"Genetic profiling of the cryoglobulinemic patient: role of BAFF promoter, FC gamma receptors and IL28B polymorphisms“

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FOCUS SULLA MALATTIA HCV
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MIXED CRYOGLOBULINEMIA

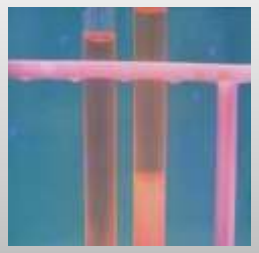
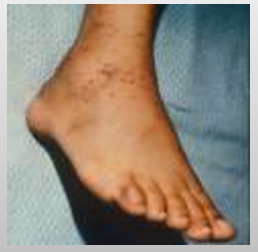
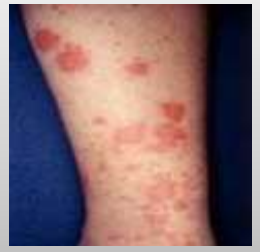
Strong association between HCV and MC:

- 80-90% MC patients are HCV+ (in Italy >95%; <5% HBV or essential)
- 40-60% of HCV patients show circulating cryoglobulins:

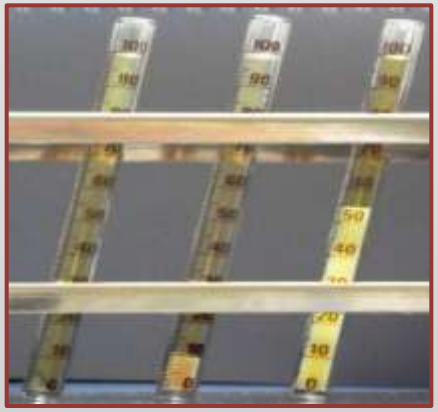
5-30% of them show a symptomatic MC: Cryoglobulinemic Syndrome (SCM)

Development of NHL in 5-10% of cases.

Defined as a systemic vasculitis caused by a intravascular deposit of ICC named Cryoglobulins (CGs) in the vessels. The CGs may be partially monoclonal (type II MC IgG-IgMK) or totally polyclonal (type III MC) immunoglobulins.

