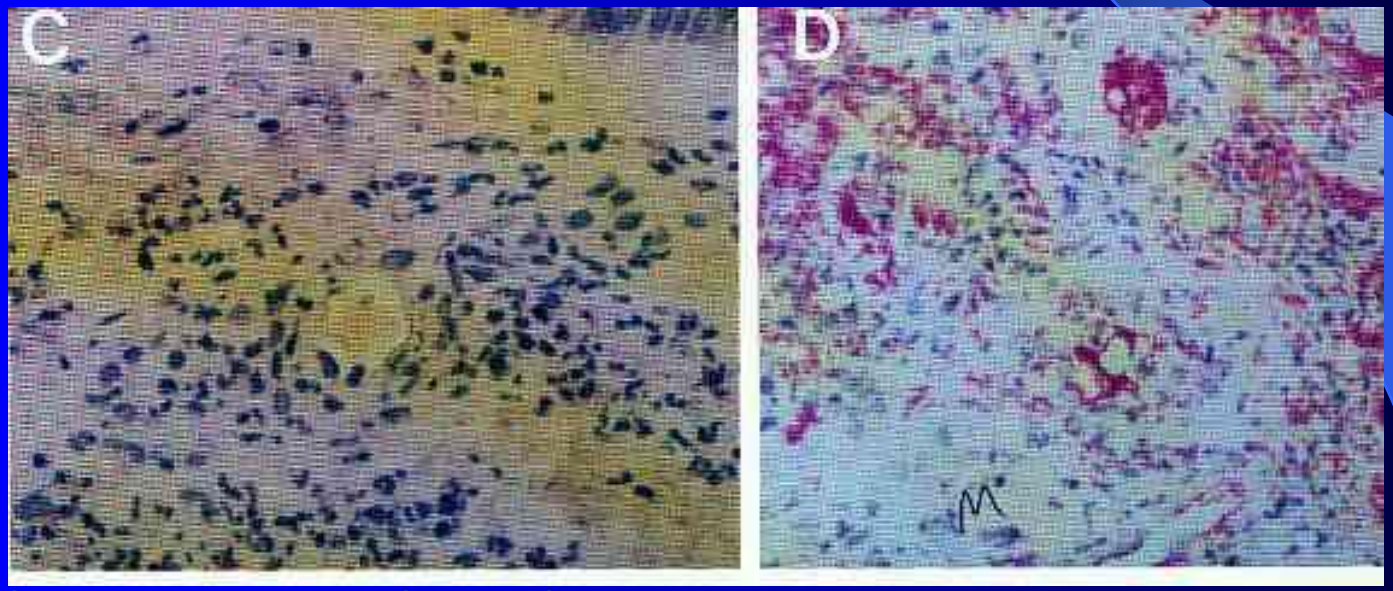
E: IgG (red staining) was detected in a similar distributation as that of IgM

F: Same methodology as above (D/E) but using anti-human IgA (No IgA was detected)

