Micro and nanoparticles as possible pathogenetic co-factors in mixed cryoglobulinemia

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Abstract

Mixed cryoglobulinemia (MC) is a rare multisystem disease whose aetiopathogenesis is not completely understood. Hepatitis C virus (HCV) infection may have a causative role, and genetic and/or environmental factors may also contribute.

To investigate the presence and possible role of environmental agents in MC.

We recruited 30 HCV-infected MC patients with different clinical manifestations and a control group of 30 healthy, sex-/age-matched volunteers. We collected serum samples from each patient and incubated at 4°C for 7 days to obtain cryoprecipitate samples.We used environmental scanning electron microscopy (ESEM) and energy dispersive X-ray spectroscopy microanalysis to verify the presence of microparticles (MPs) and nanoparticles (NPs) in serum and cryoprecipitate samples.We evaluated environmental exposure using a medical and occupational history questionnaire for each subject.

MC patients had a signi cantly higher risk of occupational exposure (OR 5.6; 95% CI 1.84–17.50) than controls. ESEM evaluation revealed a signi cantly higher concentration, expressed as number of positive spots (NS), of serum inorganic particles in MC patients compared with controls (mean NS 18, SD = 16 versus NS 5.4, SD = 5.1; $P < 0.05$). Cryoprecipitate samples of MC patients showed high concentrations of inorganic particles (mean NS 49, SD = 19).We found a strong correlation between NS and cryocrit (i.e. percentage of cryoprecipitate/total serum after centrifugation at 4°C) levels ($P < 0.001$).

In addition to HCV infection, MPs and NPs might play an important role in the aetiopathogenesis of MC.

Introduction

Mixed cryoglobulinemia (MC) is a systemic vasculitis characterized by multiple organ involvement due to the vascular deposition of circulating immune complexes, mainly mixed IgG–IgM cryoglobulins and complement [1]. Clinically, MC is considered a rare multisystem dis- ease and represents a crossroad between classical rheu- matic diseases and other autoimmune lymphoproliferative disorders. Although numerous epidemiological studies have reported the presence of circulating cryoglobulins in >50% of hepatitis C virus (HCV)-infected individu- als, MC develops in only a minority of these individuals

(<5%) [2,3]. Thus, it is possible that other unknown fac- tors may contribute to the pathogenesis of MC.

The impact of environmental pollution on health has been discussed since the middle of the 20th century [4]. In the past decade, toxicological studies have demon- strated that small organic and inorganic microparticles (MPs) and nanoparticles (NPs) can pass rapidly into the circulatory system [5]. Since there are no reports in the literature focusing on particle exposure history and MC, this preliminary study aimed to evaluate the presence and possible role of environmental agents such as MPs/ NPs in MC patients.

Methods

We investigated 30 consecutive HCV-infected (with detectable serum HCV-RNA) MC patients compared to 30 healthy, sex-/age-matched volunteers (Table 1). The pre-clinical study was approved by the University of Modena and Reggio Emilia Ethics Committee. All study subjects (cases and controls) were resident in the Italian province of Modena and recruited at the Policlinico Hospital of the University of Modena and Reggio Emilia. We interviewed all subjects using a structured questionnaire (Appendix, available as Supplementary data at *Occupational Medicine* Online) based on a previ- ous model [6] and we categorized four major sources of particle pollution: occupational exposure, environmental exposure, smoking habits and prosthesis implants. After informed consent, we collected data from all subjects and

fresh blood samples in silica and metal-free polystyrene tubes (Vacutest Kima, PD, Italy), which we immediately kept at 37°C for 2h. We separated serum by centri- fuging blood samples at 37°C for 10min at 2500rpm. Thereafter, we stored serum samples at 4°C for 7 days and we quanti ed the cryoprecipitates as cryocrit, i.e. percentage of cryoprecipitate/total serum after centrifu- gation at 4°C. We analysed serum and cryoprecipitate samples by environmental scanning electron microscopy (ESEM Quanta200; Fei, Netherlands) using the method previously described by Fassina *et al*. [7].We scanned all samples inside a selected area of each sample at ×40. Inside this area, we analysed 64 non-continuously and non-overlapping zones at ×400. We counted the num- ber of positive spots (NS) containing MPs or NPs in the analysed zones of each sample for MC patients and con- trols. We investigated particle chemical composition by energy dispersive X-ray spectroscopy (EDS) microanaly- sis (INCA; Oxford Instruments, UK).

