Follow-up del Paziente con Patologie Extraepatiche

Anna Linda Zignego

Università degli Studi di Firenze

Dipartimento di Medicina Sperimentale e Clinica-Sez. Medicina Interna

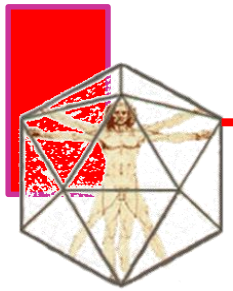
Centro Interdipartimentale di Epatologia MASVE

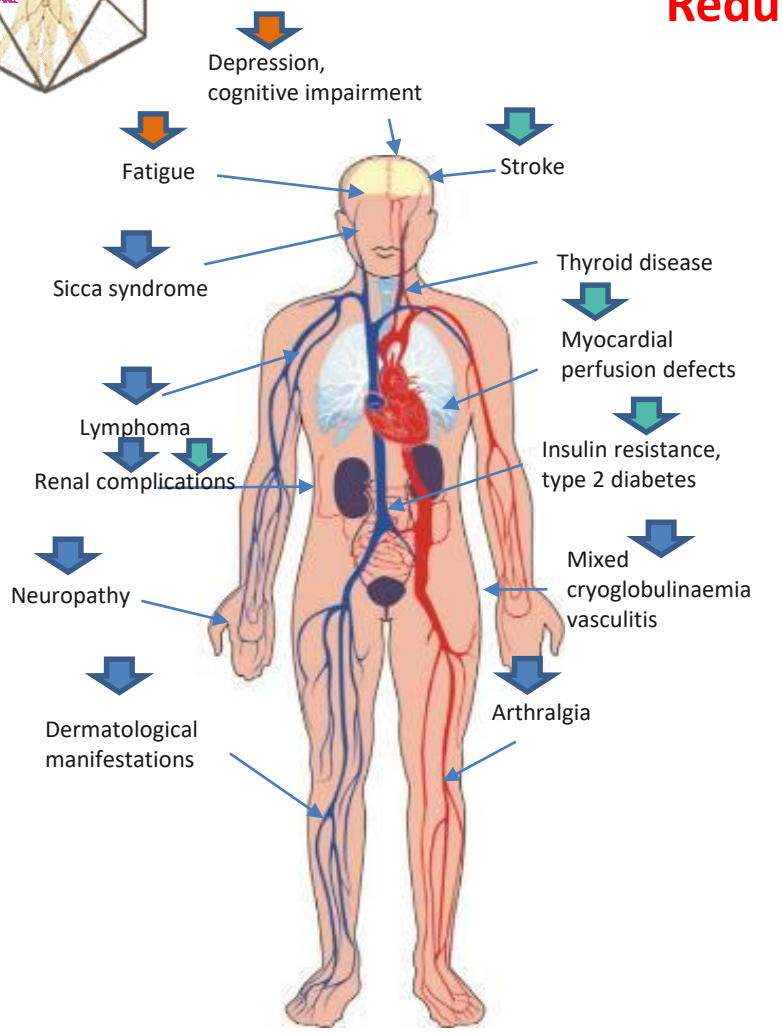
Centro di Ricerca ed Innovazione Aziendale CRIA-MASVE AOU Careggi

HCV DISEASE

a combination of Hepatic and Extrahepatic disorders with variable presentation

Extrahepatic benefits of HCV eradication: Summary





Reduced all-cause mortality

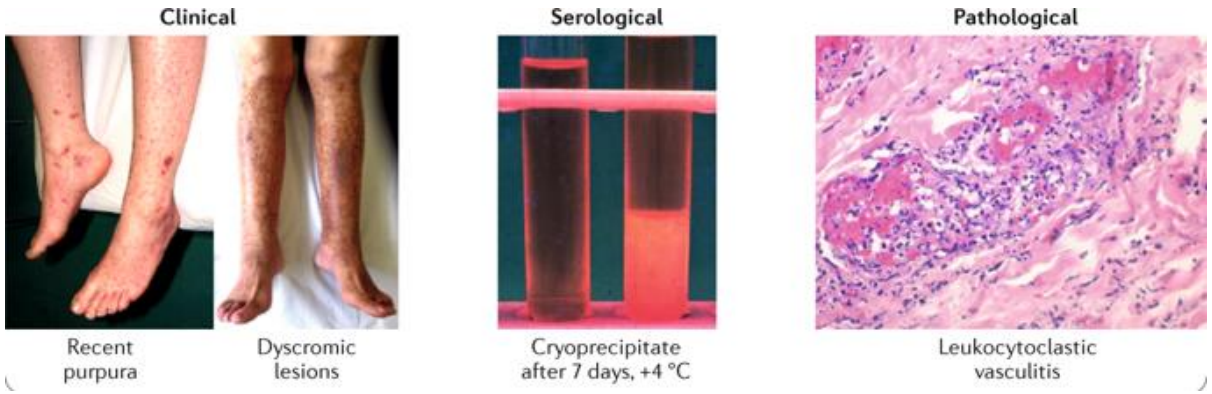
Autoimmune/B-cell LPDs=
- resolution of MC manifestations
- regression/remission of HCV-associated lymphoma

Cardiovascular diseases/Diabetes and insulin resistance=
- improvement in myocardial perfusion defects
- reduced incidence of stroke
- reduced renal and cardiovascular outcomes in diabetics
- reduced risk of insulin resistance
- reduced risk of type 2 diabetes

Cognitive and functional=
- improved cognitive performance
- reduction in fatigue
- gains in quality of life

Mixed Cryoglobulinemia-MC

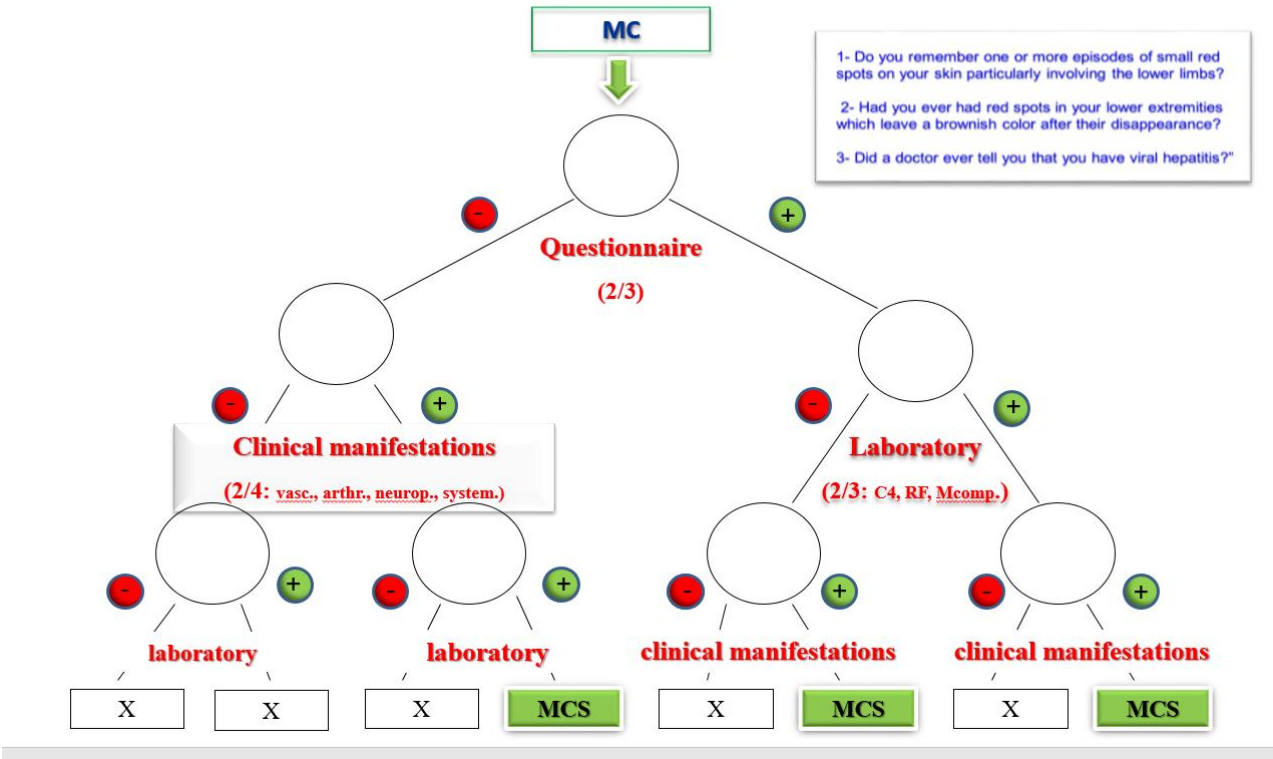
- MC is the most frequent HCV-EHD
- autoimmune/lymphoproliferative disorder more frequently observed in woman and in advanced age
- Cryoglobulins can cause systemic vasculitis in the small/medium-sized vessels affecting many organs and tissues
- Clinical manifestations of MC are due to the vasculitis: cryoglobulinemic vasculitis (CV) and MC syndrome (MCS) are generally used as synonyms
- Diagnosis of MCS/CV should be performed according to well defined criteria; among these, the presence of a combination of typical symptoms and laboratory markers including, first, low C4 and RF, is requested