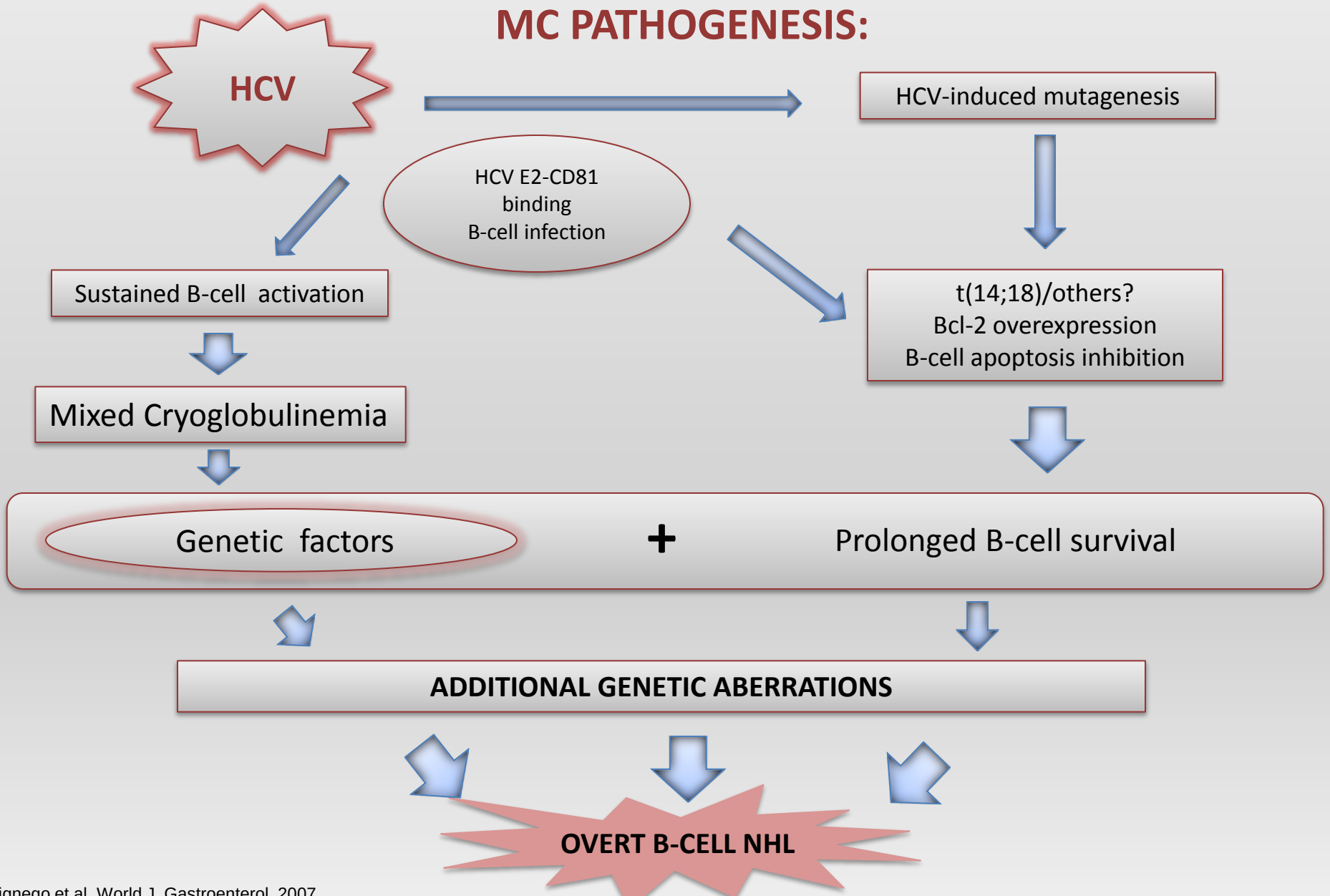




MIXED CRYOGLOBULINEMIA



- The IgM has a RF activity;
- MC is due to a clonal expansion of RF producing B cells;
- RFs are low specificity antibodies and considered as part of the *innate immunity*;
- The monoclonal component, represented by RF molecules, is characterized by the same cross-reactive idiotype, called WA;
- The expansion of RF B-cell autoreactive clones is caused by a still not completely understood event in MC pathogenesis.

MC PATHOGENESIS:



Zignego et al. World J. Gastroenterol. 2007
Zignego et al. Clin.Liver Dis. 2008

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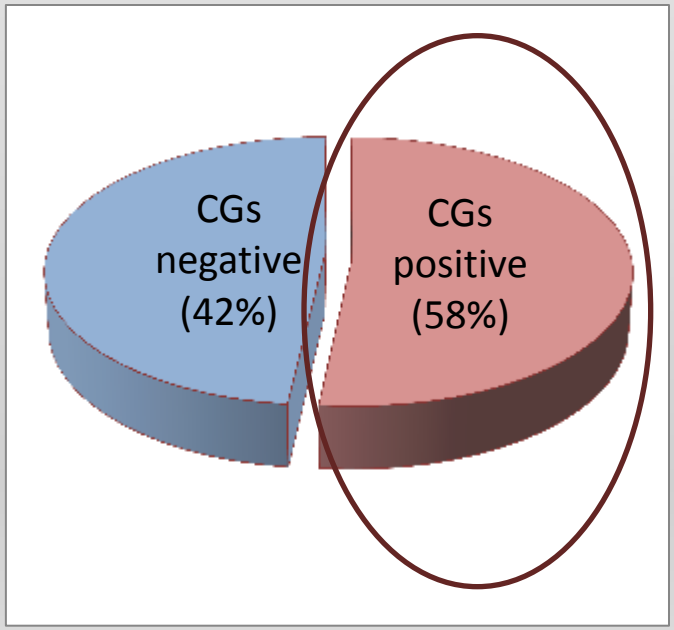
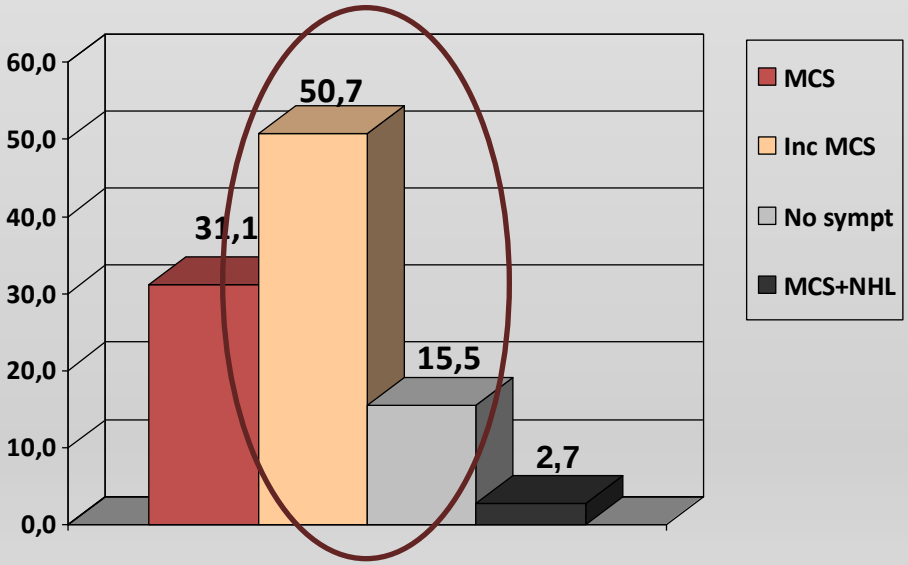


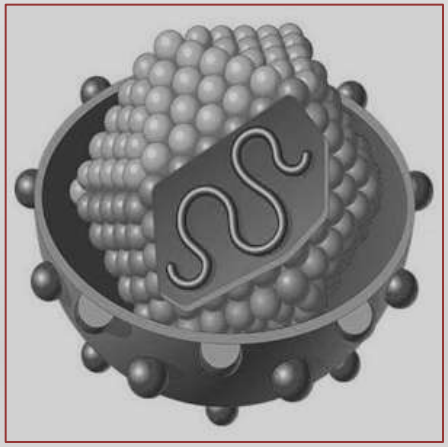
Prevalence of CGs (565 HCV+ pts)

"GREY ZONE"



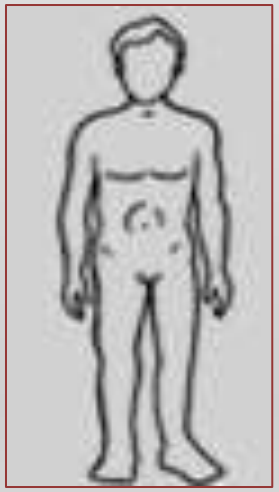
Prevalence of SCM symptoms and MCS-associated NHL (328 HCV+ pts with CGs)

MIXED CRYOGLOBULINEMIA



viral factors

?



host factors

MC AND VIRAL FACTORS

Starting from the 90s several studies have failed to demonstrate a definite role of viral factors in HCV-related MC pathogenesis

"HCV genotyping showed that multiple genotypes are involved in pathogenesis of MC… Therefore, the distribution of HCV types in MC in the current study reflects the prevalence of different genotypes in our geographic area."

