European Review for Medical and Pharmacological Sciences 2020; 24: 8067-8074

Biomarkers in HCV-related mixed cryoglobulinemia patients with non-Hodgkin lymphoma

F. GULLI[1], M. MARINO[2], C. NAPODANO[3], K. POCINO[3], F. PANDOLFI[3], A. GASBARRINI[3], G.L. RAPACCINI[3], U. BASILE[4]

[1]Laboratorio di Patologia Clinica, Ospedale Madre Giuseppina Vannini, Rome, Italy
[2]Dipartimento di Medicina Traslazionale, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy
[3]Dipartimento di Scienze Mediche e Chirurgiche, UOC Gastroenterologia, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy
[4]Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

Francesca Gulli and Mariapaola Marino equally contributed to this work

Abstract. – OBJECTIVE: Chronic Hepatitis C virus (HCV) infection can cause severe extrahepatic manifestations, such as mixed cryoglobulins (MC), up to the development of B cell nonHodgkin's lymphoma (B-NHL). Mechanisms transforming of HCV infection into lymphoproliferative and/or autoimmune disorders are still poorly understood. In course of HCV infection, the sustained virus-driven antigenic stimulation may probably induce a B-cell clonal expansion. Measurements of serum free light chains (FLCs) levels, considered as a direct marker of B cell activity, are analyzed with increasing interest in clinical practice, for diagnosis, monitoring and follow-up of plasma cell dyscrasia. Syndecan-1 (CD138) is a transmembrane heparan sulfate proteoglycan expressed and actively shed by most myeloma cells. Membrane CD138 represents the major receptor protein for HCV attachment to the hepatocyte surface and high levels of circulating sCD138 levels are detected in patients at early stage of B-cell chronic lymphocytic leukemia. This study is aimed to evaluate sCD138 and FLC levels as diagnostic biomarkers of HCV-related MC with B-NHL.

PATIENTS AND METHODS: We enrolled 35 HCV-MC-NHL patients, characterized for the specific type of cryoglobulins, and 25 healthy blood donors (HBD) as negative control. Serum sCD138 levels were determined using ELISA kits specific for human sCD138. Serum FLCs were assessed by means of the turbidimetric assay.

RESULTS: We found that serum levels of sCD138, as well as FLCs, were significantly higher in patients than in HBD ($p<0.001$).

CONCLUSIONS: In agreement with the definition of HCV-driven lymphoproliferative disorders as the consequence of a multifactorial and multistep pathogenetic process, we suggest that sCD138 and FLCs could be considered putative independent markers of worsening progression of the disease.

Key Words:
Hepatitis C virus, Mixed cryoglobulins, B cell non-Hodgkin's lymphoma, Biomarkers, Syndecan-1 (CD138), FLC.

Abbreviations

Hepatitis C virus (HCV), mixed cryoglobulinemia (MC), B cell non-Hodgkin's lymphoma (B-NHL), cryoglobulins (CGs), Immunoglobulin (Ig), Rheumatoid Factor (RF), marginal zone lymphoma (MZL), diffuse large B cell lymphoma (DLBCL), kappa (k), lambda (λ), free light chains (FLCs), systemic autoimmune rheumatic diseases (SARD), Syndecan-1 (CD138), soluble CD138 (sCD138), World Health Organization (WHO), C-reactive protein (CRP), healthy blood donors (HBD), immunofixation electrophoresis (IFE), minimal residual disease (MRD). .

Introduction

Hepatitis C virus (HCV) is a RNA hepatotropic and lymphotropic virus affecting over 180 million people, all over the world, by chronic infection[1]. In most cases, HCV infection of liver is followed by the development of extrahepatic

Corresponding Author: Cecilia Napodano, Ph.D; e-mail: cecilia.napodano@gmail.com

manifestations whose most frequent hallmark is an immune dysregulation: mixed cryoglobulinemia (MC), characterized by cutaneous vasculitis, nephritis, peripheral neuropathy and clonal Bcell lymphoproliferations[2]. Epidemiologic studies, mainly from Italian and Japanese centers, have associated HCV infection with an increased risk of B cell non-Hodgkin's lymphoma (B-NHL), with a prevalence range from 0.5% to 25%[3-5]. These studies suggested that other important genetic and/or environmental co-factors may be involved in the pathogenesis[6]. Mechanisms transforming of HCV infection into lymphoproliferative and/or autoimmune disorders are still poorly understood, although frequently many actors interconnected with each other, seem to contribute to their pathogenesis and progression. The chronic nature of the HCV infection led to speculate that a sustained virus-driven antigenic stimulation could drive B cell clonal (initially polyclonal) expansion. HCV-related lymphoproliferative disorders are characterized by the clonal expansion of B cell, above all in liver and, less frequently, in bone marrow or blood[6]. The hepatic areas of lymphatic proliferation, characteristic of HCV infection, are reminiscent of lymphatic follicles present in almost all the cases of MC, and therefore, they are considered an important site of B cell clonal growth[6]. Reactivity against specific viral antigens can be identified through the presence of the circulating cryoglobulins (CGs) representing the first step of activation of B cell clones. However, even when immune system is compromised or overwhelmed, it will still endeavor to regain and maintain tolerance to self[7,8].