Mixed cryoglobulinaemia vasculitis

Clinical	Serological
• Purpura	• Mixed cryoglobulins
• Weakness	• RF+
• Arthralgias	• Low C4
• Liver involvement	**Pathological**
• Renal involvement	• Leukocytoclastic vasculitis
• Skin involvement	• B cell expansion
• Peripheral neuropathy	

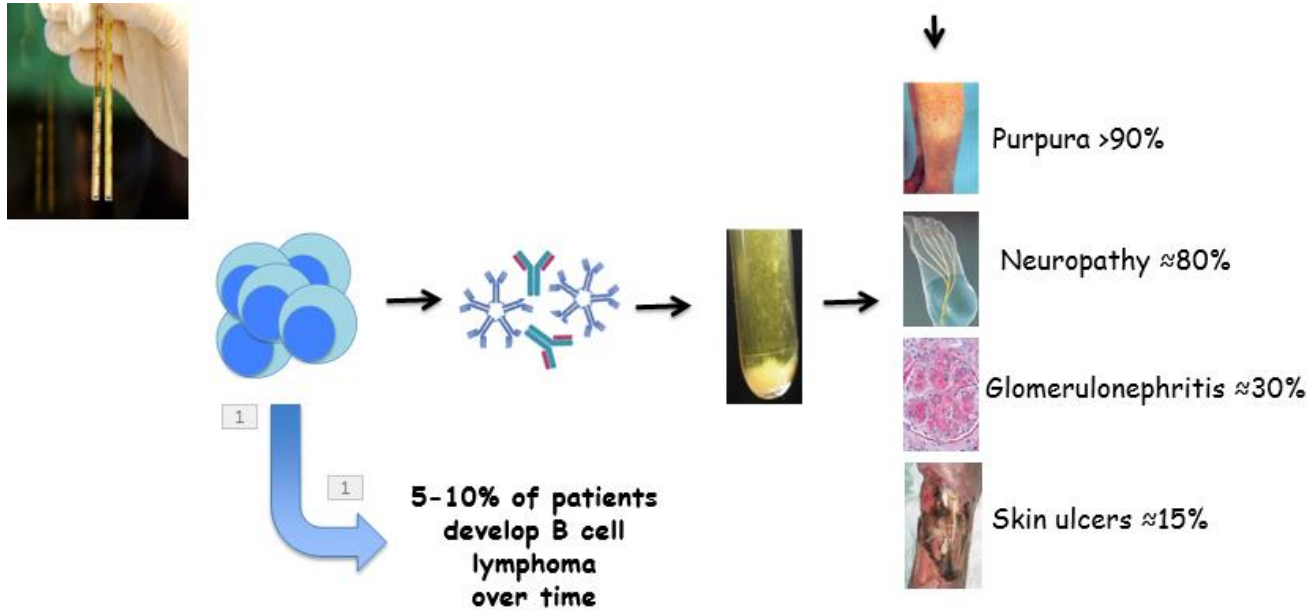
Schematic representation of the classification tree for the CV



De Vita et al, 2011

Mixed Cryoglobulinemia-MC

- Cryoglobulins are immune complexes that precipitate from serum under laboratory conditions of cold
- Types II and III cryoglobulinemia are termed “mixed” cryoglobulinemias, because they are comprised of both IgG and IgM components
- HCV induces monoclonal expansion of B cells producing RF that forms these cryoprecipitable immune complexes
- CV is caused by HCV in approximately 90% of the cases; HCV proteins/antigens contributing to CV
- 40-60% of HCV+ patients harbor cryoglobulins; 5-30% with MCS/CV manifestations



IFN-Free treatment in MCS

Authors, year	No. Patients	Treatment	SVR	Clinical (MC) Response	RC/RV
Sultanik et al, 2015	1	SOF+RBV then SOF+DAC	1 (100%)	n.a.	
Saadoun et al, 2015	24	SOF+RBV	74%	86.9%	1.17
Sise et al, 2016	12	4 SOF+RBV, 8 SOF+ SIM	83%	33.3% complete, 33.3% partial	0.8
Flemming et al, 2016	1	SOF+LDV+RBV	100%	n.a.	
	7	OMB/PAR/DAS+RTV SOF+RBV	100%	14.3%	0.14

Long-term Efficacy of IFN-Free Antiviral Regimens in Patients With HCV–Associated CV

Cacoub et al, Clin Gastroenterol Hepatol, 2019

CV Patients: 148 including 13 with NHL
Median follow-up time: 15.3 months
Primary end point: CV clinical response at week 12 after EOT

Complete response (improvement of all organs involved at baseline and the absence of clinical relapse): 106 patients (72.6%)
Partial response (improvement in some but not all organs involved at baseline): 33 patients (22.6%)
No response: 7 patients (4.8%)

>95% of patients with HCV-CV have a full or partial clinical response to DAA treatment, <5% stop therapy prematurely and <3% die
Disappearance of cryoglobulinemia: 53.1% including 97.2% achieving SVR

Factors associated with no or partial CV response:
severe form of CryoVas ($P = .03$)
peripheral neuropathy ($P = .02$)

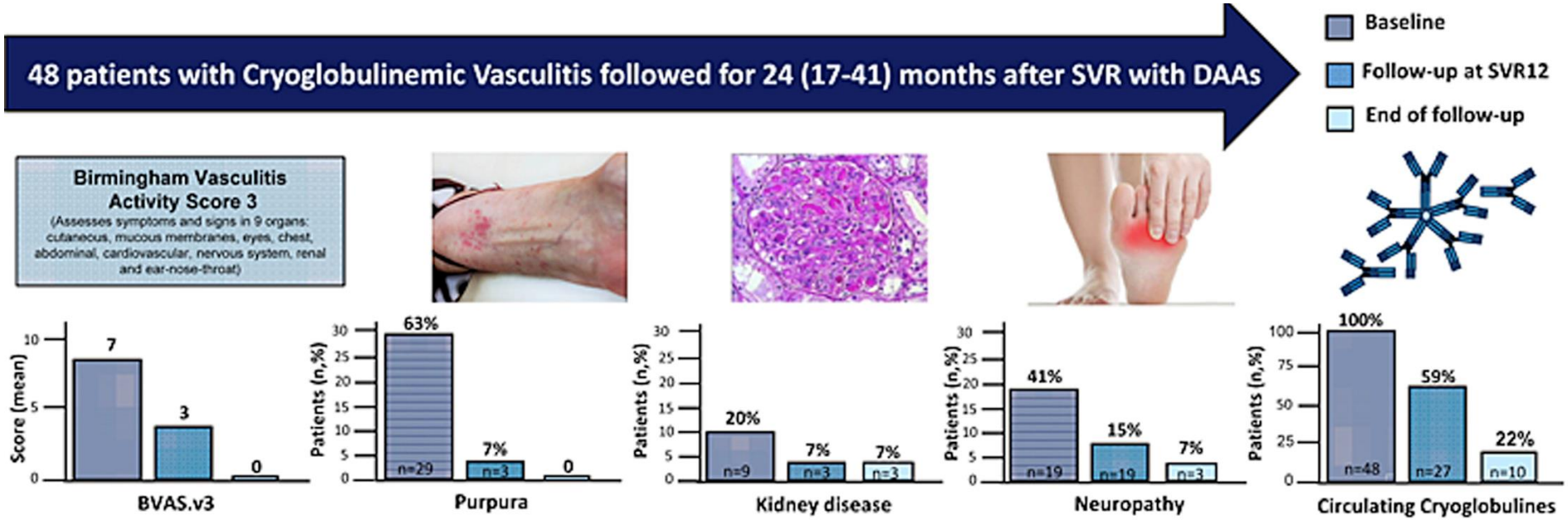
Long-Term Outcomes of Patients With HCV-Associated CV After Virologic Cure