Pozzato G. et al. Blood 1994

"… a difference in genotype prevalence was not found between HCV-related EMC and chronic hepatitis C without clinical manifestations of EMC"

Willems M. et al. J Med Virol. 1994

"In patients with hepatitis C virus infection, cryoglobulinemia is not strongly associated with a particular HCV genotype or subtype."

Frangeul L. et al. J Hepatol. 1996

MC AND VIRAL FACTORS

Role of HVR1 & HVR2 variants viral in MC pts VS associated controls

"A 385 insertion in the hypervariable region 1 of hepatitis C virus E2 envelope protein is found in some patients with mixed cryoglobulinemia type 2."

Gerotto M et al. Blood 2001

"Association of HCV-related mixed cryoglobulinemia with specific mutational pattern of the HCV E2 protein and CD81 expression on peripheral B lymphocytes."

Hofmann W.P. et al Blood 2004

"Genetic heterogeneity of the hypervariable region I of Hepatitis C virus and lymphoproliferative disorders."

Rigolet A. et al Leukemia 2005

….cryoglobulinemia may arise by virtue of as-yet-unidentified **host- rather than virus-specific factors**. Specific changes in HCV envelope sequence distribution are unlikely to be directly involved in the establishment of pathological B-cell monoclonal proliferation.

Bianchettin et al. J. Virol. 2007

MC AND HOST FACTORS

An early attempt to investigate genetic predisposition to MC has been published even before HCV discovery in 1981 by Migliorini P. et al who found no association between MC and either class I or class II HLA molecules.

Migliorini P. et al, Arthritis Rheum. 1981

Different authors reported an association of specific HLA clusters with a higher risk of developing MC and concomitant lymphoma

Lenzi M. et al Blood 1998; De Re V et al Ann NY Acad Sci 2007; De Re V et al Tissue Antig. 2010

A recent study of Fabris and collegues suggests that particular SNPs of fibronectin gene could define a higher risk of developing a frank lymphoma in MCS patients.

Fabris M. et al Ann Rheum Dis 2008

MC AND HOST FACTORS

"Ethnic difference in the prevalence of monoclonal B-cell proliferation in patients affected by hepatitis C virus chronic liver disease."

… suggests that HCV is able to determine a B-cell expansion only in the presence of, presently undetermined, host factors.

Pozzato G. et al J. Hepatol. 1999

Imbalance between CGs clearance/production in the pathogenesis of MC?

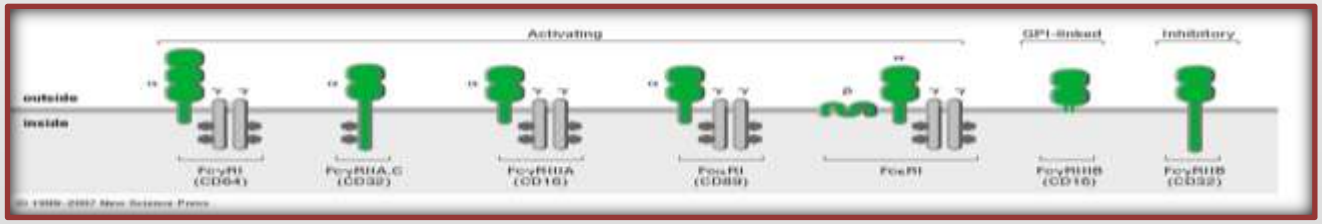
REDUCED UPTAKE AND CLEARANCE OF Igs BY MACROPHAGES OF THE RETICULOENDOTHELIAL SYSTEM

EXCESSIVE PRODUCTION OR SECRETION OF Igs BY B-CELLS

FcγR - Fc gamma receptors -

BAFF -B cell-activating factor-

FcG receptors



FcGR I - **high affinity** - binding of monomeric IgG

FcGR II and III - **low affinity** - binding of multiple IgG
- FcGR2A, FcGR3A and FcGR3B (activatory effects)
- FcGR2B (inhibitory effects)

IMMUNE RESPONSE: balance of

ACTIVATORY RECEPTORS

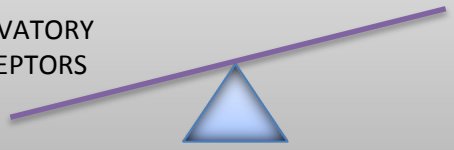
INHIBITORY RECEPTORS

AUTOIMMUNITY

(POLYMORPHIC VARIANTS REDUCING THE AFFINITY OF FCGRs)

INHIBITORY RECEPTORS

ACTIVATORY RECEPTORS



- present on leukocytes, mainly on phagocytes
- clearance of immunocomplexes
- phagocytosis
- recruitment to inflammatory lesions
- antibody-dependent cellular cytotoxicity (ADCC)
- release of inflammatory mediators
- regulation of B cell activation

BAFF: B cell-activating factor

- TNF-family member
- Mainly produced by monocytes/macrophage lineage
- Key regulator of B-cells differentiation, survival and Ig secretion

BAFF 3D MOLECULAR STRUCTURE

Overexpression associated with several AUTOIMMUNE DISORDERS:

- Sjoegren syndrome,
- Systemic Lupus Erythematosus
- Rheumatoid Arthritis

(Cheema, 2001; De Vita, 2008)

BAFF overexpression in monocytes from patients with Rheumatoid Arthritis and familial lymphoproliferative disorders associated with the presence of a T at the polymorphic site -871C/T of its promoter.

