Diagnosis and Treatment of Cryoglobulinemic Vasculitis

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Classification of Cryoglobulinemia

Type I
(10%)

B cell lymphoproliferative diseases

Type I
(10%)

IgG
IgM

Monoclonal Ig
(IgM > IgG > IgA)

Type II
(65%)

Chronic infections (HCV)
B cell lymphoproliferative diseases
Autoimmune diseases
Essential, mixed cryoglobulinaemia

Type II
(65%)

Monoclonal Ig (IgMx)
+ polyclonal Ig

Type III
(25%)

Polyclonal IgM
+ polyclonal IgG

Type I/II Mixed Cryoglobulinemia

AGENDA

- Cryoglobulinemic glomerulonephritis
- Pathogenesis, presentation and prognosis
- Anti-viral therapy
- Standard immunosuppression
- The impact of B cell depletion therapy
- International therapeutic guidelines
Prevalence of cryoglobulinaemia in patients with chronic HCV infection

about 40% of patients with chronic hepatitis C have asymptomatic circulating cryoglobulins

Ramos Casals M, Lancet 2012
A cryoglobulinemic syndrome develops in less than 5% of cases, and nephritis in 0.5% of HCV infected persons.

Genetic background probably important.

Extrarenal signs of MC vasculitis usually precede the kidney manifestations, but in a minority of cases kidney manifestations appear first.

Biopsy mandatory when kidney involvement is suspected.

PAS 20x: membrano-proliferative pattern with endocapillary proliferation and deposits in capillary lumina
Granular IgM and IgG + C3 subendothelial space and mesangium
Monocyte/macrophages are often associated with deposits, mainly in subendothelial location, interposed between GBM and endothelial lining.
Microtubular 10-25 nm, and annular structures and rings 30 nm wide are pathognomonic
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Pathogenesis

Putative target for therapy

Demographic distribution of MC nephritic patients

<table>
<thead>
<tr>
<th></th>
<th>#</th>
<th>F</th>
<th>M</th>
<th>Onset age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephritis pts</td>
<td>146</td>
<td>83</td>
<td>63</td>
<td>52.2 ±13</td>
</tr>
<tr>
<td>HCV+ve non-nephritis pts</td>
<td>34</td>
<td>20</td>
<td>14</td>
<td>56.7±9.6</td>
</tr>
</tbody>
</table>

Roccatello & Fornasieri on behalf of the Italian group of immunopathology AJKD, 2007
Roccatello & Fornasieri on behalf of the Italian group of immunopathology AJKD, 2007
EXTRARENAL SYMPTOMS

**Clinical**
- Recent purpura
- Dyscromic lesions

**Serological**
- Skin involvement
- Renal involvement
- Peripheral neuropathy
- Liver involvement
- Arthralgias
- Weakness
- Purpura

**Pathological**
- Leukocytoclastic vasculitis
- B cell expansion
- Mixed cryoglobulins
- RF+
- Low C4

**Mixed cryoglobulinaemia vasculitis**

**Serological**
- Mixed cryoglobulins
- RF+
- Low C4

**Clinical**
- Purpura
- Weakness
- Arthralgias
- Liver involvement
- Renal involvement

**Serum MCs alone**
- Possible preclinical condition with or without RF and/or low C4
- Careful clinical evaluation of possible underlying infectious (HCV or HBV) autoimmune and/or haematological and/or neoplastic disease
- Monitoring without treatment

PROGNOSTIC FACTORS
Leukocytoclastic vasculitis as a histopathological hallmark

Nat. Rev. Dis. Primers doi.org/10.1038/s41572-018-0009
AGENDA

- Cryoglobulininemic glomerulonephritis
- Pathogenesis, presentation and prognosis
- **Anti-viral therapy**
- Standard immunosuppression
- The impact of B cell depletion therapy
- International therapeutic guidelines
The new drugs target the 3 non structural proteins:
- NS3 serine protease and its cofactor NS4A
- NS5A
- NS5B RNA polymerase
Advances in HCV and cryoglobulinemic vasculitis in the era of DAAs: are we at end of the road?

...several studies have shown positive effects of DAA therapy in pts with MPGN or other HCV related renal manifestations...