Bonacci et al, Gastroenterology, 2018

Patients with HCV-CV have high rates of clinical remission after treatment with DAAs, but circulating cryoglobulins persist, and vascular disorders reappear in some patients after DAA treatment ends

Patients: 46 HCV CV and 42 HCV MC treated with DAAs
Follow-up; 24 months (range 17-41 months)

Immunological response: -in 66% HCV-CV and 70% in MC
-persisting in 20%
Clinical CV response: 91%
Clinical relapse: 5 (11%) HCV-CV (4/5 cirrhosis)



Persistence or Relapse of MC in SVR patients

RHEUMATOLOGY

Sollima et al, 2016

Retrospective analysis: in 1/7 MCS relapsed after SVR

RHEUMATOLOGY

Visentini et al, 2018

-4 patients had relapse of MCS 1.0, 5.0, 1.8, 2.5, and 13.0 years after SVR that were closely associated with respiratory infections or a new diagnosis of lung cancer

Patient	Age/sex at diagnosis	HCV GT	Antiviral therapy	Vasculitis at diagnosis	Cryocrit at diagnosis (%)	Year of relapse (after SVR)	Vasculitis at relapse	Cryocrit at relapse (%)	Disease concomitant to relapse
1	69/F	1b	SOF/LED	Kidney disease, skin ulcers, neuropathy	3	1.5	Kidney disease, skin ulcers	10	Upper respiratory tract infection
2	52/M	1b	SOF/SIM	Neuropathy, purpura, arthralgia	4	1.8	Neuropathy, purpura, kidney disease	1	Flu-like syndrome
3	82/F	1b	SOF/SIM	Neuropathy, purpura, skin ulcers, kidney disease	7	2.5	Purpura	2	Respiratory infection of unknown aetiology
4	38/F	1b	PEG-IFN/ RIBA	Neuropathy, purpura	3	13	Purpura	2	Unresectable lung adenocarcinoma

Clinical and Experimental RHEUMATOLOGY

Sollima et al, 2018

-1 patient had self-limited relapse of MCS after SVR in coincidence with influenza vaccination.

Antiviral Therapy in patients with MCS: PRELIMINARY RESULTS

PITER
Piattaforma Italiana per lo studio della Terapia delle Epatiti viRali.

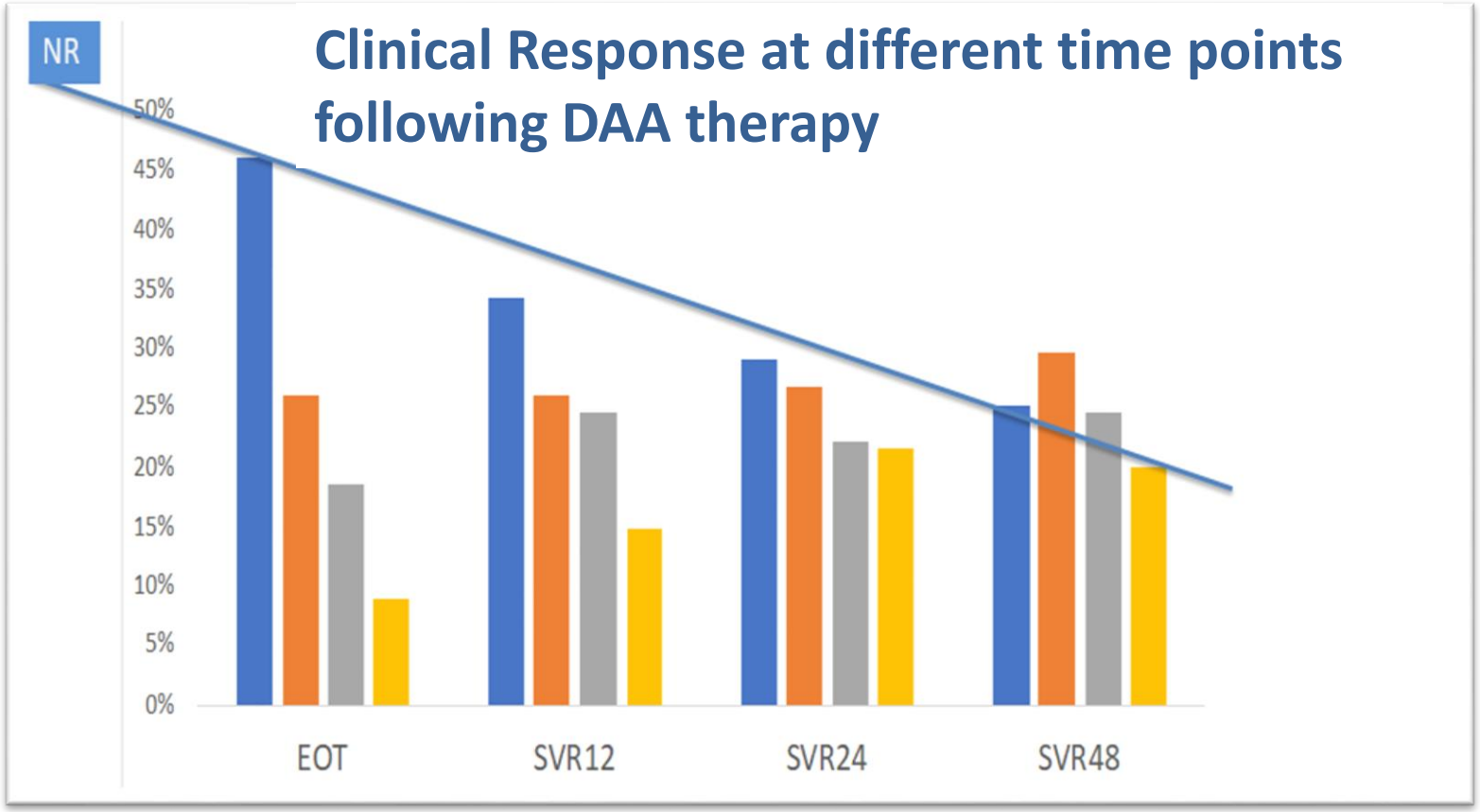
No. DAA-Treated MCS/CV patients with available data: 373
Virological Response: 97% and 96% in MC and MCS, respectively
Clinical Response: about 80% including FCR, CR, PR, NR

Full complete response (FCR)
Disappearance of all Symptoms
(*restitutio ad integrum*)