The -871T allele induced a 3-fold overexpression of the *baff* gene in a in-vitro model.

(Kawasaki, 2002; Novak, 2006)

BAFF high levels in sera of HCV (+) patients

(Toubi, 2006; Fabris, 2007; Sene, 2007)

PATIENTS AND SAMPLING

210 HCV patients

102 consecutively recruited pts. with HCV+ Mixed Cryoglobulinemia Syndrome

108 consecutively recruited pts. with HCV chronic infection without MC, MC-related stigmata (i.e.: RF, complement consumption, purpura, SS, etc: Grey Zone), or other DLPs

SAMPLES

FRESH PERIPHERAL BLOOD

SERUM

PBMC

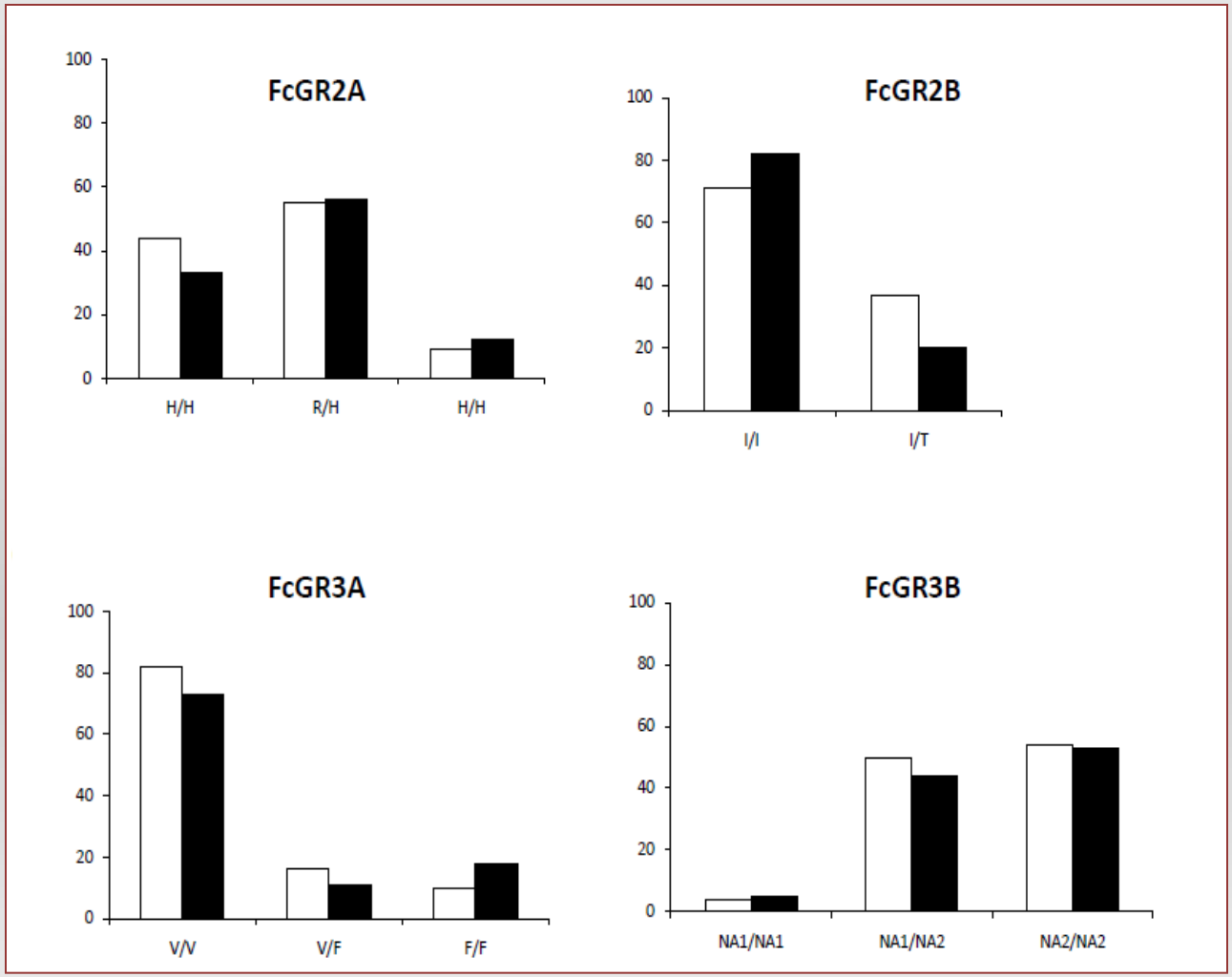
GENOTYPING AND ELISA ASSAY

GENOTYPING

- ***FCGR*** →
  - FCGR2A TaqMan SNP Genotyping Assay
  - FCGR2B FRET technology
  - FCGR3A nested PCR + RFLP analysis
  - FCGR3B allele-specific PCR
- ***BAFF promoter*** → PCR + RFLP analysis
- ***IL28B*** → TaqMan SNP Genotyping Assay (rs 12979860)