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>N</th>
<th>DAA regimens</th>
<th>RTX (n)</th>
<th>SVR (%)</th>
<th>Clinical response (%) at 12 week post-treatment</th>
<th>Complete cryoglobulin reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saadoun et al. (2016)</td>
<td>24</td>
<td>SOF/RBV × 24 week</td>
<td>4</td>
<td>74</td>
<td>87</td>
<td>46</td>
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<td>Sise et al. (2016)</td>
<td>12</td>
<td>SOF/SM (n = 8)</td>
<td>4</td>
<td>83</td>
<td>33</td>
<td>33 (n = 4/9)</td>
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<tr>
<td>Bonaccó et al. (2016)</td>
<td>35</td>
<td>3D (n = 10)</td>
<td>0</td>
<td>94</td>
<td>71</td>
<td>14</td>
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<tr>
<td>Gragnani et al. (2017)</td>
<td>17</td>
<td>3D (n = 5)</td>
<td>-</td>
<td>100</td>
<td>30 (week 8)</td>
<td>50 (week 8) 35 (week 8)</td>
</tr>
<tr>
<td>Gragnani et al. (2016)</td>
<td>44</td>
<td>SOF/RBV (n = 18)</td>
<td>2</td>
<td>100</td>
<td>66</td>
<td>27</td>
</tr>
<tr>
<td>Saadoun et al. (2017)</td>
<td>41</td>
<td>SOF/DAC (n = 32) × 12 week</td>
<td>2</td>
<td>100</td>
<td>90</td>
<td>10</td>
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<tr>
<td>Hegazy et al.</td>
<td>35</td>
<td>SOF/RBV (n = 13) × 24 week</td>
<td>-</td>
<td>100</td>
<td>84–100 for each symptom (EOT)</td>
<td>-</td>
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<tr>
<td>Emery et al. (2017)</td>
<td>18</td>
<td>DAAs ± IFN</td>
<td>3</td>
<td>89</td>
<td>39</td>
<td>22</td>
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<tr>
<td>Sollima et al. (2016)</td>
<td>7</td>
<td>3D (n = 2)</td>
<td>-</td>
<td>100</td>
<td>0</td>
<td>14</td>
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<tr>
<td>Tsuge et al. (2016)</td>
<td>1</td>
<td>DAC/ASU × 24 week</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
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<tr>
<td>Obata et al. (2017)</td>
<td>1</td>
<td>DAC/ASU × 24 week</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
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</tbody>
</table>
The new drugs target the 3 non structural proteins:
- NS3 serine protease and its cofactor NS4A
- NS5A
- NS5B RNA polymerase

American Association for the Study of Liver Diseases
European Association for the Study of the Liver
both recommend prioritizing DAA treatment in patients with symptomatic MC associated with HCV infection
Advances in HCV and cryoglobulinemic vasculitis in the era of DAAs: are we at end of the road?

...several studies have shown positive effects of DAA therapy in pts with MPGN or other HCV related renal manifestations...

Renal manifestations published in non-nephrologic Journals
- Gragnani, 4 pts *Hepatology*, 2016
- Sise, 7 pts *Hepatology*, 2016
- Bonacci, 7 pts *Clin Gastroenterol Hepatol*, 2017
- Obata, 1 pt *CEN Case Report*, 2017
- Tsuge, 1 pt *Hepatol Research*, 2016

*J Clin and Exp Hepathol*, 2017
Main clinical manifestations:

- Palpable purpura (73%),
- Arthralgia (59%),
- Weakness (77%),
- Peripheral neuropathy (63%),
- Raynaud's phenomenon (32%),
- Sicca syndrome (41%),
- Skin ulcers (14%)

Renal involvement (9%),
- 1 had a renal biopsy showing membranoproliferative GN
- 1 had a nephrotic syndrome
- 2 had reduced GFR
7/12 reduction in proteinuria/eGFR improvement

BUT

- Only 2 pts had a clear sCr improvement (1 given RTX and 1 with only a clinical diagnosis)
  - Changes were negligible in 4 pts,
  - sCr increased over time in the remaining pt.
  - The only pt who had NS received also RTX.
  - Another pt with proteinuria from 1,574 to 800 mg/gCr was concomitantly given ustekinumab.
  - Proteinuria decreased from 2 to 0.4 mg/gCr in 1 pt who did not undergo renal biopsy.
  - Of the remaining 4 pts, proteinuria was negative in 1, not determined in 1 and only determined by urinalysis (1 and 3+ in 2)

sCr (hollow squares) and proteinuria (dark circles, panels A,C,G) values obtained 3 months prior to direct-acting antiviral therapy initiation, through treatment period (gray) and through last follow-up visit. Patients 1–6 (panels A-F) achieved SVR12. Patient 7 (Panel G) relapsed four weeks after completing therapy.