Localization of HCV in cutaneous vasculitis lesion, in patient with Type II CG

Several distinct patterns of **HCV** staining were present

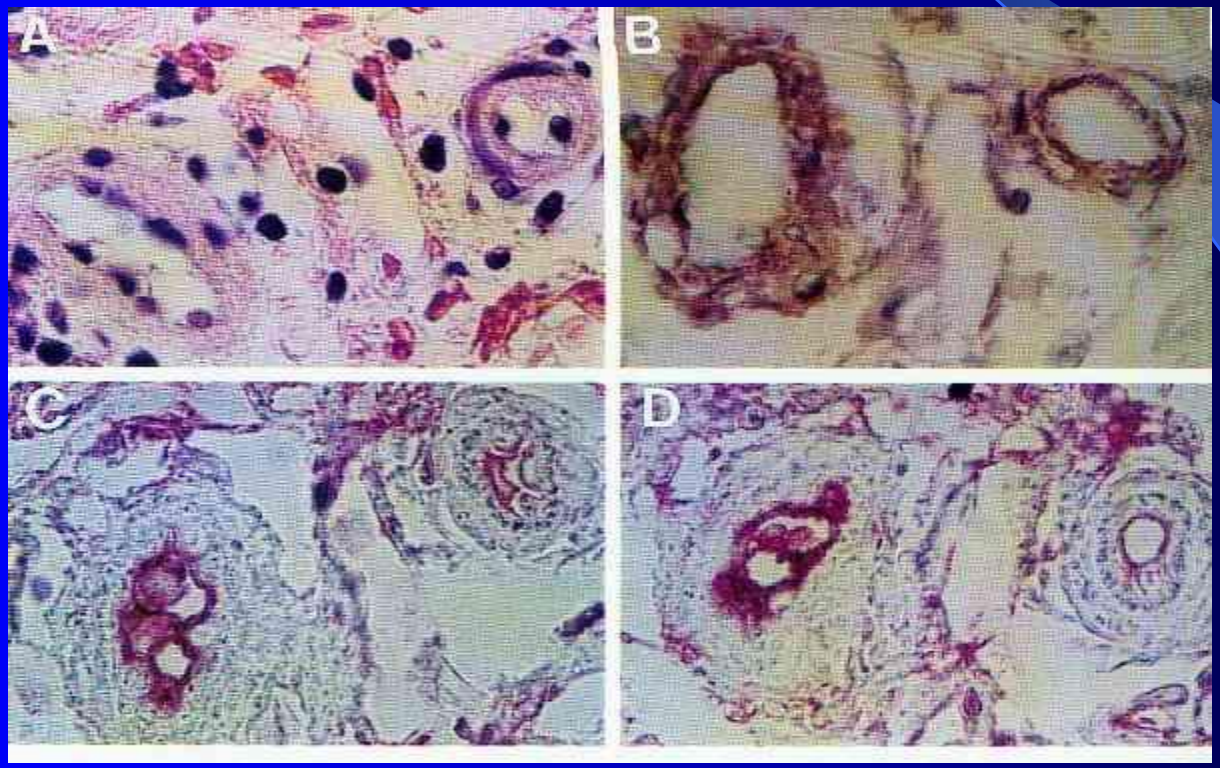
Serial sections of a skin BX from a BCV-positive patient with MCG



C: staining for the replicative form of **HCV** (using the sense probe) no replicative form

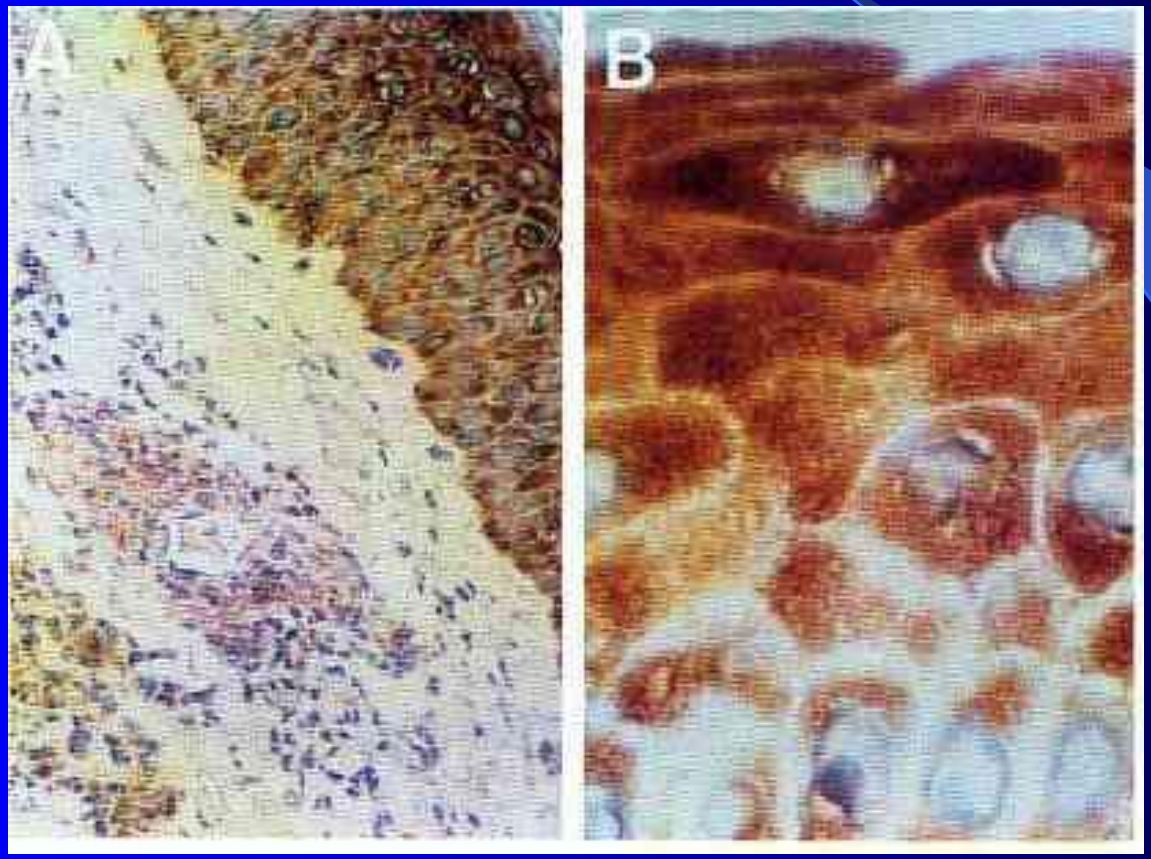
D: Demonstration of IgM (red staining) in the extra and intravascular deposits