MC syndrome, which in some cases may evolve to frank B-NHL, is the prototype of HCV-driven autoimmune and lymphoproliferative disorders[7-10]. MC implies the presence of circulating immune complexes composed of polyclonal Immunoglobulin (Ig) G (behaving as auto-antigens) and IgM with Rheumatoid Factor (RF) activity. MC is classified as type II CG that consists of Ig complexes formed by a monoclonal component (usually IgM) and polyclonal components (usually IgG, more rarely IgA) due to chronic antigenic stimulation of HCV infection. However, unlike frank malignant lymphomas, MC tends to remain unmodified for years or even decades and is followed by overt lymphoid tumors in about 10% of cases[11]. Furthermore, HCV-MC patients display a 35 times higher risk of developing lymphoma than the general population[12]. HCV could exert its oncogenic effect indirectly modulating the host immune system[13,14]. The potential pathways of malignant transformation HCV-driven process are mainly three and involve the active participation of the virus. The viral antigens stimulate B-lymphocytes proliferation through the binding to surface lymphocyte receptors; HCV replication occurs inside B cells and the oncogenic effects could be induced by HCV intracellular proteins; the "hit and run" theory which means permanent damage of B cell, a direct mutagenic action of the virus on the B cells that induces a mutated phenotype, triggering error-prone[15,16]. HCV-associated B-NHL correlates with extended infection duration (15 years) and more frequent presentation of extra-nodal disease[17]. Despite frequently reported in different studies, histotypes of B-NHL frequently associated with HCV are marginal zone lymphoma (MZL), particularly splenic zone lymphoma, lymphoplasmacytic lymphoma, and diffuse large B cell lymphoma (DLBCL)[18,19]. Therefore, the study and evaluation of B cell proliferation in HCV infected patients seem to be a useful tool in order to monitor and prevent the appearance of a B cells neoplastic event. Measurements of serum free kappa (k) and lambda (λ) light chains (FLCs) levels are consistently encountering increasing interest in clinical laboratory procedures and many guidelines acknowledge their use in clinical practice for diagnosis, monitoring and follow-up of monoclonal gammopathies[20]. FLCs have been currently deeply investigated in different systemic autoimmune rheumatic diseases (SARD)[21] but they seem to be a sensitive index of B cell activation even in organ-specific disorders[22].

Serum concentrations of FLCs are dependent upon the balance between production by plasma cells and renal clearance. The malignant B cell clone produces a monoclonal excess of only one type of light chain, often paralleled by the bone marrow suppression of cognate light chain, so that the k/λ ratio becomes highly abnormal. Accurate measurement of k/λ ratios provides a quantitative biomarker of clonality[23]. Although most cases of B-NHL are light chain restricted, only a minority of patients display sFLC abnormalities; moreover, in patients without a monoclonal component, the FLC assay proved to be of prognostic relevance[24]. The mechanism underlying this association is not clear but correlated with laboratory parameters indicating an immune system/ B cell dysregulation; this might simply reflect an environment of cytokines or other immune system interactions that may trigger a malignant B cell growth[24].

Syndecan-1 (CD138) is a transmembrane heparan sulfate proteoglycan expressed and actively shed by most myeloma cells. Upon released, soluble (s) CD138 may interact with different cell types enhancing growth factor expression and bioavailability of signaling molecules, thus conditioning the tumor microenvironment. Elevated serum levels of sCD138 have been specifically associated to malignancies and correlated with worse outcome in myeloma patients, as well as a good prognostic biomarker at different phases of the disease, arising from increased cell growth and proliferation, cell survival, cell invasion and metastasis, and angiogenesis[25].

Serum sCD138 level is higher in early stage B-cell chronic lymphocytic leukemia patients than in healthy controls, correlates negatively with peripheral blood lymphocyte count, and is higher in patients with more indolent disease course[26].