BAFF serum levels → Enzyme-Linked ImmunoSorbent Assay for human BAFF

Frequencies of FcGRs genotypes



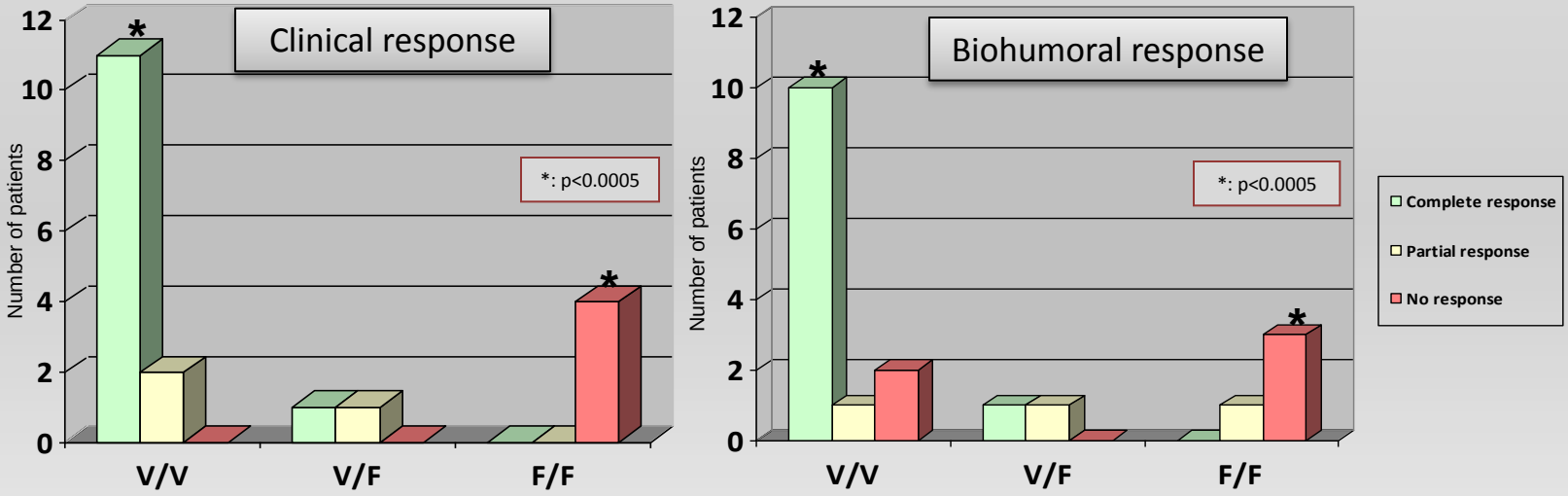
Gragnani L. et al, Arthritis Rheum. 2011

Frequencies of FcGR3A genotypes in RTX treated SCM pts

21 patients from the HCV-MCS group that were treated with anti-CD20 monoclonal antibody (**rituximab**) for severe MC syndrome