Results

The demographic and clinico-serological and virologi- cal features of MC patients are reported in Table 1. The type II/type III ratio of serum mixed cryoglobulins in MC patients was 3:1, while cryoglobulins were absent in all serum samples from controls. Clinical features of cryoglobulinemic patients included arthralgia, weakness, purpura, peripheral neuropathy, hypothyroidism, liver and renal involvement, con rming previously observed prevalence rates [1]. All patients were HCV infected and three of them were also hepatitis B virus infected.

MC was associated with a signi cantly higher preva- lence of occupational exposure compared with controls (OR 5.6; 95% CI 1.84–17.50; Table 1). All 19 exposed patients were involved in occupations with intermedi- ate or high silica exposure (ceramic, foundry and metal workers, painters and decorators).

Twenty-four of the 30 control subjects had few inor- ganic particles in their serum samples with mean NS of 5.4 (SD = 5.1).These levels were statistically lower com- pared with the mean NS of 18 (SD = 16) detected in

27/30 sera of MC patients ($P < 0.05$; Figure 1A). The cryoprecipitate samples of MC patients group had a higher NS (mean 49; SD = 19) compared with the serum samples of controls (mean 5.4; SD = 5.1; $P < 0.001$; Figure 1B). The presence of both MPs and NPs was sig- ni cantly higher in MC patients cryoprecipitate samples compared with MC patients serum samples ($P < 0.001$) (Figure 1C). We found no correlation between particles presence and cryoglobulinemia types or MC dura- tion, but there was a statistically signi cant correlation ($r = 0.923$; $P < 0.001$) between the NS and cryocrit lev- els (Figure 1D).

EDS analysis, of the elemental composition of ne particulate matter in the samples, indicated that particles had a complex composition that included several ele- ments in minor or trace amounts: Si (50%), Fe (35%),Ti (31%), Al (29%) and Mg (19%) and a lower frequency (<1%) for Cu, Zn, Mn, Ni, Cr, Ba, Sn and Zr.

Discussion

We found high or intermediate occupational silica expo- sure to be signi cantly associated with MC. ESEM analysis con rmed the presence of MPs and NPs in cryoglobulinemic subjects (Si, Al, Fe and Ti particles). NS was signi cantly correlated to cryocrit levels, sug- gesting possible involvement of MPs and NPs particles in MC immune complex composition.

Although we evaluated a limited number of patients, the comparison of MC patients with healthy controls from the same residential area and the ESEM qualitative/quantita- tive analysis of samples represent a strength of our study.

In particular, the prevalence of Si in all MC samples sug- gests a possible role of silica in MC. Previous data concerning individuals with silica exposure developing anti-neutrophil cytoplasmic antibody-associated systemic vasculitis sup- port our hypothesis [8]. Also, recent *in vitro* studies showed that silica nanoparticles can indirectly induce circulating immune complex formation by absorbing on their surface human IgM rheumatoid factor and IgG [9].

For a better understanding of the *in vivo* toxicity of particles and their actual role in the pathogenesis of MC, future research should include a biophysical approach considering the predominant elements found (Si, Al, Fe and Ti) and their possible immunological interactions. Finally, the study of MPs and NPs in a signi cant number of MC patients not infected with HCV, quite a rare dis- ease variant in Italy, might further reinforce the suggested aetiopathogenetic role of these environmental co-factors.