In HCV infection, sCD138 is the major receptor protein for virus attachment to the hepatocyte surface[27]; so, its measurement could represent a valuable tool to be used alongside FLCs quantification during the follow-up and monitoring of HCV-related MC patients undergoing treatments[10].

Moreover, sCD138 represents a non-invasive tool to estimate fibrosis, alternative to liver biopsy. In chronic inflammatory diseases, sCD138 levels do not increase and could discriminate against patients eligible for liver biopsy, excluding patients with other liver diseases[28].

The use of biomarkers in oncology has evolved from research to more routine clinical use, such as in diagnosis, monitoring of disease status and treatment efficacy. The aim of this study is the evaluation of sCD138 and FLC as serological biomarker to assess the status of HCV-related MC with B-NHL.

Patients and Methods

Patients

In this retrospective study were consecutively enrolled 35 patients (16 female and 19 males, mean age 60.4 ± 1.7) with HCV infection and a diagnosis of DLBCL, from January 2013 to December 2016 referring at the Department of Gastroenterology, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, in Rome (Italy). At the time of NLH diagnosis, all subjects checked for routine screening resulted HCV-RNA positive, cryocrit positive, without any evidence of autoimmune disorders, normal/low levels of C4 and C3, in absence of antiviral therapy and HIV or co-infection HBV. Liver disease stage was assessed by liver elastography using FibroScan (Echosens, Paris, France), and the resulting stiffness value has been converted in the corresponding METAVIR score. NHL diagnosis was recorded according to the World Health Organization (WHO) 2008 classification[29].

All the patients with inflammatory diseases with elevated levels of C-reactive protein (CRP) or co-infections (HIV and HBV) and with a monoclonal gammopathy in the supernatant were excluded. Renal function was available for all the enrolled patients and patients with renal failure (estimated glomerular filtration rate was <60 mL/min/1.73 m^2) were excluded.

Furthermore, we also included 25 (13 female and 12 males, mean age 52.5±11.8) healthy blood donors (HBD) negative for monoclonal gammopathy research, absence of HIV or HBV infection.

The main demographic features of patients and HBD are reported in Table I.

Laboratory Testing

For CGs detection, 10 mL of peripheral blood were collected and immediately stored at 37°C in pre-warmed tubes without anticoagulant for 30 min to enable complete blood clotting. Serum was transferred in Wintrobe tubes and stored for at least 7 days at 4°C to evaluate the presence of precipitates and flocculation. Before immunologic testing, the cryocrit percentage was assessed, the supernatant was removed and stored for subsequent analyses. The remaining cryoprecipitate was recovered and washed 3 times. Cryoprecipitate was re-suspended with an appropriate volume of 3% PEG 6000 solution and re-solubilized for 30 min at 37°C[30]. CGs were characterized by immunofixation electrophoresis (IFE) with G26 Fully automated system (Interlab, Rome, Italy)[31,32].

Serum sCD138 levels were determined using ELISA kits specific for human sCD138 (Diaclone Research, Besançon, France). The assay was performed according to the manufacturer's instructions and serum concentrations of sCD138 were expressed as ng/mL. sCD138 levels were measured at least twice for each patient, with reproducible results.

Serum FLCs were assessed by means of turbidimetric assay (Freelite TM Human Kappa and Lambda Free Kits, The Binding Site, Birming-

Table I. Demographic, clinical, and virologic correlates of HCV MC-related NHL patients and HBD.

Correlates	HCV MC-NHL (n. 35)	HBD (n. 25)
Age	60.4 (± 1.7)	52.5 (± 11.8)
Gender (M/F)	19/16	12/13
Metavir score		
F2	20	
F3	15	
ALT (U/L)	98.4 (± 10)	18.0 (± 5)
AST (U/L)	84 (± 8)	14 (± 7)
Total Bilirubin (mg/dl)	0.96 (± 0.05)	0.65 (± 0.08)
Conjugated Bilirubin (mg/dl)	0.42 (± 0.02)	
Cryocrit (%)	6.2 (± 0.2)	
RF (UI/ml)	40.5 (± 1.7)	< 10
C4 (mg/dl)	10 (± 0.05)	25 (± 18)
HCV-RNA (IU/ml × 10^6)	2.6 (± 0.48)	
HCV genotype		
1a	3	
1b	20	
2	4	
3	6	
4	2	

ham, UK) and performed on the OPTILITE instrument (The Binding Site, Birmingham, UK). Samples were tested according to the manufacturer's instructions and serum dilutions, where necessary, were performed according to the manufacturer's recommendations.

The analyses were performed by an operator without knowledge of the clinical information of the handled sample.