V/V genotype → **good response**

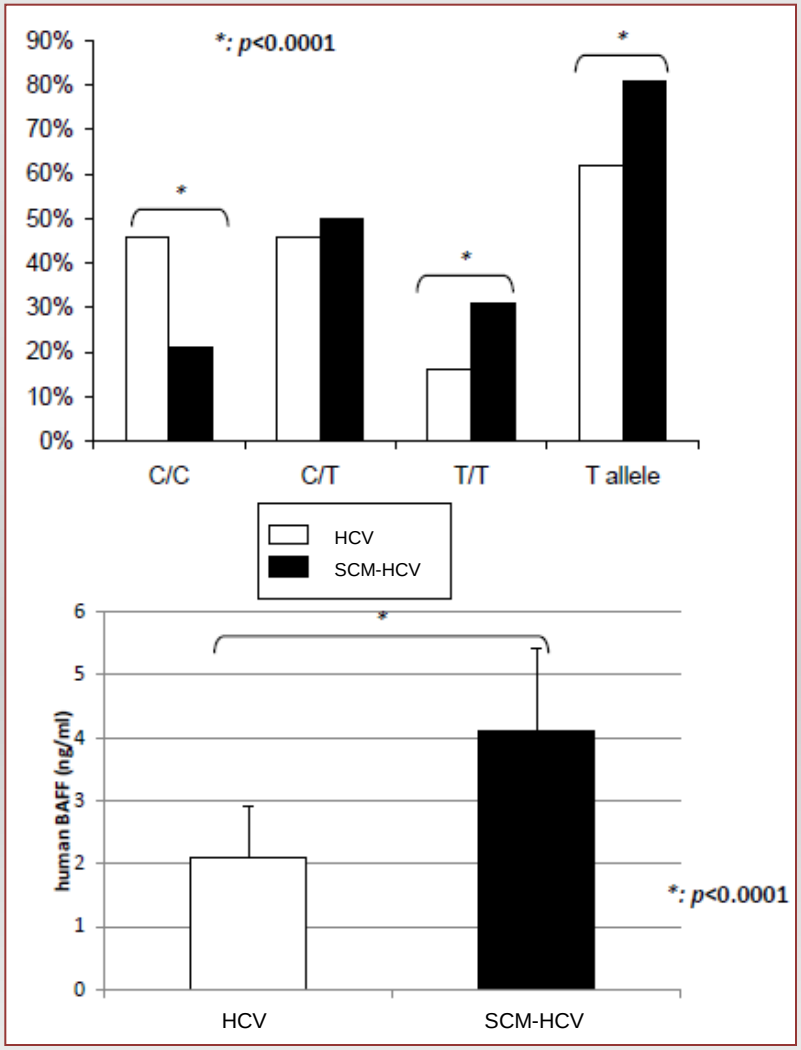
F/F genotypes → **reduced response**



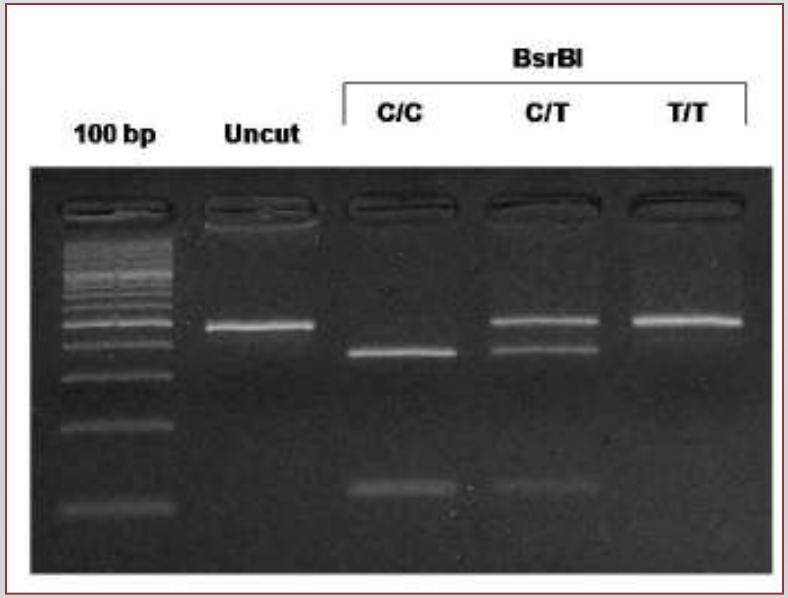
Clinical data: purpura, weakness, arthralgia, leg ulcers, neuropathy.
Biohumoral data: cryocrit, RF, C4, creatinine, proteinuria

Gragnani L. et al, Arthritis Rheum. 2011

Baff promoter polymorphism at -871



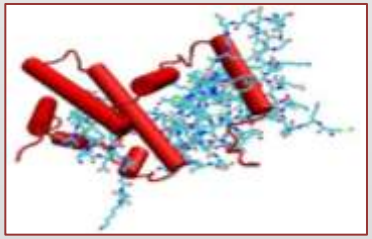
RFLP analysis for *baff* promoter genotyping



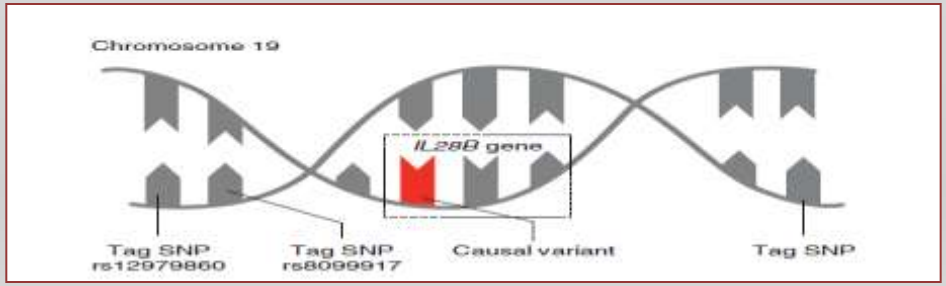
Gragnani L. et al, Arthritis Rheum. 2011