Sise, Hepatology, 2016
Prospective international multicenter study on 148 patients with HCV-CryoVas

Cryoglobulinemia..........................132/148
Previous GS, RTX, PE, IS............. 62/148
Low C4........................................61/148
Renal involvement.......................25/148

As in all trials using DAAs in CV, a half of patients remained positive for cryoglobulins.

A severe form of CryoVas and peripheral neuropathy were associated with a lack of response of HCV-CryoVas to DAA.
Clinical case #1

55-year-old man

January 2015

- Proteinuria 4.5 g/day
- sCrs 1.7 mg/dl
- Microscopic haematuria ++
- C3: 75 mg/dl, C4: 5 mg/dl
- IgM 484 mg/dl

- HCV RNA: 539265 UI/ml
- Genotype 4
- Cryocrit 0.5%
- Cryoglobulins IgG and IgM polyclonal
- RF activity 122

Renal Biopsy
Light microscopy: mesangial proliferation and endocapillary hypercellularity with segmental distribution

IF: IgM +++ C3 ++, IgG ++, C1q ++, IgA + intramembranous/subendothelial
Treatment with *Ribavirin* and *Sofosbuvir* for six months (February 2015-August 2015)

At the end of the therapy (August 2015):

- HCV RNA negative
- sCr 2 mg/dl
- Proteinuria 3.4 g/day
- Microscopic haematuria

After 18 months (February 2017):

- HCV RNA negative
- sCr 1.9 mg/dl
- Proteinuria 5.7 g/day
- Microscopic haematuria
Immunofluorescence:
C3 +++; IgM ++; C4 + and C1q +
intramembranous/subendothelial
11/18 sclerotic glomeruli.
Fig. 2: Mechanisms of HCV-related cryoglobulinaemia vasculitis.

- Virus eradication does not imply that the immunological process has been stopped.
- Several patients continue to have B lymphocyte clonal expansion after SVR.
- Persistence of B-cell clone after the cure of HCV infection also promotes disease relapse.
- DAAs therapy in HCV-MC:
  - high virological response
  - immunological, hematological and clinical responses limited

AGENDA

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- Pathogenesis, presentation and prognosis
- Anti-viral therapy
- **Standard immunosuppression**
- The impact of B cell depletion therapy
- International therapeutic guidelines
<table>
<thead>
<tr>
<th>DAA schedule</th>
<th>0.5-1 mg /Kg/day</th>
<th>Treatment Duration</th>
<th>Daniel B. Fervenza et al., eds</th>
<th>Therapy of Mixed Cryoglobulinemia Dario Roccatello and Antonello Pani</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESCALAT. 1</strong></td>
<td><strong>CYC or MMF</strong></td>
<td>Tapering 2.5-5 mg/week for 3-4 weeks</td>
<td>3 pulses IV;</td>
<td></td>
</tr>
<tr>
<td><strong>ESCALAT. 2</strong></td>
<td><strong>Plasma exchange or double filtration:</strong> one plasma volume (50 ml/Kg) per session.</td>
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Survival curves in patients randomized to receive rituximab (RTX) therapy or conventional therapy (non-rituximab [non-RTX]), consisting of glucocorticoids, azathioprine, cyclophosphamide, or plasmapheresis.
BVAS and duration of remission in patients with HCV–associated MC vasculitis randomized to receive RTX or control therapy (maintenance or increased immunosuppressive therapy)

Sneller MC, ARTHRITIS & RHEUMATISM 2012
MC patients treated with Rituximab with a mean follow-up of 72.5 months

31 pts, 29 HCV-infected, mean age years 59.8 years; range, 35-78 years
intolerance to standard therapy 12
resistance to standard therapy 9
severe BM lymphocyte infiltration 5
front-line therapy 5

16 with severe renal involvement (diffuse MPGN or renal vasculitis)
29 peripheral neuropathy
9 large skin ulcers (necrotizing in 7)

Rituximab: 4 plus 2 protocol (Roccatello, NDT 2004): 375 mg/m² on days 1, 8, 15 and 22 with two more doses administered 1 and 2 months later.

