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 contain 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
<table>
<thead>
<tr>
<th>Types of CGB</th>
<th>Immunochemical composition</th>
<th>Associated diseases</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type I</strong>: Single Monoclonal IG</td>
<td>IgG</td>
<td><strong>B-cell dyscrasis:</strong></td>
<td><strong>Cryoglobulin concentration is usually HIGH (&gt; 5mg/ml)</strong></td>
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<tr>
<td></td>
<td>IgA</td>
<td>• Myeloma</td>
<td></td>
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<tr>
<td></td>
<td>Monoclonal L-Chain</td>
<td>• W.Macroglobulinemia</td>
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<tr>
<td></td>
<td>IgM</td>
<td>• CLL</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Angioimmunoblastic</td>
<td></td>
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<td></td>
<td></td>
<td>• Lymphadenopathy</td>
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<td>• Hairy Cell Leukaemia</td>
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<tr>
<td>Type of CGB</td>
<td>Immunochemical composition</td>
<td>Associated Diseases</td>
<td>Comments</td>
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<tr>
<td>Type II: Mixed Monoclonal Ig</td>
<td></td>
<td></td>
<td>Circulating immune complexes</td>
</tr>
<tr>
<td></td>
<td>IgM-IgG</td>
<td></td>
<td>CGB con.</td>
</tr>
<tr>
<td></td>
<td>IgG-IgG</td>
<td></td>
<td>Usually(5mg/ml)</td>
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<tr>
<td></td>
<td>IgA-IgG</td>
<td>B- Cell dyscasias:</td>
<td>Mainly associated with monoclonal paraproteins</td>
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<tr>
<td></td>
<td></td>
<td>• Myeloma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• W. Macroglobulinaemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CLL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mixed essential CGB</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• HCV</td>
<td></td>
</tr>
<tr>
<td>Type of CGB</td>
<td>Immunochemical composition</td>
<td>Associated Diseases</td>
<td>Comments</td>
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</tbody>
</table>
| 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 symptomatic 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

<table>
<thead>
<tr>
<th>Symptomatology</th>
<th>TYPE I</th>
<th>TYPE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud’s</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Gangrene</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Purpura</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Nephritis</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
</tbody>
</table>
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 up to 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)

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>Incidence (%)</th>
</tr>
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<tbody>
<tr>
<td>Cutaneous</td>
<td>80%</td>
</tr>
<tr>
<td>Vascular purpura</td>
<td>60%</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>50%</td>
</tr>
<tr>
<td>Arthritis/Arthralgia</td>
<td>35%</td>
</tr>
<tr>
<td>Nephritis</td>
<td>17%</td>
</tr>
<tr>
<td>Neurologic</td>
<td>14%</td>
</tr>
<tr>
<td>Distal necrosis</td>
<td>10%</td>
</tr>
<tr>
<td>Urticaria/livedo/haemorrhage/acrocyanosis</td>
<td>4%</td>
</tr>
<tr>
<td>Leg ulcers/arterial thrombosis/abdominal pain</td>
<td></td>
</tr>
</tbody>
</table>
### Classification of cryoglobulins

<table>
<thead>
<tr>
<th>TYPE</th>
<th>FREQUENCY (%)</th>
<th>% AT CONC OF:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 1 mg/ml</td>
</tr>
<tr>
<td>Simple (type 1)</td>
<td>5-38</td>
<td>10</td>
</tr>
<tr>
<td>- IgG, IgM, or IgA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Ig light chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>14-72</td>
<td>20</td>
</tr>
<tr>
<td>- Monoclonal (type 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Polyclonal (type 111)</td>
<td>23-54</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYPE</td>
<td>DISEASE</td>
<td>CLINICAL</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>111</td>
<td>Chronic infections, autoimmune disease</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>11</td>
<td>HCV infection, CLL, Lymphoma, Sjogrens syndrome, macroglobulinemia</td>
<td>Purpura, neuropathy, keratoconjunctivitis</td>
</tr>
<tr>
<td>1</td>
<td>Macroglobulinemia, myeloma</td>
<td>Necrosis, Raynaud’ phenomenon, acrocyanosis</td>
</tr>
</tbody>
</table>
CRYOGLOBULINAEMIA
GLOMERULONEPHRITIS

PAS
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 manifestations of the disease.
Involvement of GIT (up to 20%) with EMC

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

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 CRYOGLOBULINEMIA

- 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.
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
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

E: IgG (red staining) was detected in a similar distribution 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 keratinocytes

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 (37°C)
- Fill a specific designed glass cryoglobulin tube that has been warmed to 37°C with a minimum volume of 6mL of blood
- The tube must remain at 37°C until the blood clots
- The serum is separated from the clot at 4°C.
ANALYSIS PROCEDURE

- A white precipitate (cryoglobulin) appears in the serum after 24-72 hours of storage at 37°C
- The cryoglobulins can be quantitated by:
  - Measuring the cryoglobulin protein spectrophotometrically by absorbance at 280nm
- Component of cryoglobulins can be determined by:
  - Immunoelectrophoresis
  - Isoelectric focusing in association with immunofaxitation
FALSE NEGATIVE RESULTS

- Anticoagulant tubes are used for specimen collection
- The syringe is not warmed to 37°C
- The sample is not kept at 37°C until clotting is completed
- The sample is centrifuged at temperature below 37°C
- The sample is not stored at 4°C for 72 hours
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
Leg ulcer related to cryoglobulinemia (left). Precipitated cryoglobulins in collection bag after plasmapheresis (middle). Ulcer site 7 months later (right). See page 498.
REFERENCES

- V. Rieu, P. Cohen, Characteristic and outcome of 49 patients with symptomatic cryoglobulinaemia. *Rheumatology* 2002;41:290-300