IL28B

•regulates the innate immune response;



• a tag-SNP located in a region close to the IL28B gene has been demonstrated to have a predictive value for the outcome of antiviral therapy of HCV chronic infection.



Could a particular IL28B variant have some sort of implication in MCS onset predisposition???

Thomas D.L., Nature 2009; Ge D. Nature 2009

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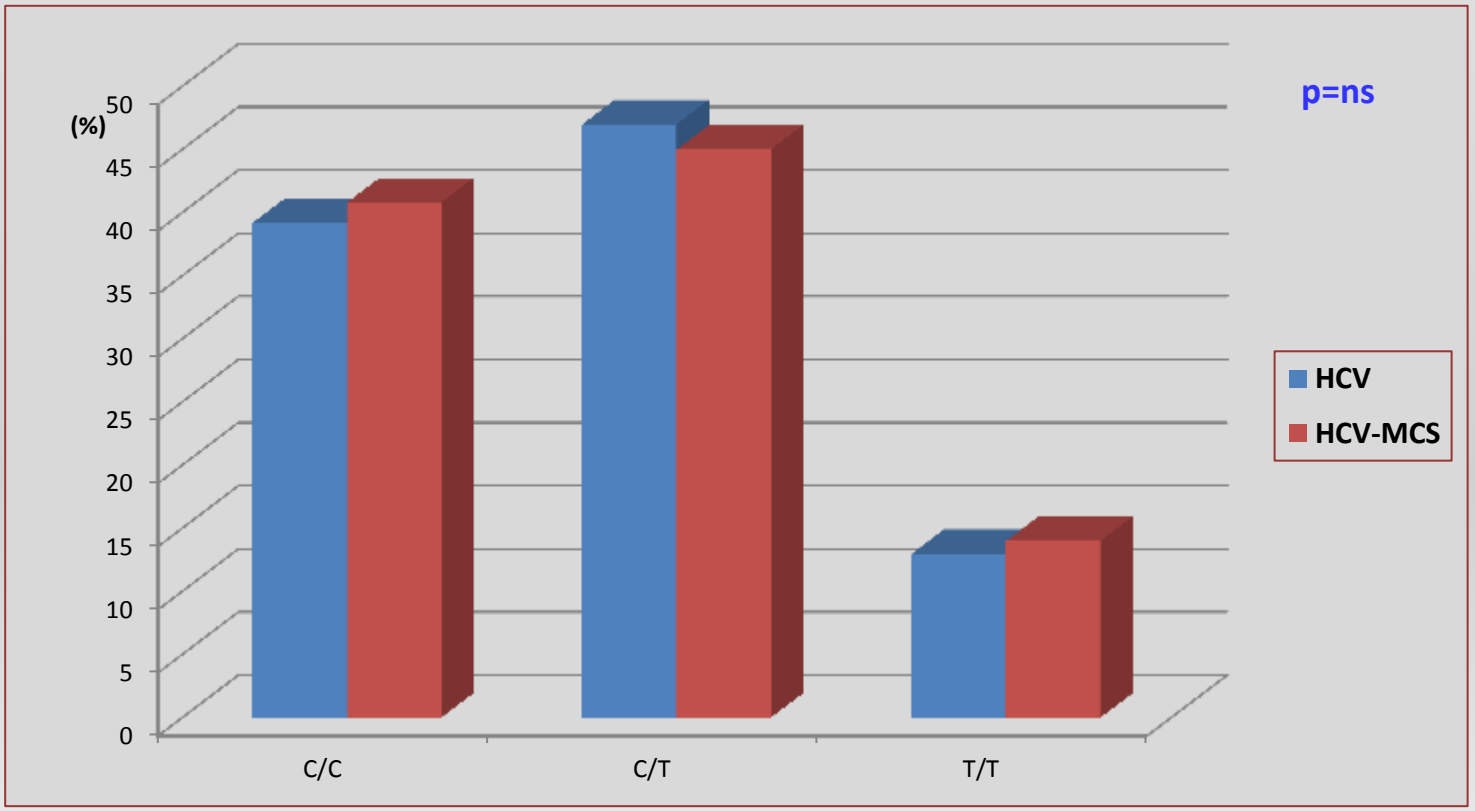
IL28B (rs12979860) allele distribution in HCV patients with and without MCS



