

Pro-inflammatory status is not a limit for longevity.

Case report of a Sicilian centenarian

Anna Aiello^{1*}, Giulia Accardi^{1*}, Stefano Aprile¹, Rosalia Caldarella², Giuseppe Cammarata³,
Ciriaco Carru⁴, Calogero Caruso¹, Marcello Ciaccio^{2,5}, Caterina Maria Gambino⁵, Mattia Emanuela
Ligotti¹, Sonya Vasto, Giuseppina Candore¹

¹Laboratory of Immunopathology and Immunosenescence, Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy; ²Department of Laboratory Medicine, “P. Giaccone” University Hospital, Palermo, Italy; ³Institute for Biomedical Research and Innovation, National Research Council of Italy, Palermo, Italy; ⁴Department of Biomedical Sciences, University of Sassari, Sassari, Italy; ⁵Unit of Clinical Biochemistry, Clinical Molecular Medicine, and Laboratory Medicine, Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy; Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy.

*These authors contributed equally

Address correspondence to:

Calogero Caruso M.D.

Professor Emeritus

Laboratory of Immunopathology and Immunosenescence

Department of Biomedicine, Neuroscience and Advanced Diagnostics

University of Palermo

Corso Tuköry 211

90134, Palermo, Italy

E-mail: calogero.caruso@unipa.it

Introduction

Most studies on centenarians represent them as the best model of ageing. They have been defined “delayers” because of not exhibit age-related diseases, “survivors” if they have shown clinically demonstrable diseases prior to the age of 80 years old, and “escapers” when they attain their 100th year of life without any common age-associated pathologies. However, the extreme longevity is often characterized by a not unique and unequivocal phenotype, as demonstrated by the centenarian population worldwide. Not all centenarians are alike, but everyone can represent a model of “positive biology” [1].

The present paper shows the interesting case of CC, a female centenarian living in Sicily (Italy). CC has become centenarian in seemingly good health despite the presence of sub-optimal biochemical parameters.

To study the peculiar features of this subject anamnestic, cognitive, biochemical, genetic, and epigenetic data were collected. None age-related diseases have been detected with the exception of a supposed mild cognitive impairment. CC showed some clinical signs of atrophic gastritis and a clear chronic status of inflammation, but her level of some microRNAs (miRNAs), with a role in the control of innate immunity and inflammation, were higher than other centenarians (unpublished data).

Therefore, CC can be considered a case appealing and useful to provide new insights into the extreme phenotype represented by extreme longevity.

Case report

The recruitment of the subject was conducted within the project “Discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and comorbidities (DESIGN)”, funded by the Italian Ministry of Education, University and Research [2].

On December 12th, 2017, well-trained nutritionists and physicians from University of Palermo administered to the centenarian a detailed questionnaire. It includes nine sections: main pathologies, drugs, smoking, cognitive status (mini-mental state examination, MMSE), geriatric depression scale (GDS), activities of daily living (ADL), instrumental activity of daily living (IADL), sleep, and eating habits. The authors also investigate the family history. The informed consent was obtained from the centenarian assisted by her daughter, also for the release of the sensitive family data. She underwent venipuncture, after a fasting period of 12 hours, in the morning (10 a.m.). The blood was collected in specific tubes containing EDTA or no additives for different analyses, including haematochemical parameters, autoantibodies, oxidative and inflammatory markers, miRNAs, and other genetic tests. Lastly, to obtain informative and

reproducible body composition analysis, weight and height were measured, and single-frequency bioelectrical impedance analysis (BIA) was performed in the supine position, with light clothes and barefoot.

CC was born on October 2nd, 1917. Her father and her mother died respectively at age of 93 and 92 years old (unknown causes). She received the primary school diploma and was a housewife. She was a widow at a young age and lived in a flat with her daughter and her son-in-law. They had a quite high income. She never smoked, slept 10 hours per night and was treated with anti-hypertensive and anxiolytic drugs as well as antiplatelet agents, *i.e.*, low-dose aspirin. It was possible to administer both the GDS and MMSE, receiving a score of 10 out of 15 (absence of depression) and 18 out 30 (mild cognitive impairment or low level of education) respectively. For the assessment of ADL (*e.g.*, personal hygiene, dressing, toileting/continence, ambulating, and eating) she was autonomous. Conversely, for IADL (*e.g.*, food preparation, financial administration, housekeeping, use of telephone, responsibility for own medication) she demonstrated a decrease in performing these activities, in the last years.

About eating habits, during young age, CC followed a Mediterranean dietary pattern characterized by a daily consumption of milk and dairy products, cereals like bread and pasta, and fruits and vegetables. She assumed legumes and red meat twice a week and rarely eggs and fishes. During the last month before the interview, concerning food group frequency intake, it was reported a daily consumption of carbohydrates as pasta, bread, and cereal derivatives like rusks, dressing such as extra-virgin olive oil, vinegar, and lemon, only whole milk as dairy products, and seasonal fruits and vegetables. She assumed once a week others carbohydrate like rice and potatoes, meat red only, eggs, legumes, two or three times a week honey as sweeteners, and mature and cream cheese as dairy products, rarely or never fishes, white meat, seafood, sugar, and desserts.