Serial sections of a Bx specimen of a perivascular lymphocytic infiltration



A: shows vessel edema but no infiltrate B: Virion form of **HCV** , in the wall of the vessel
C: IgM, intravascular wall but not in the vessel wall C: IgG, similar to IgM

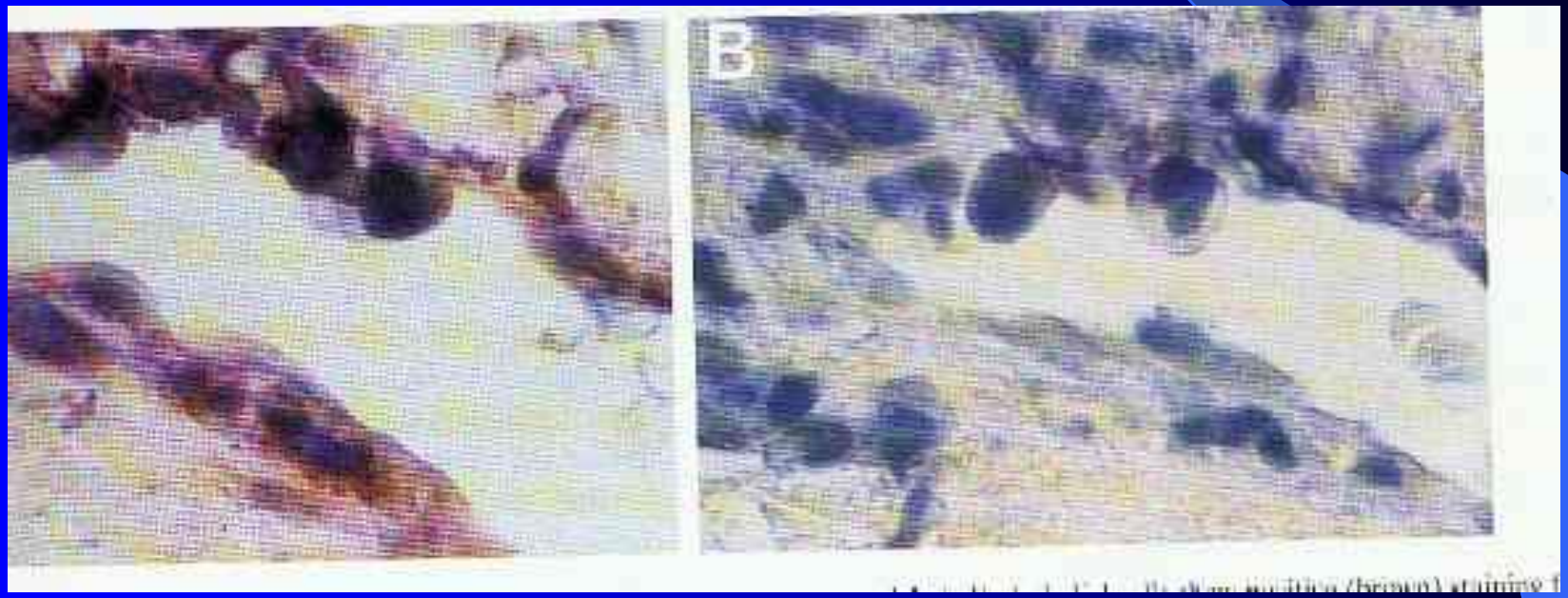
Demonstrating the virion form of HCV in the keatinocytes