Ethics and Consent

The study protocol, including benefits and harms, was explained for study participants and written informed consents were obtained. The study was approved by the Ehical Committee of

Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "A. Gemelli" – IRCCS, Rome. The study protocol was carried out in accordance with the Declaration of Helsinki, as revised in Seoul 2008.

Statistical Analysis

Comparison of mean values was performed by Student's *t*-test; *p*-values<0.05 were considered significant. A correlation analysis was carried out using the Pearson correlation coefficient.

Results

Serum levels of sCD138, free k and λ chains were evaluated in HCV-NHL patients and HBD. The mean values (± standard deviation) of sCD138, free k and λ and k/λ ratio are reported in Table II. The statistical analysis revealed significant differences between patients and HBD. Considering the group of patients, the high serum levels of sCD138 ranged from 156 to 745 ng/ml with a mean value that was significantly higher if compared with HBD group ($p<0.001$, Figure 1A), that included a series of values ranged up to 100 ng/ml. Similarly, significant differences were detected FLC profile between patients and controls (Figure 1B). The levels of free k were above the cut-off in all patients with a mean value that was significantly higher than in HBD ($p<0.001$). The levels of λ-free chain

Table II. Mean values (± standard deviation) of sCD138, free k and λ and k/λ ratio in patients and controls.

Subjects	sCD138 ng/mL	k mg/L	λ mg/L	k/λ
NHL (35)	398.03 ± 139.95	64.53 ± 29.81	28.67 ± 14.83	2.67 ± 1.59
HBD m (25)	52.76 ± 29.90	10.04 ± 4.27	11.5 ± 5.61	0.91 ± 0.27

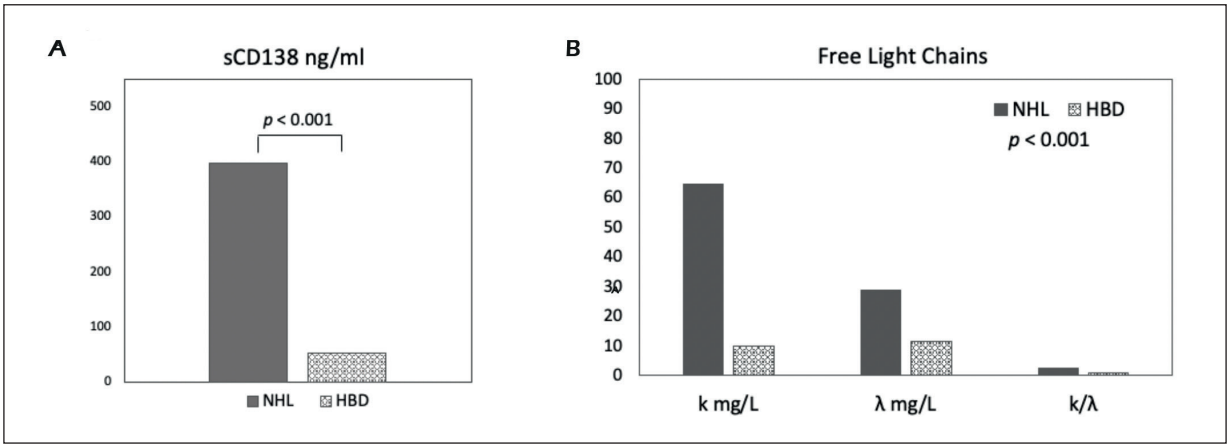

Figure 1. A, Distribution of serum levels of sCD138 between patients and controls. **B,** Distribution of serum levels of FLC between patients and controls.

were above the cut off in 19/35 patients with a mean value that was higher and statistically significant than in HBD (p<0.001). As result of this abnormal increment of FLC, the k/λ ratio was above the cut-off in 28/35 HCV NHL patients, with a mean value that was significantly higher than in HBD (p<0.001). We evaluated if levels of serum sCD138 in HCV positive-NHL with MC patients correlated with an abnormal FLC profile. We found that both free k and λ and the k/λ ratio were inversely related with sCD138, with a r<0 (Figure 2).