Complete response (CR)
Improvement of all Symptoms

Partial response (PR)
nprovement of more than 50% of symptoms

Non-response (NR)
Remaining conditions



Antiviral Therapy in patients with MCS: PRELIMINARY RESULTS

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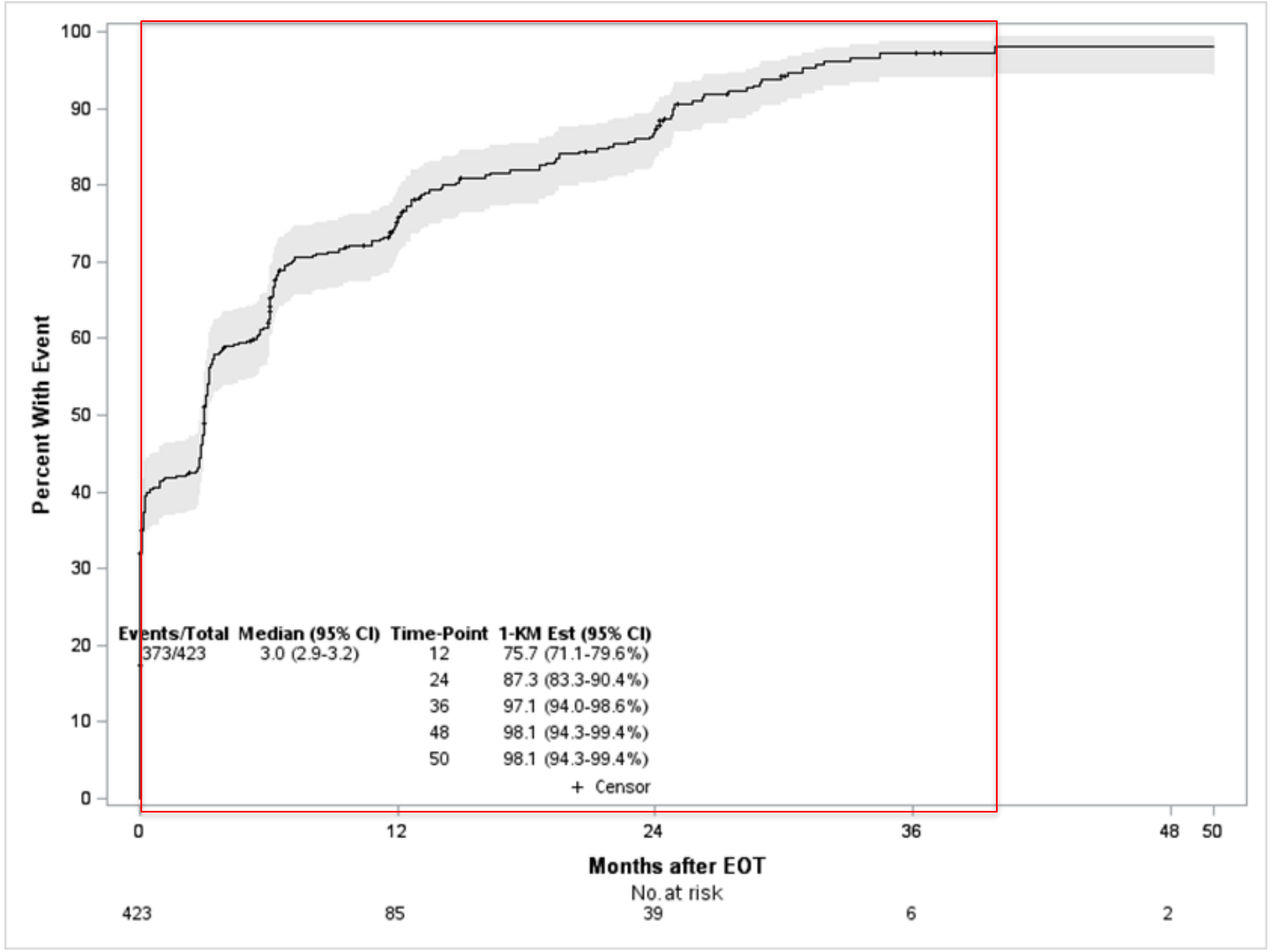
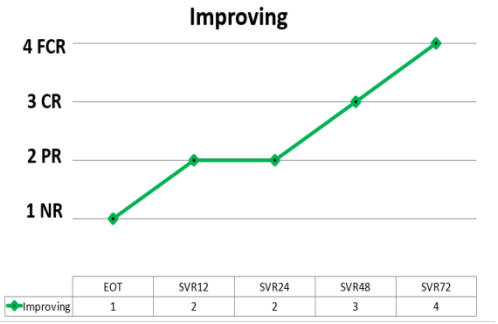
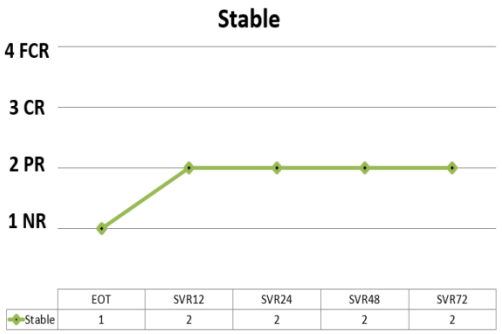
Piattaforma Italiana per lo studio della Terapia delle Epatiti viRali.

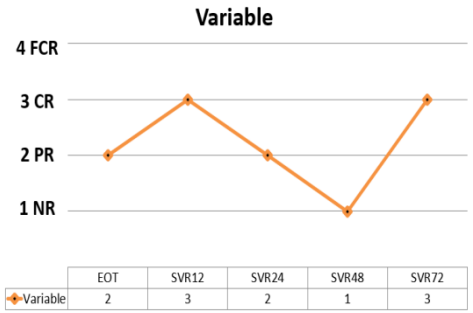
FIRST TIME OF CLINICAL RESPONSE

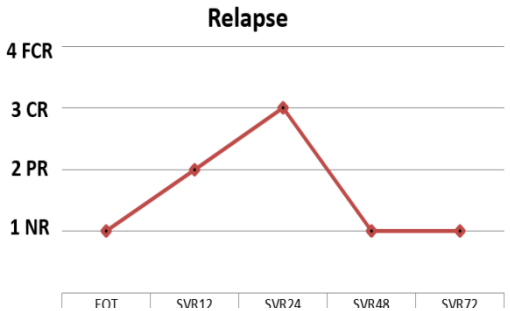


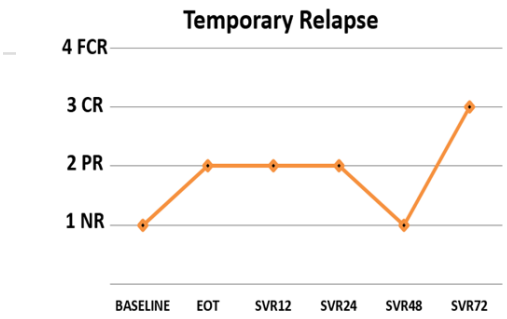
Post Treatment Symptoms in SVR patients= Observed patterns

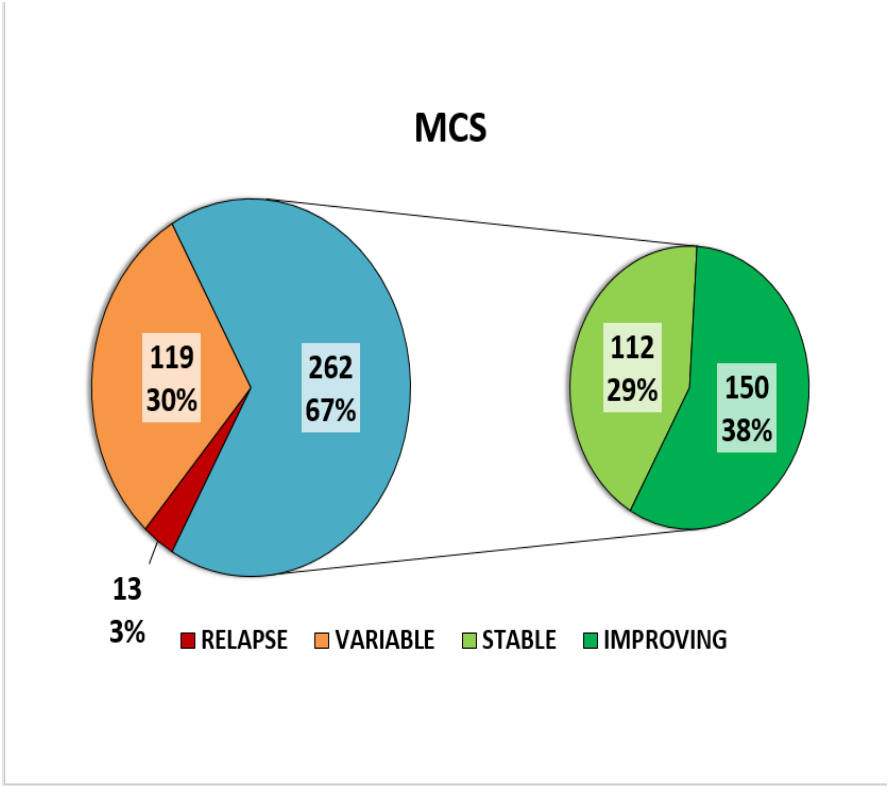


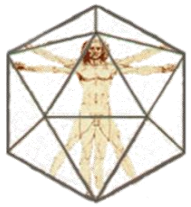












HCV-MC

after the euphoric phase of a simplified and very effective etiological treatment several problems remain complicating the clinical management of patients