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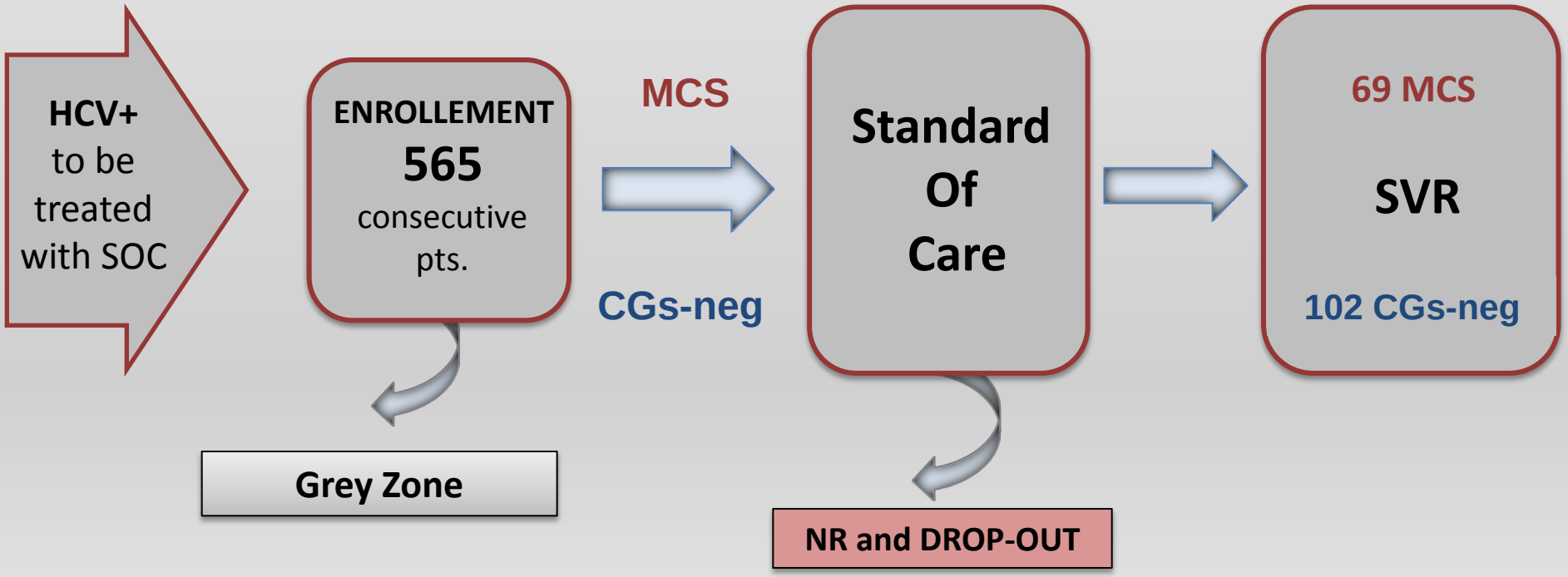
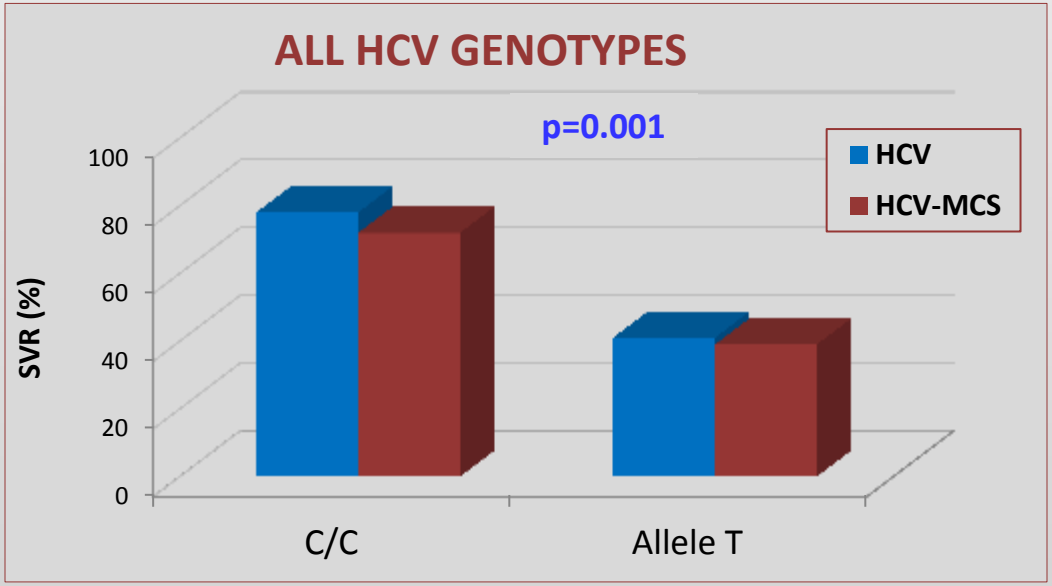
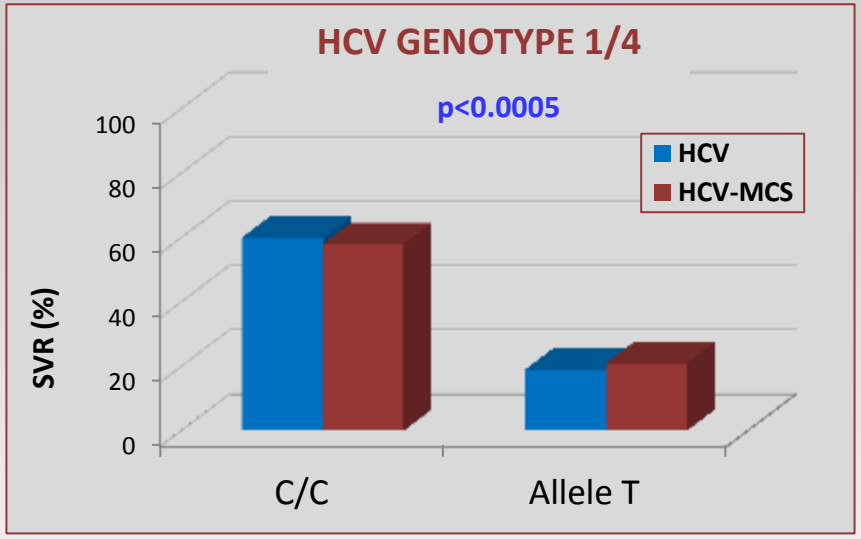
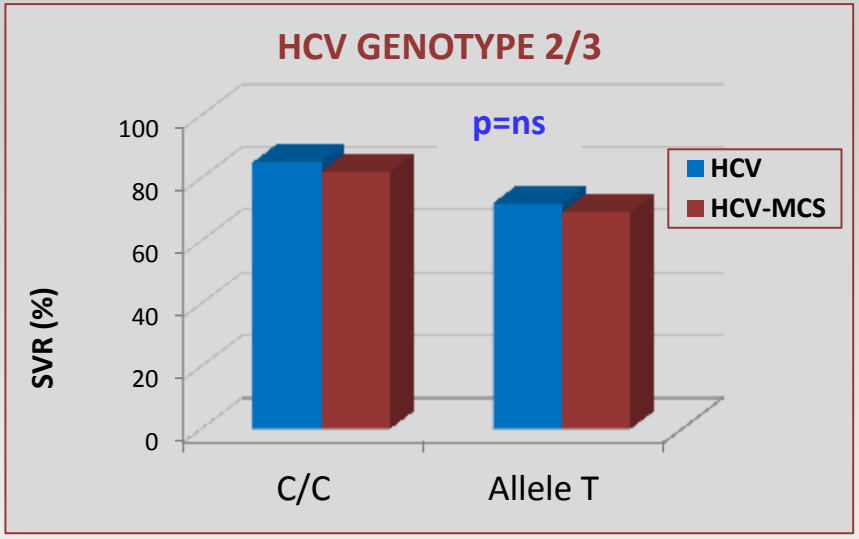


Predictive value of IL28B in HCV patients with and without MC syndrome







SUMMARY

- Genetic variants of the 4 major types of low-affinity FcGRs seem to be not directly involved in SMC pathogenesis. However, the analysis of patients treated with anti-CD20 mAb for a severe MC syndrome strongly suggests the importance of the allelic status for the FcγR3A-158V/F polymorphism.

- A polymorphic variant of the *baff* promoter was strongly associated with the presence of MCS and with elevated BAFF serum levels, emphasizing the potential contribution of the genetic background in the development of HCV-related lymphoproliferative disorders.

- A similar distribution of IL28B alleles was observed in patients with and without MCS suggesting that the genetic variants associated with the rs12979860 do not play a major role in MCS pathogenesis. However…

SUMMARY

- In spite of the immunologic changes associated with MC -especially involving innate immunity-, it does not affect the association between SNPs near the *IFN lambda-gene cluster* and clearance of HCV.

- IL28B genotyping should be considered as part of the treatment decision algorithm in this difficult-to-treat population

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