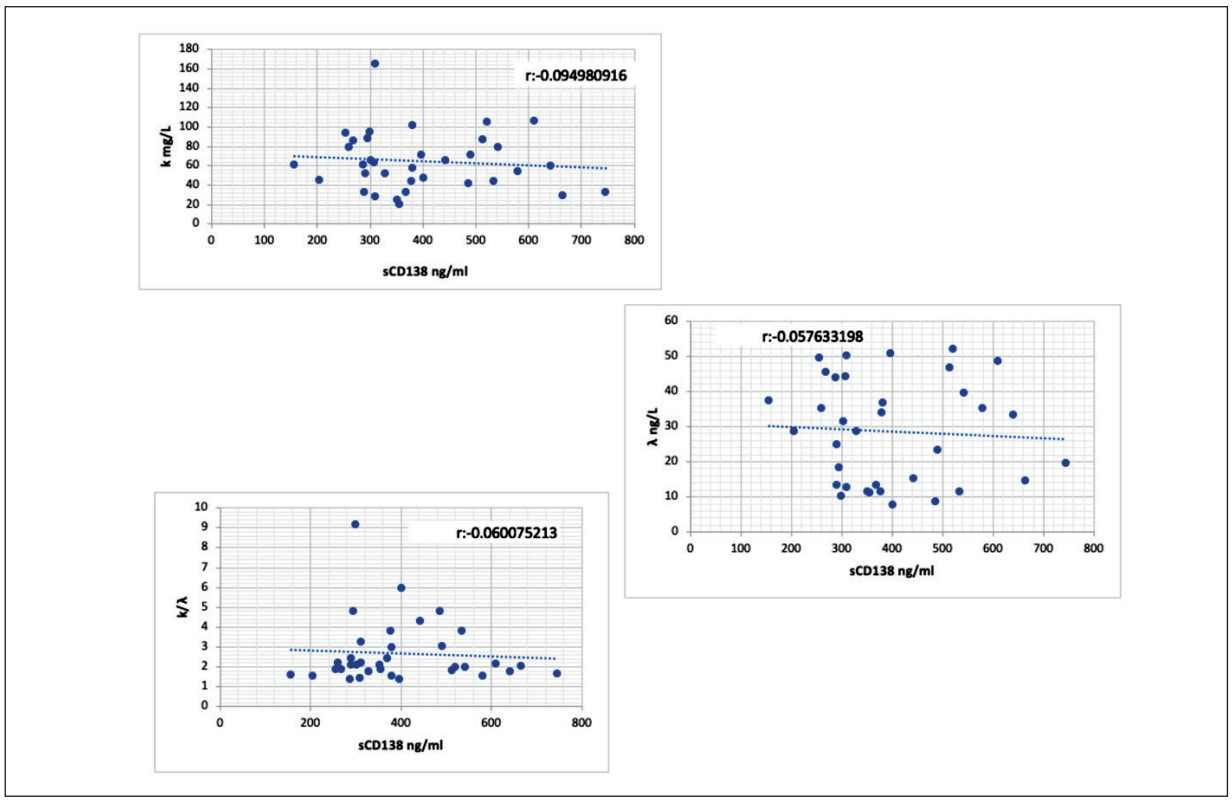

Figure 2. Correlations between serum sCD138 and FLC in HCV positive-NHL with MC patients.

Discussion

The measurement of serum tumor markers levels is an economic and noninvasive diagnostic assay often requested to get information about the presence or absence of disease, and minimal residual disease (MRD). Despite in clinical practice, the real usefulness for patient monitoring during follow up as well as response to therapy evaluation in case of advanced cancer is frequently debate. In particular, the ideal serum tumor marker should be able to early detect diseases, predict response or resistance to specific therapies and monitor patients after primary therapy. Expression of markers within the same tumor can change depending on the specific site or stage of cancer. Despite this complexity and variability, most types of cancer are treated with the same generic therapies.

In a previous study[33] we showed early prognostic biomarkers in low CGs, correlation between vascular endothelial growth factor, IgG subclasses and FLCs representing a multistage inflammation of acquired immune system[9]. Moreover, we correlated serum biomarkers with clinical and laboratory response suggesting high levels of FLCs, IgM Heavy Chain and VEGF could represent the activity to identify MRD indicative of possible relapse or worsening outcome[34]. In the present research, we analyzed the potential value of sCD138 and FLCs as serum biomarker to be implemented for further use in the context of HCV-NHL with a type II CG.

sCD138 is released at high levels in some pathological states, such as inflammations or microbial infections, but also in tumors[26,35]. Our results show that sCD138 and FLCs levels are significantly higher in our patient group if compared with HBD reflecting the double face of this disease: a neoplastic dysregulation of B cell. When we analyzed the trends of these two parameters in our patients looking for a correlation, we found an inverse trend, suggesting that sCD138 and FLC are two independent biomarkers of disease. sCD138 reflects the high tumor burden of disease while FLC is a specific clue of the monoclonal component.

sCD138 released by membrane cell can accumulate in the extracellular matrix or spread into the circulation where it remains in soluble state and can continue to exercise its function as a signal molecule, reaching very high levels in patients suffering from particular cancerous forms. By acting as key regulators of "cell signaling", through their interactions with numerous growth factors and factors promoting angiogenesis, heparansulfates mediate a dramatic transformation of the microenvironment that supports the growth of the tumor as a real "organ" and promote the "aggressive" phenotype. By conditioning the extracellular microenvironment, CD138 amplifies the signal of cell growth factors thus supporting tumor growth[36,37].