At the time of the visit, she weighed 45 kg with a height of 147 cm and a body mass index of 20.8 Kg/m². The analysis of body composition by bioimpedance assay led to the conclusion that the centenarian presented a loss in body cell mass (BCM). At the same time, it was observed an hyperhydration due to the ratio between extracellular mass (ECM) and BCM (ECM/BCM>1). ECM contains all the metabolically inactive tissues, whereas BCM contains all the metabolically active tissues of the body. Conventionally, this value of ECM/BCM ratio is representative for malnutrition, but in this case, due to reasonable physical condition of the subject, we can speculate that this datum does not correspond to the physical examination. In fact, it was not observed a cachectic status. It is important to note that BIA presents limitations when applied to centenarians because current reference values have been developed starting from analyses in healthy subjects [3].

About genetic tests, she was genotyped for Forkhead box O3A (FOXO3A) rs2802292 and Apolipoprotein (Apo)E. She carried, in heterozygosity (G/T), the G single nucleotide polymorphism of FOXO3A gene known to be associated with longevity in different populations. Concerning ApoE alleles, she carried the neutral $\epsilon 3$ in homozygote state ($\epsilon 3/\epsilon 3$) [2,3].

For all the rest of the results and acronyms, see Table 1. Regarding haematochemical values, CC displayed levels of total cholesterol, LDL, creatinine, urea, and glycaemia slightly out of range. Instead, osteocalcin levels, marker of bone turnover, were more than double out of range. It is noteworthy that CRP value was in the reference range although other inflammatory markers were increased. Among haematological results, MCV and MCH were marginally out of range, suggesting a possible deficiency of vitamin B12. In roughly two-thirds of all cases of this deficiency RDW, a measure of the range of variation of red blood cell volume and size, is elevated, as it was in CC at the time of the recruitment. Accordingly, she showed very low levels of vitamin B12, although without anaemia. Possible causes are chronic inflammation of the pancreas, intestinal parasites, certain medications, genetic disorders, scarce intake, increased requirement, or decreased absorption. This is generally due to deficiency of intrinsic factor linked to gastric atrophy or surgical removal of the stomach. For this reason, autoimmunity tests were performed, demonstrating positivity of APCA and anti-intrinsic factor antibodies (both by immunoblot). This indicates the presence of IgG against H^+/K^+ -ATPase and intrinsic factor respectively, suggesting the possibility of atrophic gastritis with damage of parietal cells of the corpus and fundus of the stomach. The known consequence is vitamin B12 deficiency for the lower availability of intrinsic factor. However, the complete autoimmune analysis showed also a positive fluoroscopic pattern to anti-Jo-1 antigen at 1:160 dilution (by indirect immunofluorescence assay), confirmed by an immunoblotting assay, as recommended by guidelines. Anti-Jo-1 are antibodies directed against the cytoplasmic protein histidyl-tRNA synthetase which catalyses the binding of the histidine to its cognate tRNA during protein synthesis. Usually, they are associated with polymyositis and dermatomyositis. However, our centenarian did not show clinical signs related to these diseases.

About oxidative stress and inflammation, she appeared in a suffering status, except for GSH and MDA levels, the main product of the polyunsaturated fatty acids peroxidation. The anti-oxidant PON and TAU were significantly below the reference range. The increased ADMA/ARG ratio, consequently to the enhanced activity of arginine methylating enzymes, is another important index of oxidative stress. The pro-inflammatory status was demonstrated by high levels of KYN, a marker of immune system activation, and of KYN/TRP ratio, a sensitive biomarker of systemic inflammation. Moreover, levels of HCY above the range were also observed as well as CYS values

due to the accumulation of HCY [3]. However, no noteworthy inflammatory pathologies have been found outside the possible mild cognitive deficit. At this regard, it was performed the genotyping of methylenetetrahydrofolate reductase (MTHFR) gene. The MTHFR enzyme is involved in the conversion of 5,10-methylen-tetrahydrofolate to 5-methyl-tetrahydrofolate, a molecule that allows the remethylation of homocysteine into methionine, through the intervention of vitamin B12 as a cofactor. Notwithstanding, CC carried only one mutation at MTHFR gene position C677T, so she was heterozygous as about 30 to 40 percent of Caucasian population. In addition

Conclusion

Centenarians and supercentenarians could present the same risk factors and detrimental alleles for common diseases than the general people. Nevertheless, the interaction between genetics, epigenetics, lifestyle, and environment can positively modulate the maintenance of molecular cellular mechanisms, suggesting the presence of protective genetic and/or epigenetic factors in the long-lived individuals [1]. It is not known whether the observed seeming fair physical condition of CC, despite the abnormality of laboratory parameters, depends on the presence of protective alleles (it is noteworthy, as reported by her relatives, that at the date of March 18th, 2020, she was in a relatively good health state). However, miRNA data suggest a possible epigenetic modulation, likely with anti-inflammatory effects, that confer protection against tissue damage. In particular, decreased expression of miR-181a has been observed in the patients with coronary artery disease and it has been suggested to have an antiatherogenic effect through blocking NF- κ B activation and vascular inflammation [5].

Our data strengthen the suggestion of the possible importance of miRNA restoration or of their mimetics as therapeutic agents for inflammatory diseases.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. The Institutional Ethics Committee (“Paolo Giaccone”, University Hospital) approved the DESIGN study protocol (Nutrition and Longevity, No. 032017). The study was conducted in accordance with the Declaration of Helsinki and its amendments.

Informed Consent. Informed consent was obtained from the centenarian assisted by her daughter.

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