Although the majority of CV patients obtained a long-lasting clinical response after viral eradication, some of them experienced a symptom persistence while others showed a CV relapse after a transient improvement/healing

The vasculitis symptoms recurrence has been closely associated to events such as infections or cancer onset and even to flu-vaccination. These conditions or other unknown/unnoticed events, characterized by immune system stimulation, might reactivate persistent pathological B-cell clones

- **It may be conceivable that IFN and/or RBV treatment is a better choice than IFN/RBV- free therapy due to their antiproliferative and/or immunomodulatory effects?**
- **Is it possible to use non invasive markers able to show the risk of persistence/relapse of MCS/CV after DAAs?**
- **Is it possible to design, for HCV-related LPDs too, a clinically useful flow-chart for a more accurate follow-up of HCV-MC patients after SVR?**

Liver INTERNATIONAL

Clinico-immunological outcomes of HCV-cured MC: lower relapse rate with IFN-based than IFN-free therapy. *Colantuono S et al, 2021*

Retrospective study:
70 CV SVR patients treated with DAAs vs 39 treated with IFN
follow-up duration: 30.5 vs 48 months

DAA-treated less efficiently reduced cryoglobulins (P = .006) and circulating B-cell clones (P = .004)
DAA-treated had more frequently relapses of MCV (18% vs 3%, P = .028) and need for RTX (P = .01)

Although largely inferior on an ITT basis, IFN may be superior to DAAs on clinico-immunological outcomes possibly owing to its antiproliferative activity.

IFN-free therapy in HCV MC: a prospective, controlled, clinical and quality of life analysis

Gragnani et al, 2021

Patients: 182 including 139 cryoglobulinemic (85 with CV, 54 without MC) vs 43 controls

SVR12 total: 166 (91.2%) patients (77/85 CV, 48/54 MC, 41/43 controls)

CV response in SVR: progressively improved, reaching 96.7%, 24 weeks after treatment

QoL scores: baseline physical and mental component summaries were significantly lower in the CV group compared to the other groups. DAAs-based therapy was effective in significantly increasing QoL, suggesting its important role in improving CV's individual and social burden

Role of RBV in the treatment of HCV-associated MC with IFN-free regimens

Boglione et al. Arch. Virol. 2018

AIM principale: Valutazione della cinetica del criocrito in 132 pazienti HCV+ sottoposti a trattamento con DAA +/- RBV

Pazienti: 132 HCV+ con MC (criocrito medio: 4.5%) :

Maschi: 100 (75.8%)

Età: 54 (48-64)

Consumo C4: 11 (8.3%)

Hb: 14.7% (13.4-15.3%)

eGFR: 99.1 (83-119.6)

CM sintomatica: 57 (43.2%)

Tipo di sintomi:

Porpora: 14 (10,6%)

Artralgie: 13 (9.8%)

Parestesie: 12 (9%)

Neurop. periferica: 8 (6%)

47 tot.

Table 1 Baseline characteristics of study population

Patients characteristics	$N = 132$
Gender: male, *n* (%)	100 (75.8)
Age (years)	54 [48–64]
Ethnic group, *n* (%)	
Italian	116 (87.9)
Egyptian	8 (6)
East-Europe	8 (6)
Years of HCV infection	14.6 [8.5–26.6]
Years of HCV infection > 20 years, *n* (%)	32 (24.2)
Risk factors for HCV, *n* (%)	
Unknown	57 (43.2)
Intravenous drug use	56 (42.4)
Sexual exposure	15 (11.4)
Trasfusional	4 (3)
BMI (kg/m^2)	25.2 [23–27.8]
HCV genotypes, *n* (%)	
1a	29 (22)
1b	47 (35.6)
2	10 (7.6)
3	34 (25.8)
4	12 (9)
Liver stiffness (kPa)	16.8 [12.1–33.5]
Fibrosis stage [Metavir score], *n* (%)	
F0–F2	8 (6)
F3	35 (26.5)
F4	89 (67.4)
Cryocrit (%)	4.5 [0.5–7.5]
Reduced C4, *n* (%)	11 (8.3)
Insuline resistance, *n* (%)	33 (25)
Alanine aminotransferase (ALT) [IU/L]	79.5 [50.5–122.7]
Aspartate aminotransferase (AST) [IU/L]	56.2 [17–105]
Hemoglobin (g/dL)	14.7 [13.4–15.3]
eGFR (mL/min)	99.1 [83–119.6]
Albumin (g/L)	40 [35–42]
HCV-RNA [LogIU/mL]	6.1 [5.7–6.5]
Naïve, *n* (%)	72 (54.5)
Experienced, *n* (%)	60 (45.5)
Previous treatment	
PEG-IFN and RBV	48 (36.4)
PEG-IFN and TLV/BOC	12 (9)
Previous treatment outcome, *n* (%)	
Null responder	35 (26.5)
Partial responder	6 (4.5)
Relapser	19 (14.4)
Presence of NS3 RAVs, *n* (%)	13 (12.6)
Symptomatic crioglobulinemia, *n* (%)	57 (43.2)
Type of symptoms, *n* (%)	
Palpable purpura	14 (10.6)
Arthralgias	13 (9.8)
Paresthesias	12 (9)
Peripheral neuropathy	8 (6)

Role of RBV in the treatment of HCV-associated MC with IFN-free regimens

Boglione et al. Arch. Virol. 2018

Virological response		
SVR12	132 (100)	
Immunological response		
Complete response	71 (53.8)	
Partial response	44 (33.3)	
Null response	17 (12.8)	
Clinical response		
Persistence of symptoms	15 (11.4)	*(26.3%)*
Improvement of symptoms	31 (23.5)	*(54.4%)*
Worsening of symptoms	1 (0.8)	*(1.7%)*
Resolution of symptoms	10 (7.6)	*(17.5%)*