Figure 1

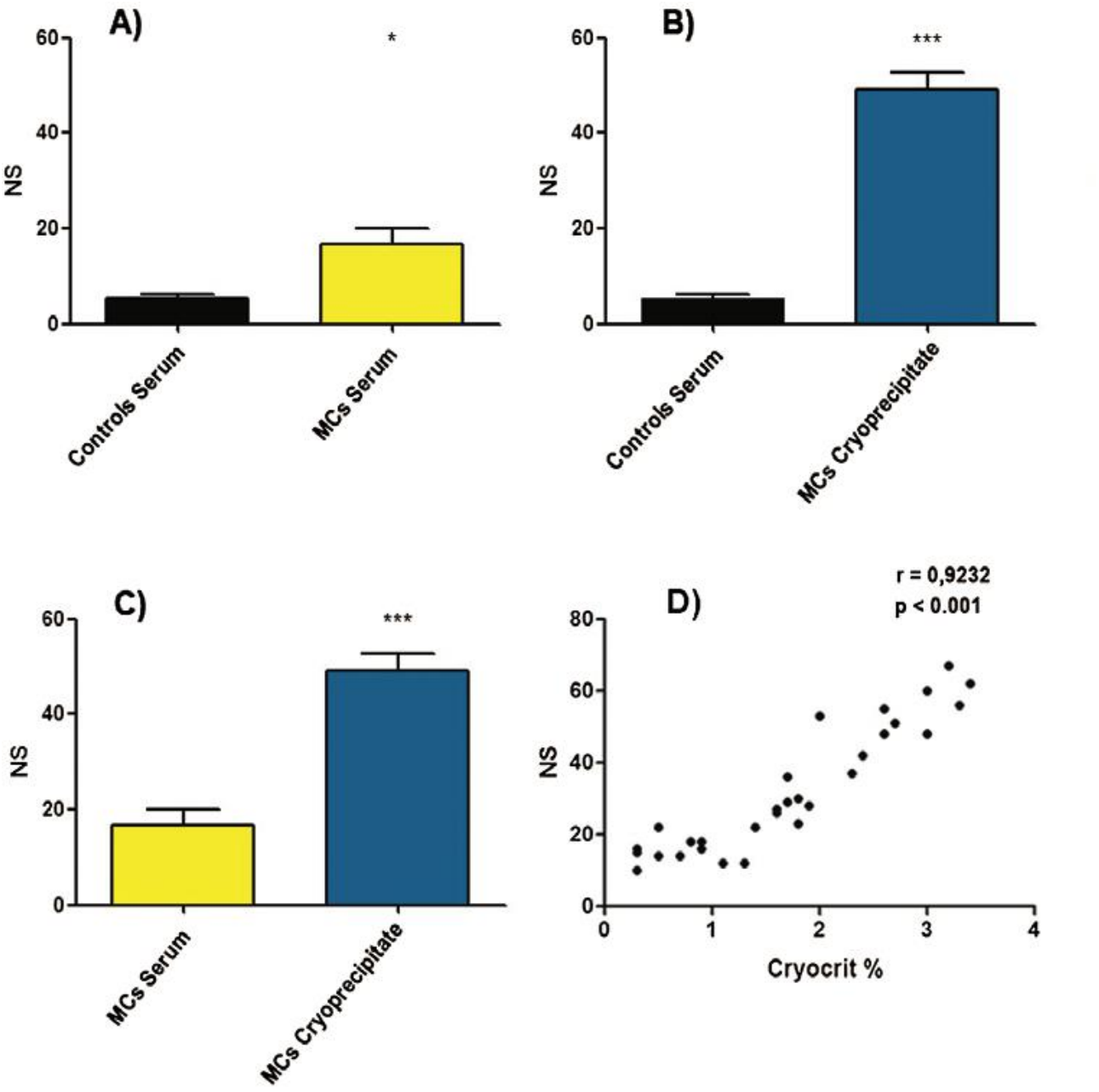

Figure 1. (A) NS containing MPs or NPs in controls serum samples versus MC serum samples (*$P < 0.05$); (B) NS in controls serum samples versus MC cryoprecipitate samples (***$P < 0.001$); (C) NS in MC serum samples versus MC cryoprecipitate samples (***$P < 0.001$). NS are reported as a mean ± SD. (D) Correlation between NS containing particles and cryocrit levels (%) of MC patients.

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