A: Perivascular deposits

B: stronger staining of the suprabasal keratinocytes

Endothelial cells shows positive staining for the virion form RNA of **HCV** in situ hybridization



SPECIMEN REQUIRED

- **COLLECT :- Whole blood must be drawn in pre-warm (37oC) syringe and kept at 37oC.**
- Immediately after blood has been obtained, transfer sample to a pre-warmed (37oC).
- Plain red- top vacutainer and keep sample at 37oC until clotting is complete (min: 6 mL)
- **TRANSPORT :-** 3mL serum at 20- 25oC
- **PEDIATRIC COLLECTION/ TRANSPORT :-** 1 mL serum at 20-25oC

SPECIMEN REQUIRED (con't.)

- **REMARKS :-**
  - Let clot for one hour at 37oC . Separate serum from cells using a 37oC centrifugate, if possible
  - Fasting samples recommended.
  - Donot refrigerate or freeze at any time
- **UNACCEPTABLE :-**
  - Refrigerated or frozen samples
  - Samples collected in serum separated tubes
- **REFERANCE INTERVAL :-**
  - Negative at 72 hours
- **STABILITY :-**
  - Ambient 7 days

COLLECTION PROCEDURE

- Collect 6-10 mL of blood in a warm syringe (37oC)
- Fill a specific designed glass cryoglobulin tube that has been warmed to 37oC with a minimum volume of 6mL of blood
- The tube must remain at 37oC until the blood clots
- The serum is separated from the clot at 4oC.

ANALYSIS PROCEDURE

- A white precipitate (cryoglobulin) appears in the serum after 24-72 hours of storage at 37oC
- The cryoglobulins can be quatitated by:

  Measuring the cryoglobulin protein spectrophotometrically by absorbance at 280mm
- Component of cryoglobulins can be determine by:
  - Immunoelectrophoresis
  - Isoelectric focusing in asociation with immunofaxition

FALSE NEGATIVE RESULTS

- Anticoagulant tubes are used for specimen collection
- The syringe is not warmed to 37oC
- The sample is not kept at 37oC until clotting is completed
- The sample is centrifuge at temperature below 37oC
- The sample is not stored at 4oC for 72 hours

THERAPEUTIC MODALITIES

- Subcutaneous interferon alpha (IFN) at the dose of 3MU thrice weekly for 6 or 12 months, with or without plasma exchange (PE) schedule as follows:
  - 60 ml/kg bodyweight was removed and replaced with gelatin or human albumin three times/week for 2 weeks
  - Two times/ for 2 weeks
  - One time/week for 3 weeks
- Corticosteriods (with visceral manifestations)
- Care must be given to ensure replacement fluids are warmed before infusion

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MAY 1996

Leg ulcer related to cryoglobulinemia (left). Precipitated cryoglobulins in collection bag after plasmapheresis (middle). Ulcer site 7 months later (right). See page 498.

REFRENCES

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