IMMUNOLOGICAL RESPONSE ACCORDING TO RBV AND AVT HISTORY

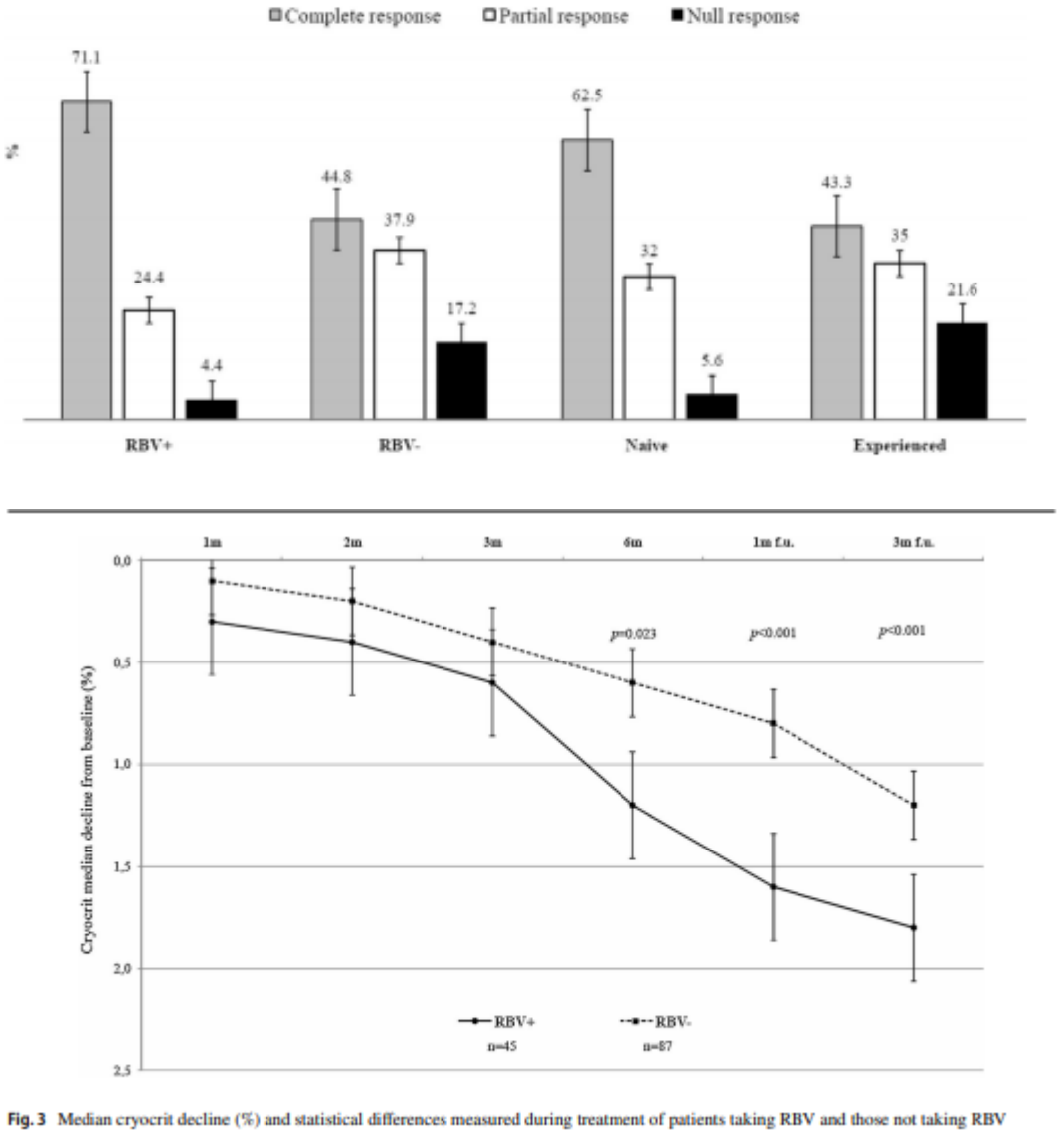


Fig. 3 Median cryocrit decline (%) and statistical differences measured during treatment of patients taking RBV and those not taking RBV

Long-lasting persistence of large B-cell clones in hepatitis C virus-cured patients with complete response of mixed cryoglobulinaemia vasculitis. Liver Int 2019
Visentini et al, 2019

Analysis of persistence of B-cell clonality through cytofluorimetry techniques

45 CV treated with DAAs including 8 lymphoma
follow-up: from 9 to 38 months after EOT Amongst the 45 CV patients

Circulating B-cell clone persisted in all of the lymphoma patients and in 27% of other CV pts
3 patients with clinical relapse: 2 showed the persistence of a B-cell clone and 1 relapsed during pneumonia

DAAs for HCV-MC: dissociated virological and haematological responses.
Pozzato et al, 2020

67 HCV+ MC including:
6 patients with lymphoma
30 with MBL

Virological response: 95%
Persistent MBL at F-U week 24: in 7/30 (23%);
CV complete clinical response in 60% (including 1/6 patients with NHL)

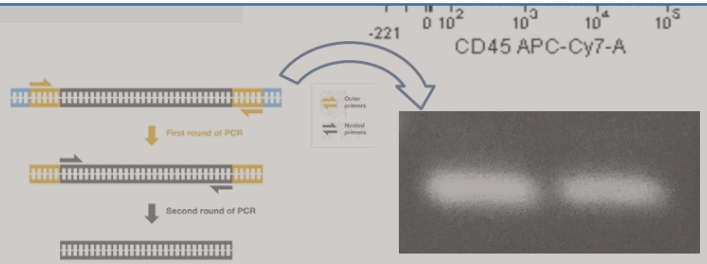
Conclusions: DAAs were non able to eliminate the clonal alterations induced by HCV in a large proportion of cases

Haematological and genetic markers in the rational approach to HCV SVR patients with or without persisting cryoglobulinemic vasculitis. *Gragnani et al, submitted paper*

98 HCV-MCS SVR after DAAs: (35 m/63 f, mean age 67 (58-73)
Median post therapy F-U= 12 months (6-24)
Group A: 52 (53%) (no evidence of post-DAAs MCS symptoms)
Group B: 46 (47%) (persistence or recurrence of post-DAAs MC symptoms)
The main demographical, clinical and virological characteristics were homogeneous in the two groups except for the RF level that was higher in the group B

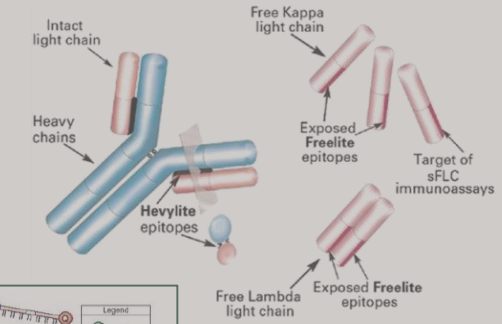
T(14;18) translocation

the B-cell specific translocation was detected in PBMCs through "nested" PCR (MBR bcl-2/JH PCR) on genomic DNA (Zignego et al, Ann Intern Med. 2002). Sensitivity was approximately one rearranged cell in 10^5



Free light chains (FLCs)

FLCs and their ratio were assessed by immunoassay (Turbidimetric assay on Optilite instrument). A ratio of κ /λ <0.26 or >1.65 is reputed abnormal. Serum samples were retrospectively tested at baseline (T0) and at the EOFU



Genetic analysis

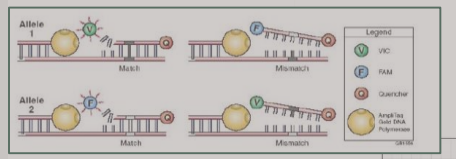
Some SNPs (on PBMCs DNA) from different genes/gene systems previously associated with a higher risk of LPD during HCV infection including:

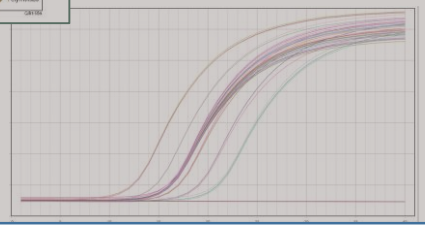
- The baff (B-cell Activator Factor) system: gene rs12428930/promoter rs9514828 /receptor rs61756766;

Giannini et al, Blood 2008; Gragnani et al, Arthr & Rheum 2011; Lake-Bakaar et al, Clin Exp Immunol. 2012; Ayad MW et al, Mol Diagn Th 2015

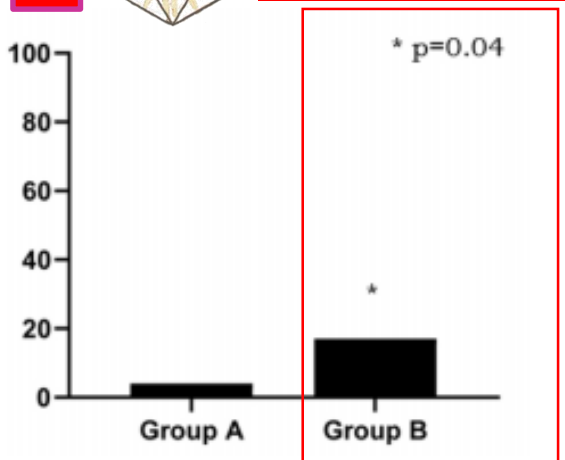
- The NOTCH4: rs notch4 rs2071286 and HLA class II rs9461776