Follow-up: 15-36 months 8 pts
36-60 months 6 pts
60-100 months 10 pts
>100 months 6 pts
1 pt lost from the follow-up after 60 months

Roccatello et al. Current Opinion in Rheumatology 2019
RTX dosage and CD19+ count

[Graph showing the comparison of RTX dosage and CD19+ count before and after each dose.]
Laboratory profiles of 16 patients with cryoglobulinemic nephritis undergoing a 4 plus 2 infusion protocol of RTX

Serum creatinine (mg/dl)  
Proteinuria (mg/24 hrs)

- Mean follow-up 54.3 (12-96) months.
- Re-inductions in 9 cases (after a mean of 31.1 months, range 12-54).
- Dose prednisone at the last observation:
  - 10 pts without maintenance treatment
  - 3 pts with 2.5 mg/d
  - 2 pts with 5 mg/d prednisone

HCV RNA serum load

P = 0.04
Cryoglobulinemic polyneuropathy in 26 pts: EMG changes after anti-CD 20 MoAb

Roccatello et al. Current Opinion in Rheumatology 2019

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>POST</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPE ampl</td>
<td>mV 1.11±1.25</td>
<td>1.53±1.49</td>
<td>0.047</td>
</tr>
<tr>
<td>SPE MCV</td>
<td>m/s 43.79±8.9</td>
<td>42.99±8.45</td>
<td>n.s.</td>
</tr>
<tr>
<td>SPE Lat</td>
<td>ms 4.07±1.65</td>
<td>4.28±1.56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sural ampl</td>
<td>( \mu )V 0.77</td>
<td>6.79</td>
<td>0.079</td>
</tr>
<tr>
<td>Sural SCV</td>
<td>m/s 36.87±1.12</td>
<td>49.32±6.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Paresthesia 26 pts to 4
Burning feet  in 7 to 2.
Muscle asthenia in 19 to 3
“4 plus 2” Rituximab protocol: effects of therapy
“4 plus 2” Rituximab protocol: duration of effects

After 6 years the survival rate was 75% and the probability of remaining symptom-free for 10 years without any therapy was 60% after a single “4 plus 2” infusion cycle,

Reinduction

9 pts after 31.1 months with likelihood of living symptom-free for 5 years after relapse was 80%

Roccatello Current Opinion in Rheumatol 2019
Causes of death in MC patients

- Cardiovascular
- Hepatic failure
- Sepsis

- Tarantino 1995: KI, #105 pts
- Roccatello 2007: AJKD, #156 pts
- Roccatello 2012: ASN #31 pts
The case #1 of the 55-year-old man with chronic hepatitic C and cryoglobulinemic nephritis

Post DAA biopsy

Post DAA lab data

HCV RNA neg
sCr 1.9 mg/dl
Proteinuria 5.7 g/day
Microscopic haematuria
Treatment with *Rituximab 375 mg/mq* every one week for four doses plus two more doses 375 mg/m2 after 1 and 2 months

- **Rituximab**
- **Ribavirin**
- **Sofosbuvir**

**HCV RNA negative**
**sCr 2 mg/dl**
**Proteinuria 1.4 g/day**
**Microscopic haematuria neg**
**C3: 105 mg/dl, C4: 16 mg/dl**

**Proteinuria (g/day)**
**sCr (mg/dl)**
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<th>Renal presentation</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Stable kidney function and/or nonnephrotic proteinuria</td>
<td>Direct-acting antiviral therapy</td>
</tr>
<tr>
<td>Cryoglobulinemic flare, nephrotic syndrome, or rapidly progressive kidney failure</td>
<td>Direct-acting antiviral therapy with immunosuppressive treatment, with or without plasma exchange</td>
</tr>
<tr>
<td>Histologically active HCV-associated glomerulonephritis that does not respond to direct-acting antiviral therapy</td>
<td>Rituximab as first-line immunosuppressive treatment</td>
</tr>
</tbody>
</table>
DAAs plus RTX: which sequence?

At the same time

**Advs:** lowering autoimmune response while lowering viral load (and no significant differences in virologic responses compared to the administration of DAA alone)

**Cons:** potential hematologic toxicity

Sequential **DAA before**
Mild/moderate arthralgia and purpura might benefit of anti-viral therapy alone

Sequential **RTX before (mandatory in MPGN)**
RTX could further increase the odds of achieving SVR by depleting B cells which are a potential reservoir for the virus

Consider the entity of viral load and clinical manifestations
CMID & Nephrology and Dialysis Division  
(ERK-net Member)  
Universitary Center of Research of Nephrology, Rheumatology,  
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Interregional Coordinating Center of the Network of Rare  
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- Laura Solfietti

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- Roberto Cavallo (Neurology)
- Osvaldo Giachino (Transfusion Medicine)
- Vittorio Modena (Rheumatology)
- Valerio Veglio (Infectious Diseases)

---

**Rheumatology team**
- Mirella Alpa
- Simone Baldovino
- Carla Naretto
- Daniela Rossi
NEXT WEBINAR

Enrico Vidal

“Non-infectious complications of peritoneal dialysis in children”.

OCTOBER 29, 4 PM