In patients with DLBCL the prognostic performance of FLC assay is altered by the presence of monoclonal IgM, while patients without a monoclonal component FLC assay result to be of prognostic relevance. The mechanism underlying this association is not still clear. Elevated FLC correlates with laboratory testing that indicate the immune system or B cell activation, reflecting an environment of cytokines or other immune system interactions favorable for malignant B cell growth[24].

Our HCV-MC patients with B-NHL display a free k/λ ratio that is specifically higher then HBD, and mostly altered considering our previous papers analyzing patients at precedent stage to B-NHL[9,33].

FLCs assays rely on the assumption that the assessment of an unbalanced production of FLC in a monoclonal gammopathy yields an altered FLC k/λ ratio. This is considered a reliable tool to define the disease grade of B-NHL. B-NHL is worsened by monoclonal IgM k type II CG, when the HCV-driven pathogenetic process leads the lymphomagenesis towards a no-return point making it progressively independent from the etiologic agent. Considering the chronic nature of HCV infection, we suggest that a sustained virus-driven antigenic stimulation could induce B cell clonal mostly in the liver and, less frequently, in the bone marrow or blood[6]. Clonal B cell expansion could be correlated to the presence of extrahepatic manifestations of HCV, high levels of CG and FLCs, and overt B cell of NHL. The well investigated strength association between FLC and autoimmunity focused the questions regarding their role in the pathogenesis and diagnosis of human diseases. Different reports showed a correlation between FLC levels and disease activity suggesting that FLCs could be considered bioactive molecules rather than a secondary product of the synthesis of immunoglobulins, acting as pathogenic mini-autoantibodies in different disease-settings[21,38,39].

Conclusions

The hepato- and lympho-tropism of HCV may be responsible for the variety of hepatic and extra-

hepatic immune-mediated and neoplastic disorders, such as mixed cryoglobulinemia syndrome and B-cell NHL. Precision medicine devoted to neoplastic disease is an evolving approach for the management of cancer patients that aims to target the new knowledge on pathogenesis to more precise tailored therapy. Many challenges are still open to identify clinically relevant markers for disease susceptibility and treatment efficacy. In the era of biological drugs, starting from our results, upon further deep analysis and validation on a large number of patients, the employment of sCD138 and FLC might change the profile of the HCV related syndromes, for prevalence, clinical characteristics and prognosis with an improvement of clinical investigation.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This research and its publication have been funded from Università Cattolica del Sacro Cuore Fondazione Policlinico Universitario Agostino Gemelli IRCCS (Rome, Italy) as a part of its programs on promotion and dissemination of scientific research (Linea D1 to MM).