Zignego AL et al, Genes & Immunity 2014, Gragnani L *et al*, Oncotarget 2017; Artemova M, Dig Liver Dis. 2018

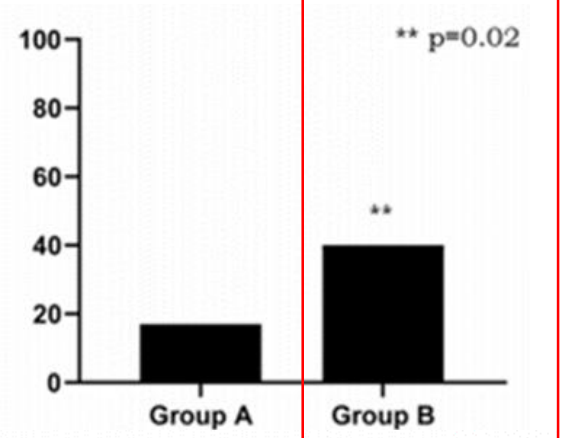




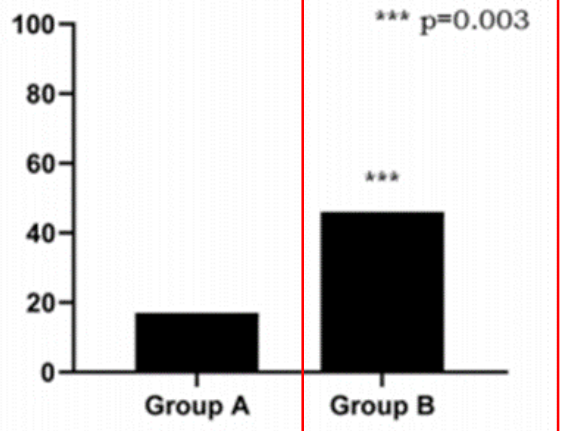
Clonality markers at the EOFU (prospective analysis)



Monoclonal B-cell lymphocytosis (MBL: B cell surface k/λ ratio) in 2/52 (4%) of Group A vs 8/46 (17%) of Group B patients (4% vs 17% p=0.04 RR=2.841 95% CI=0.8116-9.945)
MBL + patients presented with all three phenotypes: 4/10 (40%) had NON-LLC phenotype (CD5- CD20+), 2/10 (20%) had atypical-LLC phenotype (CD5+ $CD20^{BRIGHT}$) and 4/10 (40%) had LLC-like phenotype, (CD5+$CD20^{DIM}$)

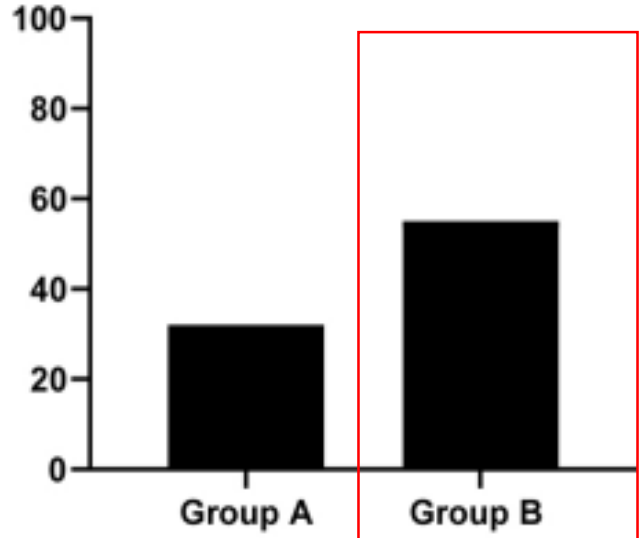


T(14;18) in Group A and in Group B patients: (17% vs 40% p=0.02 RR=1.900 95% CI=1.030-3.503)



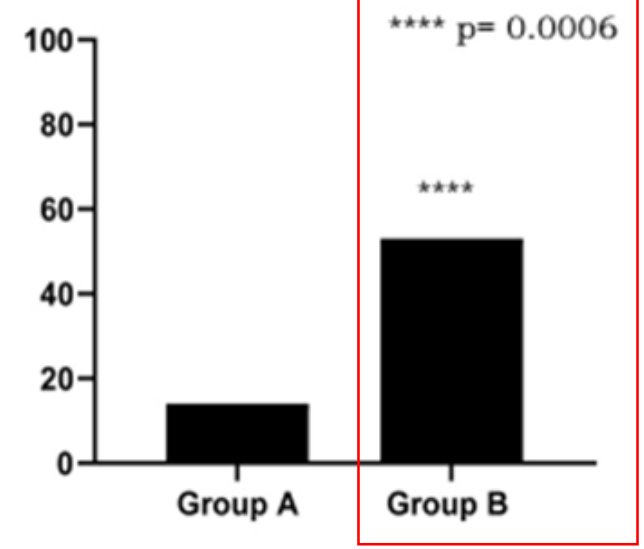
serum FLC k/λ ratio above the upper limit of 1.65 in Group A and in Group B patients (16% vs 46% p=0.003 RR=2.222 95% CI=1.202- 4.110)

Clonality markers in pre-therapy available samples



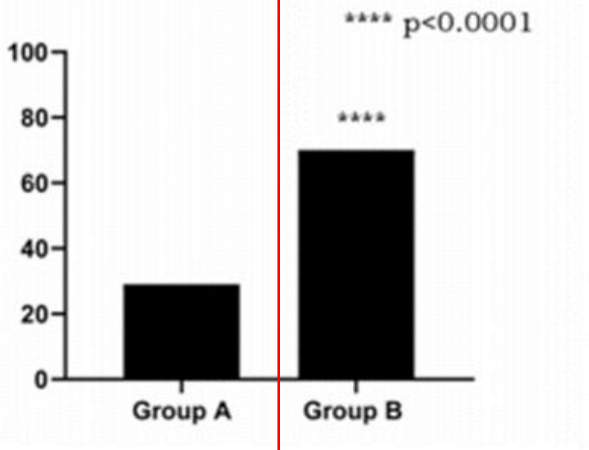
Presence of t(14;18) in Group A and in Group B patients (32% vs 55%)

B



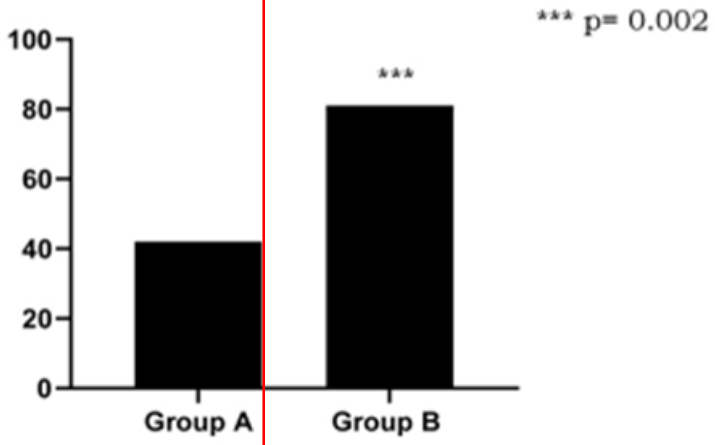
Abnormal serum FLC k/λ ratio in Group A and in Group B patients (14% vs 53% p=0.0006 OR= 6.667 95% CI= 2.130-20.87)

Presence of at least one clonality marker after and before DAAs



POST-DAAs end of F-U:

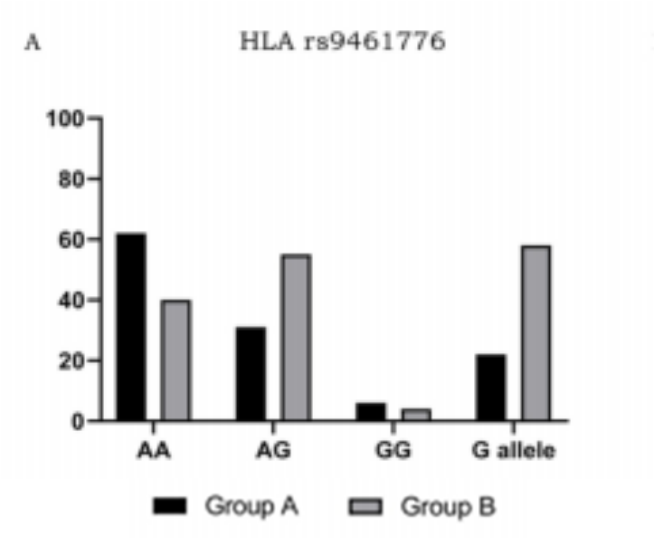
Presence of at least one clonality marker in 29% Group A and in 70% Group B patients (p<0.0001)



PRE-DAAs:

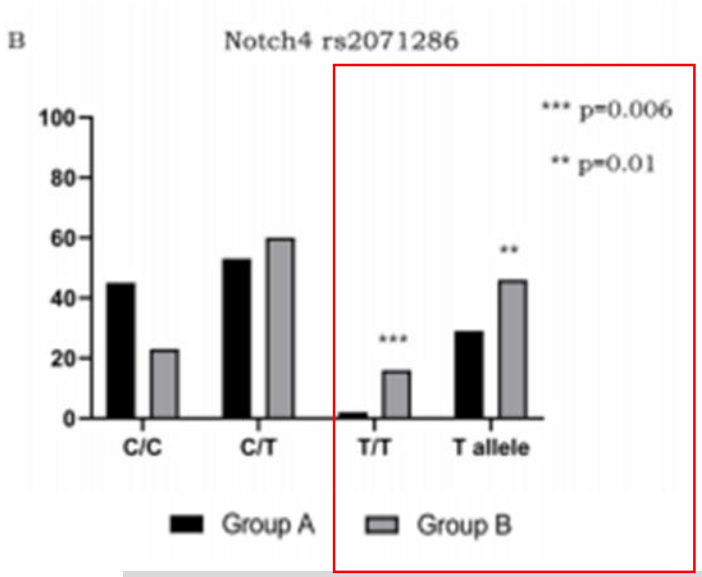
Presence of at least one clonality markers in 43% Group A and in 79% Group B patients (p=0.002 OR= 4.922 95% CI= 1.830-13.76)

Genotyping analysis of HLA class II rs9461776 and Notch4 rs2071286



Distribution of polymorphic variant of the HLA class II rs9461776.

The heterozygous genotype A/G and G minor allele were more frequent in Group B than in Group A (respectively 56% vs 31% and 33% vs 22%). The homozygous minor genotype G/G showed similar frequency between the two groups (6% in Group A vs 4% in Group B)



Distribution of polymorphic variant of the Notch4 rs2071286.

Significant association between rs2071286 SNPs and Group B patients with the dominant and recessive model of penetrance (C/C vs C/T+T/T for the dominant model of penetrance: p=0.04 OR=0.37, 95% CI:0,1434-0,9158; for the recessive model of penetrance: T/T vs C/T+C/C p=0.02 OR=9.33 95% CI: 1,099-79,28).

Homozygous haplotype T/T: 2% Group A vs 16% Group B p=0.006. T minor allele frequency: 29% Group A vs. 47% Group B (p=0.01 OR=2.17 95% CI=1.18-3.9)

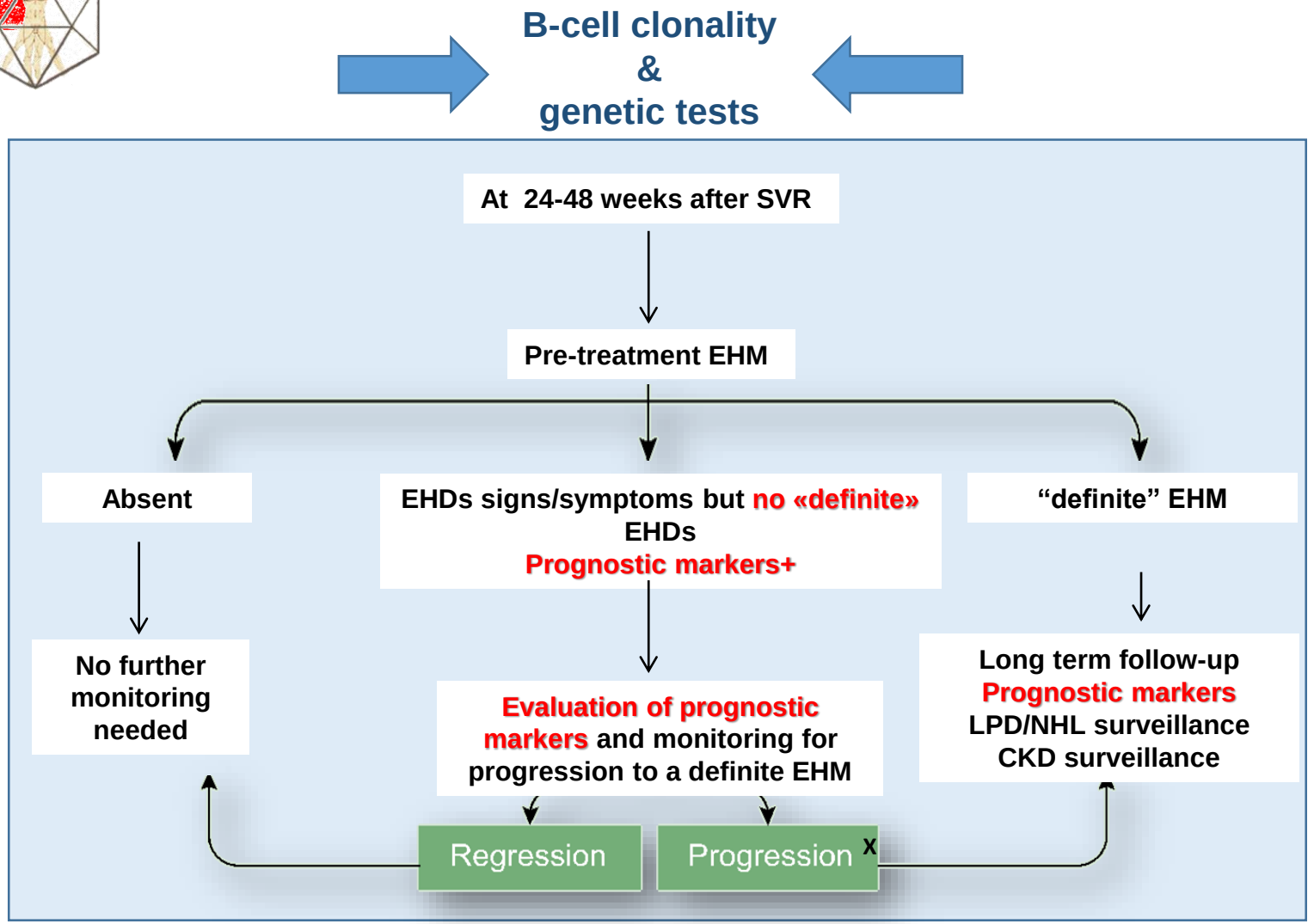
Role of Notch Receptors in Hematologic Malignancies. Gragnani L, Lorini S, Marri S, Zignego AL.Cells. 2020 Dec 24

The baff system SNPs analysis did not show any differences between the groups.

KEY TAKE-AWAY SLIDE

- **Take-away point 1:** At the moment, there are no clinical clues that could predict CV maintenance/recurrence after DAAs;
- **Take-away point 2:** Our results suggest that either bio-humoral or genetic tests could be useful in predicting the clinical outcome of CV after anti-HCV therapy;
- **Take-away point 3:** Clinical response outcome predictors could be precious for the assessment of a rational flow-chart to manage CV patients during the follow-up, thereby selecting the best therapeutic strategy and preventing any further evolution towards malignancy.

Taylored Post AVT Follow-Up of HCV-MC Patients



*Consider RTX treatment in persisting B-cell clonality