References

1) Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009; 49: 1335-1374.
2) Gasparotto D, De Re V, Boiocchi M. Hepatitis C virus, Bcell proliferation and lymphomas. Leuk Lymphoma 2002; 43: 747-751.
3) Ferri C, Caracciolo F, Zignego AL, La Civita L, Monti M, Longobardo G, Lombardini F, Greco F, Capochiani E, Mazzoni A. Hepatitis C virus infection in patients with nonHodgkin's lymphoma. Br J Haematol 1994; 88: 392-394.
4) Silvestri F, Barillari G, Fanin R, Pipan C, Falasca E, Salmaso F, Zaja F, Infanti L, Patriarca F, Botta GA, Baccarani M. Hepatitis C virus infection among cryoglobulinemic and noncryoglobulinemic Bcell nonHodgkin's lymphomas. Haematologica 1997; 82: 314-317.
5) Izumi T, Sasaki R, Miura Y, Okamoto H. Primary hepatosplenic lymphoma: association with hepatitis C virus infection. Blood 1996; 87: 5380-5381.
6) Zignego AL, Gragnani L, Piluso A, Sebastiani M, Giuggioli D, Fallahi P, Antonelli A, Ferri C. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. Expert Rev Clin Immunol 2015; 11: 15-31.
7) Basile U, Gulli F, Gragnani L, Pocino K, Napodano C, Miele L, Santini SA, Marino M, Zignego AL, Rapaccini GL. Different biochemical patterns in type II and type III mixed cryoglobulinemia in HCV positive patients. Dig Liver Dis 2018; 50: 938-943.
8) Gulli F, Basile U, Gragnani L, Fognani E, Napodano C, Colacicco L, Miele L, De Matthaeis N, Cattani P, Zignego AL, Rapaccini GL. Autoimmunity and lymphoproliferation markers in naïve HCV-RNA positive patients without clinical evidences of autoimmune/lymphoproliferative disorders. Dig Liver Dis 2016; 48: 927-933.
9) Basile U, Marino M, Gragnani L, Napodano C, Gulli F, Pocino K, Lorini S, Santini SA, Basile V, Miele L, Zignego AL, Rapaccini GL. Sentinel biomarkers in HCV positive patients with mixed cryoglobulinemia. J Immunol Methods 2020; 476: 112687.
10) Basile U, Gulli F, Isgrò MA, Napodano C, Pocino K, Santini SA, Gragnani L, Conti L, Rossi E, Cordone I, Zignego AL, Rapaccini GL, Cigliana G, Berruti F, Todi L, Marino M, Di Stasio E. A novel biomarker score for the screening and management of patients with plasma cell proliferative disorders. Eur Rev Med Pharmacol Sci 2019; 23: 4293-4302.
11) Ferri C, Zignego AL, Pileri SA. Cryoglobulins. J Clin Pathol 2002; 55: 4-13.
12) Monti G, Pioltelli P, Saccardo F, Campanini M, Candela M, Cavallero G De Vita S, Ferri C, Mazzaro C, Migliaresi S, Ossi E, Pietrogrande M, Gabrielli A, Galli M, Invernizzi F. Incidence and characteristics of non-Hodgkin lymphomas in a multicenter case file of patients with hepatitis C virus-related symptomatic mixed cryoglobulinemias. Arch Intern Med 2005; 165: 101-105.
13) Ferri C, La Civita L, Zignego AL, Pasero G. Viruses and cancers: possible role of hepatitis C virus. Eur J Clin Invest 1997; 2: 711-718.
14) Zhao LJ, Chen F, Li JG, Yin R, Zhang XH, Huang SM, Liu F. Hepatitis C virus-related mixed cryoglobulinemic endocapillary proliferative glomerulonephritis and B-cell non-Hodgkin lymphoma: a case report and literature review. Eur Rev Med Pharmacol Sci 2015; 19: 3050-3055.
15) Machida K, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, Lai MY, Lai MMC. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci U S A 2004; 101: 4262-4267.
16) Peveling-Oberhag J, Arcaini L, Hansmann ML, Zeuzem S. Hepatitis C-associated B-cell non-Hodgkin lymphomas. Epidemiology, molecular signature and clinical management. J Hepatol 2013; 59: 169-177.
17) Khaled H, Abu-Taleb F, Haggag R. Hepatitis C virus and non-Hodgkin's lymphomas: a minireview. J Adv Res 2017; 8: 131-137.
18) Arcaini L, Paulli M, Boveri E, Vallisa D, Bernuzzi P, Orlandi E. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting fea-

tures but similar morphologic and phenotypic profiles. Cancer 2004; 100: 107-115.

19) Torres HA, Mahale P. Most patients with HCV-associated lymphoma present with mild liver disease: a call to revise antiviral treatment prioritization. Liver Int 2015; 35: 1661-1664.

20) Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, Palumbo A, Jagannath S, Blade J, Lonial S, Dimopoulos M, Comenzo R, Einsele H, Barlogie B, Anderson K, Gertz M, Harousseau JL, Attal M, Tosi P, Sonneveld P, Boccadoro M, Morgan G, Richardson P, Sezer O, Mateos MV, Cavo M, Joshua D, Turesson I, Chen W, Shimizu K, Powles R, Rajkumar SV, Durie BG; International Myeloma Working Group. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009; 23: 215-224.

21) Gulli F, Napodano C, Marino M, Ciasca G, Pocino K, Basile V, Visentini M, Stefanile A, Todi L, De Spirito M, Rapaccini GL, Basile U. Serum immunoglobulin free light chain levels in systemic autoimmune rheumatic diseases. Clin Exp Immunol 2020; 199: 163-171.

22) Basile U, Marino M, Napodano C, Pocino K, Alboini PE, Gulli F, Evoli A, Provenzano C, Bartoccioni E. Serological immunoglobulin-free light chain profile in myasthenia gravis patients. J Immunol Res 2018; 2018: 9646209.

23) Cigliana G, Gulli F, Napodano C, Pocino K, De Santis E, Colacicco L, Cordone I, Conti L, Basile U. Serum free light chain quantitative assays: dilemma of a biomarker. J Clin Lab Anal 2018; 32: e22243.

24) Maiolo E, Alma E, Napodano C, Gulli F, Bellesi S, Cuccaro A, Pocino K, D'Alo' F, Iachini M, Martini M, Larocca LM, De Stefano V, Basile U, Hohaus S. The prognostic impact of monoclonal immune globulin and free light chain secretion in diffuse large B cell lymphoma (DLBCL). Leuk Lymphoma 2020; 61: 1133-1139.

25) Cigliana G, Torti E, Gulli F, De Santis E, Dell'Abate MT, Colacicco L, Pisani F, Conti L, Basile U. Relationship between circulating syndecan-1 levels (CD138s) and serum free light chains in monoclonal gammopathies. J Exp Clin Cancer Res 2015; 34: 37.

26) Wołowiec D, Dybko J, Wróbel T, Urbaniak-Kujda D, Jaźwiec B, Tomaszewska-Toporska B, Kapelko-Słowik K, Potoczek S, Kuliczkowski K. Circulating sCD138 and some angiogenesis-involved cytokines help to anticipate the disease progression of early-stage B-cell chronic lymphocytic leukemia. Mediators Inflamm 2006; 3: 42394.

27) Shi Q, Jiang J, Luo G. Syndecan-1 serves as the major receptor for attachment of hepatitis C virus to the surfaces of hepatocytes. J Virol 2013; 87: 6866-6875.

28) Zvibel I, Halfon P, Fishman S, Penaranda G, Leshno M, Or AB, Halpern Z and Oren R. Syndecan1 (SCD138) serum levels: a novel biomarker in predicting liver fibrosis stage in patients with hepatitis C. Liver Int 2009; 29: 208-212.

29) Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica 2010; 102: 83-87.

30) Gulli F, Santini SA, Napodano C, Bottoni P, Pocino K, Rapaccini GL, Basile U. Cryoglobulin test and cryoglobulinemia hepatitis c-virus related. Mediterr J Hematol Infect Dis 2017; 9: e2017007.

31) Napodano C, Pocino K, Gulli F, Colacicco L, Santini SA, Zuppi C, Basile U. Comparison of fully automated and semiautomated systems for protein immunofixation electrophoresis. J Clin Lab Anal 2017; 31: e22027.

32) Gulli F, Basile U, Gragnani L, Napodano C, Pocino K, Miele L, Santini SA, Zignego AL, Gasbarrini A, Rapaccini GL. IgG cryoglobulinemia. Eur Rev Med Pharmacol Sci 2018; 22: 6057-6062.

33) Basile U, Napodano C, Pocino K, Gulli F, Santini SA, Todi L, Marino M, Rapaccini GL. Serological profile of asymptomatic HCV positive patients with low level of cryoglobulins. Biofactors 2019; 45: 318-325.

34) Basile U, Gulli F, Napodano C, Pocino K, Basile V, Marrapodi R, Colantuono S, Todi L, Marino M, Rapaccini GL, Visentini M. Biomarkers of minimal residual disease in rituximab-treated patients with mixed cryoglobulinemia. Biotechnol Appl Biochem 2020. doi: 10.1002/bab.1929. Online ahead of print.

35) Alonci A, Allegra A, Bellomo G, D'Angelo A, Penna G, Cannavò A, Musolino C. Soluble CD138 serum levels are not associated with other poor prognostic markers in patients with B-chronic lymphocytic leukaemia. Med Oncol 2010; 27: 1336-1339.

36) Gotte M, Kersting C, Ruggiero M, Tio J, Tulusan AH, Kiesel L, Wülfing P. Predictive value of syndecan-1 expression for the response to neoadjuvant chemotherapy of primary breast cancer. Anticancer Res 2006; 26: 621-627.

37) Nguyen TL, Grizzle WE, Zhang K, Hameed O, Siegal GP, Wei S. Syndecan-1 overexpression is associated with nonluminal subtypes and poor prognosis in advanced breast cancer. Am J Clin Pathol 2013; 140: 468-474.

38) Napodano C, Pocino K, Rigante D, Stefanile A, Gulli F, Marino M, Basile V, Rapaccini GL, Basile U. Free light chains and autoimmunity. Autoimmun Rev 2019; 18: 484-492.

39) Basile U, Gulli F, Gragnani L, Napodano C, Pocino K, Rapaccini GL, Mussap M, Zignego AL. Free light chains: eclectic multipurpose biomarker. J Immunol Methods 2017; 451: 11-19.